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(54) Title: NOVEL PEPTIDE VACCINES AND USES THEREOF



Figure 4C

(57) Abstract: Novel pharmaceutical compositions comprising immunogenic peptide and method of using these compositions for immunomodulation, specifically, in prevention and treatment of infections by SARS-CoV-2 virus.



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NOVEL PEPTIDE VACCINES AND USES THEREOF

FIELD OF THE INVENTION

The invention relates to peptide vaccines and method of using the same. Specifically, the invention related to pharmaceutical compositions comprising immunogenic peptides for prevention and treatment viral infections.

BACKGROUND OF THE INVENTION

Vaccination is an effective way to improve public health by building up adaptive immunity to a target pathogen [52].

However, it takes considerable time to screen vaccine targets for clinical validation and production of a vaccine. Advances in bioinformatics and next-generation sequencing technology, immunoinformatics and reverse vaccinology can minimize the time for screening antigens from protein sequences of pathogens and utilitarian advantage in the search of potential new vaccine targets [53, 54].

A new coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 or COVID-19) has recently emerged as a human pathogen that causes fever, pulmonary disease and pneumonia [1-3]. Following an outbreak that initiated in China, human-to-human infection has spread rapidly across the world. The COVID-19 global pandemic is more severe than the previous coronavirus related outbreaks of the severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle-East respiratory syndrome (MERS-CoV) [4-6]. By June 2, 2021, more than 171M people were infected, and more than 3.5M people died globally from COVID-19.

The current global emergency of the COVID-19 outbreak prompts the urgent need of a vaccine against SARS-CoV-2 [56]. Currently, people keep dying from COVID-19 or remain incapacitated due to post-Covid complications. Understanding the part of the SARS-CoV-2 protein sequence that can generate an immune response in

humans, thus facilitate design and production of novel safe and efficacious vaccines against this viral pathogen, which, remains an urgent and unmet need for the whole world population.

SUMMARY OF THE INVENTION - WILL BE FINALIZED ONCE CLAIMS ARE

5 APPROVED

It is a principal object of the present invention to provide effective and safe vaccines for prevention and treatment of COVID-19 infection.

10 The invention provides a pharmaceutical composition comprising an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No.25, or a fragment, or a homologue thereof and at least one pharmaceutically acceptable carrier.

15 The invention provides a pharmaceutical composition comprising an isolated immunogenic peptide having amino acid sequence set forth as SEQ ID No. 33 to SEQ ID No.45, or a fragment, or homologue thereof and at least one pharmaceutically acceptable carrier.

20 The invention provides an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 25, or a fragment, or a homologue thereof, wherein the immunogenic peptide is selected from a synthetic, a semi-synthetic or a recombinant peptide, and wherein immunogenic peptide has at least 85% homology to at least one epitope of
25 SARS-CoV-2 proteome.

The invention provides an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 33 to SEQ ID No. 45, or a fragment, or a homologue thereof, wherein the immunogenic peptide is selected from a synthetic, a semi-synthetic or a recombinant peptide, and wherein immunogenic
30

peptide has at least 85% homology to at least one epitope of SARS-CoV-2 proteome.

The invention provides method of treating a condition associated with an infection by SARS-Cov-2 in a subject in need of such treatment, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 25, or a fragment, or a homologue thereof, to thereby effectively treat the condition associated with an infection by SARS-Cov-2.

The invention provides a method of preventing infection with SARS-Cov-2 in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition comprising an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 25, or a fragment, or a homologue thereof, to thereby effectively prevent infection with SARS-Cov-2.

The invention provides a method of immunomodulation comprising administering to a subject an amount of a pharmaceutical composition comprising an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 25, or a fragment, or a homologue thereof.

The invention provides a method of vaccinating a subject against infection by SARS-CoV-2, the method comprising:

- a. providing a pharmaceutical composition according to the embodiments of the invention,
- b. administering the pharmaceutical composition to the subject,

c. testing the subject for a presence of antibodies that bind to a peptide having amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 25.

BRIEF DESCRIPTION OF THE DRAWINGS

5 **Fig.1** is a schematic view of computational approaches used to identify potential immunodominant epitopes from the proteome of SARS-CoV-2;

Fig.2 is an exemplary embodiment of the Potential immunogenic regions mapped onto the SARS CoV-2 proteins. (A) Nucleocapsid phosphoprotein: residues 176-191 (orange), 240-264 (salmon), 268-286 (lime), 292-330 (sand), 360-375 (sky-blue). (B) Membrane glycoprotein: residues 61-70 (orange), 157-187 (salmon). (C) spike glycoprotein: residues 327-343 (orange), 663-680 (yellow), 817-833 (wheat), 891-918 (green), 1019-1041 (lime), 1060-1068 (red), 1157-1209 (cyan), 1254-1273 (sky-blue). In all these regions, both B-cell and T-cell epitopes are mapped. (D) The region Val1060-Val1068 (orange beta-strand) of the spike glycoprotein (green cartoon) is mostly composed of hydrophobic residues (VVFLHVTYV) which are not exposed to solvent; (E) Residues Asp663-Leu680 (DIPIGAGICASYHTVSL, blue) of the spike glycoprotein (green cartoon) are mostly solvent-exposed, with the exception of Cys671 and Ala672;

Fig.3 is an exemplary embodiment of point mutations found within the potential epitopes of SARS-CoV-2 USA isolates.

25 The mutated regions are highlighted in red; and

Fig.4 is an exemplary embodiment of (A) Structures of aligned MHC (Class I) HLA-peptide complexes are fairly similar. They share the same canonical fold and peptide binding mode (B) A typical 9mer peptide molecule (orange sticks) arranged in the binding site of HLA protein (green cartoon) from MHC Class I: the N (upper side) and C (down side) termini occupy essentially the same positions in the A and F pockets, respectively (C)

Typical 13mer peptide in "bulged" conformation (orange chain) confined in the interface area between its cognate HLA-B*35:08 protein (alpha chain, green; beta-micro-globulin, blue) and the T-cell receptor (alpha chain, wheat; beta-chain, lemon). PDB ID: 2ak4 (D) The elongated 17-residue epitope (blue chain, LNKHIDAYKTFPPTEPK) bound to HLA from MHC Class I (green ribbon), with the N-terminal part arranged outside the A-pocket; the C-terminal part is bound in the F-pocket of the binding groove. The binding mode of the core epitope KTFPPTEPK (orange chain) is also shown.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is now described more fully hereinafter with reference to the accompanying examples, in which embodiments of the invention are shown. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather these embodiments are provided so that this disclosure will be thorough and complete and will fully convey the scope of the invention to those skilled in the art.

According to some embodiments, the invention provides a pharmaceutical composition comprising an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No.25, or a homologue thereof and at least one pharmaceutically acceptable carrier. As used herein, the term "immunogenic" arises from the term "Immunogenicity" - the ability of a foreign substance, such as an antigen, to provoke an immune response in the body of a human or other animal. In the context of the invention term "immunogenic peptide" refers to a peptide capable to provoke an immune response upon administration to a subject. As used herein, the term "immune response" includes T cell mediated and/or B cell mediated immune responses. Exemplary immune responses include T cell responses, e.g., cytokine production and cellular cytotoxicity, in

addition, the term immune response includes immune responses that are indirectly effected by T cell activation, e.g., antibody production (humoral responses) and activation of cytokine responsive cells, e.g., macrophages. Immune cells
5 involved in the immune response include lymphocytes, such as B cells and T cells (CD4+, CD8+, Th1 and Th2 cells); antigen presenting cells (e.g. professional antigen presenting cells such as dendritic cells); natural killer cells; myeloid cells, such as macrophages, eosinophils, mast cells, basophils, and
10 granulocytes. As used herein, the term "peptide" refers to a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically, having no specific length. The term peptide does not exclude post-translational modifications that include but are not limited to
15 phosphorylation, acetylation, glycosylation and the like. The term also applies to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally
20 occurring amino acid polymer. By an "isolated" peptide, it is intended that the peptide is essentially free from contaminating cellular components, such as carbohydrate, lipid, or other proteinaceous impurities associated with the polypeptide in nature. Typically, a preparation of isolated peptide contains
25 the peptide in a highly purified form, i.e., at least about 80% pure, at least about 90% pure, at least about 95% pure, greater than 95% pure, such as 96%, 97%, or 98% or more pure, or greater than 99% pure. One way to show that a particular protein preparation contains an isolated peptide is by the appearance
30 of a single band following SDS-polyacrylamide gel electrophoresis of the protein preparation and Coomassie Brilliant Blue staining of the gel. Alternatively, other analytical chemistry techniques such as high-performance liquid chromatography (HPLC) or mass spectrometry (MS) or any other

applicable technique may also be used to determine purity. A peptide that is the predominant specie present in a preparation is substantially purified.

5 According to some embodiments, the pharmaceutical composition comprises an isolated immunogenic peptide having amino acid sequence set forth as SEQ ID No. 33 to SEQ ID No.45, or a fragment, or homologue thereof and at least one pharmaceutically acceptable carrier.

10 According to some embodiments of the above pharmaceutical composition, the isolated immunogenic peptide comprises amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No.7 or a homologue thereof.

15 In the context of the invention "homology" is defined by amino acid sequence identity of a polypeptide to the query amino acid sequence of the invention. By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the invention, it is intended that the amino acid sequence of the subject peptide is identical to the query sequence except that the subject peptide sequence may include
20 up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a peptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% (5 of 100) of the amino acid residues in the subject sequence may be inserted, deleted,
25 or substituted with another amino acid. In the context of the embodiments of the invention, the percentage of identity is can be calculated, without limitation, using a global alignment (i.e., the two sequences are compared over their entire length). Methods for comparing the identity and homology of two or more
30 sequences are well known in the art.

In the context of the invention the term "fragment" refers, without limitation, to any part and/or portion of the peptide of the invention. The fragment can be a result of enzymatic

cleavage and/or hydrolysis and/or any other processing. Alternatively, the fragment can be chemically synthesized as a standalone peptide and/or produced as a recombinant peptide using any suitable method known in the art. Fragments, according to the embodiments of the invention, may have superior immunogenic features.

According to some embodiments of the above pharmaceutical composition, the isolated peptide may have at least 70% sequence identity with the peptide of any one of SEQ ID NO: 1 to SEQ ID No. 25. In one embodiment, the isolated peptide may have at least 70%, 75%, 80%, 83%, 85%, 87%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity with the peptide of any one of SEQ ID NO: 1 to SEQ ID No. 25 and is still able to provoke immune response in the subject in substantially the same way as the immunogenic peptide of any one of SEQ ID NO: 1 to SEQ ID No. 25.

According to some embodiments of the above pharmaceutical composition, the isolated peptide may have at least 70% sequence identity with the peptide of any one of SEQ ID NO: 33 to SEQ ID No. 45. In one embodiment, the isolated peptide may have at least 70%, 75%, 80%, 83%, 85%, 87%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity with the peptide of any one of SEQ ID NO: 33 to SEQ ID No. 45 and is still able to provoke immune response in the subject in substantially the same way as the immunogenic peptide of any one of SEQ ID NO: 1 to SEQ ID No. 45.

According to some embodiments of the above pharmaceutical composition, the isolated immunogenic peptide has at least 90% homology to at least one epitope of SARS-CoV-2 proteome. In one embodiment, the isolated immunogenic peptide has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% homology to at least one epitope of SARS-CoV-2 proteome.

As used herein, the term "SARS-CoV-2 proteome" refers, without limitation, to the entire set of proteins that is, or can be, expressed by SARS-CoV-2 at a certain time. It is the set of expressed proteins in a given type of SARS-CoV-2, at a given
5 time, under defined conditions. In the context of the invention, the term "epitope" refers, without limitation, to the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells. Epitope is also known as antigenic determinant.

10 According to some embodiments of the above pharmaceutical composition, the isolated immunogenic peptide is a synthetic peptide. In one embodiment, the isolated immunogenic peptide is a semi-synthetic peptide. In one embodiment, the isolated immunogenic peptide is a recombinant peptide. In one embodiment,
15 the immunogenic peptide comprises at least one unnatural amino acid.

Peptides of the invention may be synthesized, without limitation, in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are
20 commercially available and can be used in accordance with known protocols. Peptides of the invention may also be synthesized by solid phase peptide synthesis (SPPS) method with high efficiency and purity. The purity of any given peptide; generated through automated peptide synthesis or through recombinant methods may
25 be determined using reverse phase HPLC analysis. Chemical authenticity of each peptide may be established by any method well known to those of skill in the art. As an alternative to automated peptide synthesis, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes a peptide
30 of choice is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression as described herein below. Recombinant methods can be used, without limitation, for

producing longer peptides (polypeptides). Such a peptide may for example only comprise substitutions compared to the reference sequence. The substitutions preferably correspond to conservative substitutions. As used herein, the term

5 "conservative substitution" includes, without limitation, the use of a chemically derivatized residue in place of a non-derivatized residue. "Chemical derivative" refers to a peptide having one or more residues chemically derivatized by reaction of a functional side group. Examples of such derivatized

10 molecules include for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl

15 and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine may be derivatized to form N-im-benzylhistidine. Chemical derivatives also include peptides that contain one or more naturally-

20 occurring amino acid derivatives of the twenty standard amino acids. For examples: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for

25 lysine. The term "conservative substitution" also includes the use of non-natural amino acids aimed to control and stabilize peptides or proteins secondary structures. These non-natural amino acids are chemically modified amino acids such as prolinamino acids, beta-amino acids, N-methylamino acids, cyclopropylamino acids, alpha, alpha-substituted amino acids as

30 describe here below. These non-natural amino acids may include also fluorinated, chlorinated, brominated- or iodinated modified amino acids.

According to some embodiments of the above pharmaceutical composition, the immunogenic peptide has amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No.25.

5 According to some embodiments of the above pharmaceutical composition, the immunogenic peptide has amino acid sequence set forth as SEQ ID No. 33 to SEQ ID No.45.

According to some embodiments of the above pharmaceutical composition, the immunogenic peptide has amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No.7.

10 According to some embodiments of the above pharmaceutical composition, the composition further comprises at least one biopolymer. In the context of the invention, the term "biopolymer" refers, without limitation, to the materials occurring naturally synthesized from animals, plants, bacteria,
15 and fungi or could be the polymers which are produced chemically by using biological materials like sugars, amino acids, oils, or natural fats. Biopolymers can be divided also into two broad groups, namely biodegradable and nonbiodegradable biopolymers. Alternatively, biopolymers can be classified on their origin as
20 being either bio-based or fossil fuel-based. The bio-based biopolymers can be produced from plants, animals, or microorganisms. There are many more nondegradable bio-based biopolymers than there are biodegradable bio-based biopolymers. In the context of the invention, the biopolymers are used as a
25 delivery system. Biopolymers might be used to increase immunogenicity and to protect peptides from harmful effects of degrading enzymes and aggressive antibodies, it is generally necessary to use nanosystems such as protein, biopolymer conjugations or nanoparticles (NPs). NPs are particles that are
30 below 1000 nanometers. NPs are morphologically and physicochemically influenced by the physical and chemical properties of the starting material used. For example, the NPs used as polymeric carriers are solid colloidal structure. The

active substance can be encapsulated, absorbed or dissolved in the particle. Polysaccharides, polyanhydride, polycaprolactone, polyacrylic acid and polylactic-co-glycolic acid is also used for producing NPs and produce a co-polymer system such as, without limitation, poly(ethylene glycol) (PEG)-Nps and poly(ethylene glycol)-poly(ϵ -caprolactone) copolymers (PEG-PCL) copolymers. In addition, the copolymers of N-vinyl-2-pyrrolidone with acrylic acid (P(VP-co-AA)), PLGA, NPs loaded with the antigenic peptide can be used for vaccine prototypes.

According to some embodiments of the above composition, the immunogenic peptide and the at least one biopolymer form a peptide-loaded nanoparticle. According to some embodiments of the above composition, the immunogenic peptide and the at least one biopolymer form a peptide-biopolymer conjugate complex.

According to some embodiments of the above composition, the non-limiting list of biopolymers include liposome, NPs, lipids, poly-nucleotides, poly amino acids, and minerals.

The invention further provides the pharmaceutical composition according to the above embodiments, for use as an immunomodulator. In the context of the invention, the term "Immunomodulator" refers, without limitation, to agents and compositions that change the composition and/or function of immune system or immune cells. The term "immunomodulation" refers to modification of the immune response or the functioning of the immune system by the action of an immunomodulator.

The invention further provides the pharmaceutical composition according to the above embodiments, for use as a medicament.

The invention further provides the pharmaceutical composition according to the above embodiments, for use as a medicament.

The invention further provides the pharmaceutical composition according to the above embodiments, for use as a vaccine.

The invention further provides the pharmaceutical composition according to the above embodiments, for use in the treatment of a condition associated with an infection by SARS-CoV-2.

According to some embodiments of the above composition, the composition can be administered, without limitation, subcutaneously, intravenously, orally, transmucosally, transdermally, topically or nasally. The composition according to some embodiments of the invention can be present in the form of solution, syrup, suspension, cream, gel, foam, transdermal patch, dispersion, or powder.

According to some embodiments, the invention provides an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 25, or a fragment, or homologue thereof, wherein the immunogenic peptide is selected from a synthetic, a semi-synthetic or a recombinant peptide, and wherein immunogenic peptide has at least 85% homology to at least one epitope of SARS-CoV-2 proteome. In one embodiment, the isolated immunogenic peptide has amino acid sequence set forth SEQ ID No. 1 to SEQ ID No. 25. In one embodiment, the isolated immunogenic peptide has amino acid sequence set forth SEQ ID No. 1 to SEQ ID No. 25. In one embodiment, the immunogenic peptide has at 70%, 75%, 80%, 83%, 85%, 87%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity with the peptide of any one of SEQ ID NO: 1 to SEQ ID No. 25 and is still able to provoke immune response in the subject in substantially the same way as the immunogenic peptide of any one of SEQ ID NO: 1 to SEQ ID No. 25.

According to some embodiments, the invention provides an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 33 to SEQ ID No. 45, or a fragment, or homologue thereof, wherein the immunogenic peptide is selected from a synthetic, a semi-synthetic or a recombinant peptide, and wherein immunogenic peptide has at least 85% homology to at

least one epitope of SARS-CoV-2 proteome. In one embodiment, the isolated immunogenic peptide has amino acid sequence set forth SEQ ID No. 33 to SEQ ID No. 45. In one embodiment, the isolated immunogenic peptide has amino acid sequence set forth SEQ ID No. 1 to SEQ ID No. 25. In one embodiment, the immunogenic peptide has at 70%, 75%, 80%, 83%, 85%, 87%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity with the peptide of any one of SEQ ID NO: 33 to SEQ ID No. 45 and is still able to provoke immune response in the subject in substantially the same way as the immunogenic peptide of any one of SEQ ID NO: 1 to SEQ ID No. 25.

According to some embodiments, the invention provides an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 7, or a homologue thereof, wherein the immunogenic peptide is selected from a synthetic, a semi-synthetic or a recombinant peptide, and wherein immunogenic peptide has at least 85% homology to at least one epitope of SARS-CoV-2 proteome. In one embodiment, the isolated immunogenic peptide has amino acid sequence set forth SEQ ID No. 1 to SEQ ID No. 7. In one embodiment, the immunogenic peptide has at 70%, 75%, 80%, 83%, 85%, 87%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity with the peptide of any one of SEQ ID NO: 1 to SEQ ID No. 7 and is still able to provoke immune response in the subject in substantially the same way as the immunogenic peptide of any one of SEQ ID NO: 1 to SEQ ID No. 7.

The isolated immunogenic peptide according to the above embodiments, has at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% homology to at least one epitope of SARS-CoV-2 proteome.

According to some embodiments, the invention provides an oligonucleotide having a nucleotide sequence encoding the isolated immunogenic peptide according to the above embodiments. In one embodiment, the oligonucleotide has a nucleotide sequence

set forth SEQ ID No. 26 to SEQ ID No. 32, or a fragment, or a homologue thereof.

According to some above embodiments, the oligonucleotide of the invention has at least 70%, 75%, 80%, 83%, 85%, 87%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity with the oligonucleotide having the sequence set forth of SEQ ID No. 26 to SEQ ID No. 32. The non-limiting list of the oligonucleotides of the invention are summarized in Table 3.

Nucleotide Sequence	Epitope	SEQ ID
CCTAAAGAAATCACTGTTGCTACATCACGAACGCTTTCTTATTACAAATTG	PKEITVATSRTLSTYYKL	26
CAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGCATTTGGCATG	QFAPSASAFFGMSRIGM	27
TTCTTCGGAATGTCGCGCATTTGGCATGGAAGTCACACCTTCGGGAACGTGG	FFGMSRIGMEVTPSGTW	28
TTTATTGAAGATCTACTTTTCAACAAAGTGACACTTGCAGATGCTGGCTTC	FIEDLLFNKVTLDAGF	29
GGTGCTGCATTACAAATACCATTTGCTATGCAAATGGCTTATAGGTTTAAT	GAALQIPFAMQMAYRFN	30
CCATTTGCTATGCAAATGGCTTATAGGTTTAATGGTATTGGAGTTACACAG	PFAMQMAYRFNGIGVTQ	31
GAAATTGACCGCTCAATGAGGTTGCCAAGAATTTAAATGAATCTCTCATCGATCTCCAAGAACTTGGAAAGTATGAGCAGTAT	EIDRLNEVAKNLNESLI DLQELGKYEQY	32

Table 3: oligonucleotides encoding immunogenic peptides.

As used herein, the term "sequence" refers, without limitation to peptide, polypeptide, oligonucleotide or polynucleotide.

As used herein, the terms "oligonucleotide", "nucleic acid", "nucleic acid sequence", "nucleotide", "nucleic acid molecule" or "polynucleotide" are intended to include DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), natural occurring, mutated, synthetic DNA or RNA molecules, and analogs of the DNA or RNA generated using nucleotide analogs. It can be single-stranded or double-stranded. Such nucleic acids or polynucleotides include, but are not limited to, coding sequences of structural genes, anti-sense sequences, and non-coding regulatory sequences that do not encode mRNAs or protein products. These terms also encompass a gene. The term "gene",

"allele" or "gene sequence" is used broadly to refer to a DNA (deoxynucleic nucleic acids) associated with a biological function. Thus, genes may include introns and exons as in the genomic sequence or may comprise only a coding sequence as in cDNAs, and/or may include cDNAs in combination with regulatory sequences. Thus, according to the various aspects of the invention, genomic DNA, cDNA or coding DNA may be used.

According to some embodiments, the invention provides an expression vector comprising the oligonucleotide according to the above embodiments, operably linked to a promoter.

According to some embodiments, the invention provides a host cell comprising the expression vector according to the above embodiments.

Oligonucleotides of the invention may be produced by any technique known per se in the art, such as, without limitation, any chemical, biological, genetic or enzymatic technique, either alone or in combination(s).

In its broadest sense, the term "vector" refers to any vehicle capable of facilitating the transfer of a nucleic acid to the cells. In general, the vectors useful in the invention include, but are not limited to, plasmids, phagemids, viruses, other vehicles derived from viral or bacterial sources that have been manipulated by the insertion or incorporation of the nucleic acid sequences of interest. Viral vectors may include, without limitation, nucleic acid sequences from the following viruses such as retrovirus, such as moloney murine leukemia virus, harvey murine sarcoma virus, murine mammary tumor virus, and mouse sarcoma virus; adenovirus, adeno-associated virus; SV40-type viruses; polyoma viruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; polio virus; and RNA virus such as a retrovirus. One can readily employ other vectors not named but known to the art. Viral vectors are based on non-

cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest.

Additional vectors include plasmid vectors. Plasmid vectors are useful because they do not have safety concerns as the viral
5 vectors. These plasmids, having a promoter compatible with the host cell, can express a peptide from a gene operatively encoded within the plasmid. Some commonly used plasmids include, without limitation, pBR322, pUC18, pUC19, pRC/CMV, 5V40, and pBlueScript. Other plasmids are well known to those of ordinary
10 skill in the art. Additionally, plasmids may be custom designed using restriction enzymes and ligation reactions to remove and add specific fragments of DNA.

According to some embodiments, the invention provides a method of treating a condition associated with an infection by SARS-
15 Cov-2 in a subject in need of such treatment, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 25, or a fragment, or homologue
20 thereof, to thereby effectively treat the condition associated with an infection by SARS-Cov-2.

According to some embodiments, the invention provides a method of treating a condition associated with an infection by SARS-
Cov-2 in a subject in need of such treatment, comprising
25 administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 33 to SEQ ID No. 45, or a fragment, or a homologue thereof, to thereby effectively treat the condition associated
30 with an infection by SARS-Cov-2.

According to some embodiments, the invention provides a method of preventing infection with SARS-Cov-2 in a subject, comprising administering to the subject an effective amount of a

pharmaceutical composition comprising an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 25, or a fragment, homologue thereof, to thereby effectively prevent infection with SARS-Cov-2.

- 5 According to some embodiments, the invention provides a method of preventing infection with SARS-Cov-2 in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition comprising an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 10 33 to SEQ ID No. 45, or a fragment, homologue thereof, to thereby effectively prevent infection with SARS-Cov-2.

According to some embodiments, the invention provides a method of immunomodulation comprising administering to a subject an amount of a pharmaceutical composition comprising an isolated 15 immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 25, or homologue thereof.

According to some embodiments, the invention provides a method of immunomodulation comprising administering to a subject an amount of a pharmaceutical composition comprising an isolated 20 immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 33 to SEQ ID No. 45, or homologue thereof.

According to some embodiments of the above methods, the immunogenic peptide has amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 25.

- 25 According to some embodiments of the above methods, the immunogenic peptide has amino acid sequence set forth as SEQ ID No. 33 to SEQ ID No. 45.

According to some embodiments of the above methods, the immunogenic peptide has amino acid sequence set forth as SEQ ID 30 No. 1 to SEQ ID No. 7.

According to some embodiments of the above methods, the immunogenic peptide has at least 70%, 75%, 80%, 83%, 85%, 87%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity with the peptide of any one of SEQ ID NO: 1 to SEQ ID No. 25 and is still able to provoke immune response in the subject in substantially the same way as the immunogenic peptide of any one of SEQ ID NO: 1 to SEQ ID No. 25. In one embodiment, the immunogenic peptide comprises at least one unnatural amino acid.

According to some embodiments of the above methods, the immunogenic peptide has at least 70%, 75%, 80%, 83%, 85%, 87%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity with the peptide of any one of SEQ ID NO: 1 to SEQ ID No. 25 and is still able to provoke immune response in the subject in substantially the same way as the immunogenic peptide of any one of SEQ ID NO: 33 to SEQ ID No. 45. In one embodiment, the immunogenic peptide comprises at least one unnatural amino acid.

According to some embodiments of the above methods, the immunogenic peptide has at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% homology to at least one epitope of SARS-CoV-2 proteome.

According to some embodiments of the above methods, the administration route can be, without limitation, subcutaneously, intravenous, oral, transmucosal, transdermal, topical, or nasal and the pharmaceutical composition may be formulated, without limitation as a solution, syrup, suspension, cream, gel, foam, transdermal patch, dispersion, powder, or any other form suitable for the methods according to the embodiments of the invention.

According to some embodiments, the invention provides a method of vaccinating a subject against infection by SARS-CoV-2, the method comprising:

d. providing a pharmaceutical composition according to the embodiments of the invention,

e. administering the pharmaceutical composition to the subject,

5 f. testing the subject for a presence of antibodies that bind to a peptide having amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 25.

In the context of the invention, the step of testing the subject for a presence of antibodies can be carried by any applicable
10 method known in the art with the adjustments required to achieve specificity.

According to some embodiments, the invention provides a method for preventing and/or slowing progression of a condition associated with SARS-CoV-2 by administering to a subject an
15 effective amount of a pharmaceutical compositions according to the embodiments of the invention.

According to some embodiments of the above method, the non-limiting list of conditions associated with an infection by a virus of the Coronaviridae family includes acute respiratory
20 distress syndrome (ARDS), common cold, pneumonia, bronchitis, severe acute respiratory syndrome, and Middle East respiratory syndrome.

As used herein, the phrase "slowing and/or preventing progression" refers, without limitation, to the influence of the
25 treatment on the clinical course of the disease. For example, illness severity of SARS-CoV-2 (COVID-19) ranges from mild to critical, while mild to moderate disease is categorized as mild symptoms up to mild pneumonia; severe disease has manifestations of dyspnea, hypoxia, or more than 50% lung involvement on
30 imaging; and critical disease has manifestations of respiratory failure, shock, or multiorgan system dysfunction, which may

result in death (<https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-guidance-management-patients.html>). In the context of the invention, the proposed therapy is aimed at slowing and/or preventing the transition from mild to severe and to critical illness. The "slowing and/or preventing" progression of the condition according to the embodiments of the above method may be measured using any appropriate questionnaire, method, scale, diagnostic tool, or any other means that are known in the art or acceptable by the relevant functions and professionals. The term "preventing" might but does not necessarily mean recovery from the illness. As such, the term "preventing" relates to the situation when the patient does not present symptoms and/or signs and/or manifestations of the onset of the disease and/or the next "stage" of illness severity as defined by the appropriate and acceptable parameters for the specific disease condition. The term "slowing", or attenuating is can, without limitation, prolong the time of transition into the next "stage" of illness severity, thus providing greater window of opportunity for extensive care and recovery.

As used herein, the term "clinical manifestations" refers, without limitation, to signs and symptoms of the disease that can be either objective, when observed by a physician, or subjective, when perceived by the patient.

Any acceptable dose and administration regimen of the pharmaceutical compositions according to the embodiments of the invention can be used.

The pharmaceutical compositions according to the embodiments of the invention may be a solid composition, a liquid composition, or a semi-solid composition. In one embodiment, the pharmaceutical composition is designed for oral administration, intra-muscular administration, intravenous administration, intraperitoneal administration, intranasal administration,

intramucosal administration, or transdermal administration. In yet another embodiment, the pharmaceutical composition is in the form of a tablet, a capsule, a powder, a powder for suspension, a powder for reconstitution, granules, a syrup, a suspension, a suppository, a patch, and a dispersion.

According to some embodiments of the invention, the pharmaceutical compositions may be administered to a subject defined as a potential responder. As used herein the term "responder" is meant to be understood, without limitation, as a subject who, based on his gene expression profile, namely specific biomarkers, is likely to respond to the proposed treatment. For example, genetic profile of the Responder is characterized by upregulation and/or downregulation of certain genes, while genetic profile of the Non-Responder is characterized by different pattern of gene expression. The reason for the differentiated response can be a result of various cellular pathway and processes. The change in genetic profile may be triggered, without limitation, by administration of the proposed therapeutics and/or by the infection itself. The variability in the response of different people to viral infection and may lead to differential gene expression and different response to therapeutic tool. The opposite is also possible, while administration of similar therapeutics to different people may lead to different gene expression pattern which becomes a determinant of the clinical outcome.

According to some embodiments of the above methods, compositions isolated immunogenic peptides, a non-limiting list of clinical manifestations according to the embodiments of the invention includes fever, cough, dyspnea, hypoxia, more than 50% lung involvement on imaging, respiratory failure, shock, multiorgan system dysfunction, malaise, fatigue, sputum/secretion, neurological symptoms, dermatological manifestations, anorexia,

myalgia, sneezing, sore throat, rhinitis, goosebumps, headache, chest pain and diarrhea.

Examples

Example 1: Identification of the immunodominant epitopes from the proteins of SARS-CoV-2

Immunodominant epitopes, which can generate both antibody and cell-mediated immunity, were identified in order to generate memory cells against SARS-CoV-2. To determine the immunodominant epitopes, B-cell and T-cell epitopes were predicted together with their possible MHC alleles from the SARS-CoV-2 proteins. All the B-cell and T-cell epitopes that were predicted from different protein sequences of SARS-CoV-2 were selected for further analysis. Multiple approaches were used to predict the linear B-cell epitopes from the protein sequences of SARS-CoV-2, including three machine learning-based methods, namely, BepiPred [22], ABCpred [23], and LBtope [24]. A cutoff of 0.51 was used and all the window lengths of 10-20 were chosen for predicting B-cell epitopes by means of the ABCpred search. Then, using a combinatorial screening approach, all the predicted B-cell and T-cell epitopes (MHC-I and MHC-II) libraries of different lengths, from all the protein sequences were analyzed. The libraries of predicted B-cell epitopes vs. T-cell epitopes were compared and the epitopes which have 100% sequence coverage were selected. The lengths of the immunogenic regions were selected based on the maximum coverage of B-cell or T-cell epitopes in the mapped regions. The pipeline used in the study for detecting immunodominant epitopes is shown in Figure 1.

To understand the immunomodulatory effect of epitopes identified from immunogenic regions, their abilities as protective antigens were predicted using Vaxijen [34]. Unique epitopes were selected accordingly for further analysis. A total of 17 immunogenic regions from membrane glycoprotein, spike glycoprotein, and

nucleocapsid phosphoprotein, where both B-cell and T-cell epitopes were identified and mapped.

The 100% identical and experimentally confirmed epitopes between SARS-CoV and SARS-CoV-2 were chosen in this study. Accordingly,
5 all the epitopes predicted from the 17 regions of three proteins of SARS-CoV-2 were mapped with the experimentally validated epitopes of SARS-CoV and selected only the 100% identical epitopes. The length of the epitopes was adjusted based on the mapped experimentally determined epitopes of SARS-CoV. To define
10 the immunodominant epitopes, the core parts of both B-cell and T-cell epitopes were verified within those mapped epitope sequences. Finally, 15 potential immunogenic regions of SARS-CoV-2 that explicitly include 25 mapped immunodominant epitopes were found, which can generate the immune response for both B-
15 cells and T-cells (Table 1, Figure 2A, 2B and 2C). Mapping of immunogenic regions onto the structure of SARS-CoV-2 spike glycoprotein (Figure 2C) revealed a number of potential epitopes that were not exposed to solvent. For example, the beta-strand, Val1060-Val1068, composed of hydrophobic residues (VVFLHVTYV)
20 was not a solvent-accessible region in the multi-subunit spike glycoprotein (Figure 2D,). Indeed, the solvent-accessible surface area (SASA) was estimated to be ~0 for all residues of this epitope, with the only exception of Val1068 (SASA ~24 Å², Supplementary Table S2). This region contrasts with the nearby
25 region of another epitope, Asp663-Leu680 (DPIGAGICASYHTVSL, Table 1), which was mostly exposed to solvent (Figure 2E). This implies the "recognition-after-proteolysis" pathway of protein interactions with the immune system.

regions WO 2021/245682	Epitope sequence	VaxiJen score	World population covPCT/IL2021/050677 (%)	
membrane_glycoprotein:165-181	PKEITVATSR TSLSY YKL	0.59	95.82%	1
nucleocapsid_phosphoprotein:306-322	QFAPSASAFFGMSR IGM	0.7326	92.81%	2
nucleocapsid_phosphoprotein:314-330	FFGMSRIGMEVTPS GTW	0.756	87.42%	3
Spike_glycoprotein:817-833	FIEDLLFNKVTLAD AGF	0.5301	94.26%	4
Spike_glycoprotein:891-907	GAALQIPFAMQMAY RFN	0.8583	97.46%	5
Spike_glycoprotein:897-913	PFAMQMAYRFNGIG VTQ	1.3306	92.52%	6
Spike_glycoprotein:1182-1209	EIDRLNEVAKNLNE SLIDLQELGKYEQY	0.4662	88.57%	7
membrane_glycoprotein:61-70	TLACFVLA	1.2318	43.26%	8
membrane_glycoprotein:157-174	GRCDIKDLPKEITV ATSR	0.7029	21.39%	9
membrane_glycoprotein:172-187	TSRTLSYYKLGASQ RV	0.6212	74.10%	10
nucleocapsid_phosphoprotein:240-257	QQQGQTVTKKSAAE ASKK	0.6811	34.14%	11
nucleocapsid_phosphoprotein:292-308	IRQGTDYKHWPQIA QFA	0.5118	5.43%	12
nucleocapsid_phosphoprotein:176-191	SRGGSQASSRSSSR SR	1.0216	14.70%	13

nucleocapsid_phosphoprotein:248-264	KKSAAEASKKPRQK RTA	0.4812	11.03%	14
nucleocapsid_phosphoprotein:268-286	YNVTQAFGRRGPEQ TQGNF	0.5493	46.87%	15
Spike glycoprotein:1025-1041	AATKMSECVLGQSK RVD	0.6159	71.87%	16
Spike glycoprotein:1060-1068	VVFLHVTYV	1.5122	49.55%	17
Spike glycoprotein:1157-1173	KNHTSPDVDLGDIS GIN	1.1116	10.12%	18
Spike glycoprotein:1019-1035	RASANLAATKMSECVLG	0.4414	50.21%	19
Spike glycoprotein:1165-1181	DLGDISGINASVVN IQK	0.8314	62.76%	20
Spike glycoprotein:1254-1273	CKFDEDDSEPVLKG VKLHYT	0.8247	77.19%	21
Spike glycoprotein:327-343	VRFPNITNLCPFGE VFN	0.6787	67.84%	22
Spike glycoprotein:663-680	DIPIGAGICASYHT VSLI	0.521	48.70%	23
Spike glycoprotein:902-918	MAYRFNGIGVTQNV LYE	0.759	71.99%	24
nucleocapsid phosphoprotein:360-375	YKTFPPTEPKKDKK KK	0.4161	43.03%	25

Table 1: Potential immunodominant regions of SARS-CoV-2 and the mapped epitopes in those regions.

As a next step, based on the above epitopes, new immunogenic peptides were designed using a combinatorial approach, by linking together immunogenic epitopes via a hydrolysable linker sequences (certain examples of the combined/linked epitopes are

summarized in Table 2. The design is based on an algorithm combining immunogenic epitopes via hydrolysable linker (E1-L-E2). The rationale is to create potential "pro-peptides", namely, artificial peptides that can produce/release shorter immunogenic fragments/epitopes through *in-vivo* enzymatic cleavage. As such, artificial peptide QQQGQTVTKKSAAEASKKEIDRLNEVAKNLNESLIDLQELGKYEQYIKDLPKEITVATSR (SEQ ID 44) undergoes enzymatic cleavage into immunogenic peptides QQQGQTVTKKSAAEASKK, EIDRLNEVAKNLNESLIDLQELGKYEQY and IKDLPKEITVATSR through enzymatic cleavage. Similarly, artificial peptide YKTFPPTEPKKDKKKKEIDRLNEVAKNLNESLIDLQELGKYEQYIKDLPKEITVATSR (SEQ ID 45) undergoes enzymatic cleavage two immunogenic peptides YKTFPPTEPKKDKKKK, EIDRLNEVAKNLNESLIDLQELGKYEQY and IKDLPKEITVATSR. Thus, the artificial peptide can be a part of the pharmaceutical composition as well as each of the immunogenic epitopes which are products of the enzymatic cleavage.

WO 2021/245682	Epitope ID	Epitope sequence	VaxiJen score	World population covPCT/IL2021/050677 (%)	
	Combo_12	MAYRFNGIGVTQNVLYEPKEITVATSR TSLSYK	0.7571	99.64%	33
	Combo_21	PKEITVATSR TSLSYKLMAYRFNGIGVTQNVLYE	0.5146	98.84%	34
	Combo_123	PFAMQMAYRFNGIGVTQTLACFVLAA VVFLHVTYV	1.0994	99.64%	35
	Combo_132	VVFLHVTYVTLACFVLA AVDLGDISGINASV VNIQK	1.1585	99.64%	36
	Combo_1234	TLACFVLA AVKKSAEASKKPRQKRTAVVFLHVTYVAATKMSECVLGQSKRVD	0.8094	99.64%	37
	Combo_342	PFAMQMAYRFNGIGVTQVVFLHVTYVM AYRFNGIGVTQNVLYE	0.9435	99.64%	38
	Combo_231	VVFLHVTYVYKTFPPTEPKKDKKKKTL ACFVLA	0.8988	99.64%	39
	Combo_1A	YKTFPPTEPKKDKKKKPFAMQMAYRF NGIGVTQ	0.8454	98.84%	40
	Combo_1B	GAALQIPFAMQMAYRFNVVFLHVTYV	1.0779	99.64%	41
	Combo_3C	PFAMQMAYRFNGIGVTQYKTFPPTEPKKDKKKK	0.9606	98.84%	42
	Combo_1D	DLGDISGINASV VNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYI WLG	0.638	98.84%	43

Combo_2D	QQQGQTVTKKSAAEASKKEIDRLNEV AKNLNESLIDLQELGKYEQYIKDLPK EITVATSR	0.39	99.64%	44
Combo_3D	YKTFPPTPEPKDKKKKEIDRLNEVAKN LNESLIDLQELGKYEQYIKDLPKEIT VATSR	0.363	99.64%	45

Table 2: hydrolysable pro-peptides.

Example 2: Analysis of viral mutations within the potential epitope regions

Selection pressure of the human immune system has been shown to drive the viral point mutations that evade immune surveillance [48]. Therefore, patterns of mutational events need to be examined to understand the epitope escape that is important for the transmission of viruses between different sub-populations. The potential immunogenic epitopes with a low chance of mutations are the unspoiled candidates for effective vaccines. Particularly, mutations within the immunodominant epitopes identified in SARV-CoV-2 isolates from different geographic locations were analyzed. A number of single point mutations within the immunodominant regions of a few SARS-CoV-2 sequences isolates from USA were found (Figure 3). Despite the low number of point mutations in the immunodominant epitopes, they reflected the severity of mutated viral genomes within the USA population. Our observation highlights that the immune pressure-induced genetic drifts have an important role in the evolution of SARS-CoV-2. This may be essential for evading immune surveillance by the host.

Example 3: Population coverage of immunodominant epitopes

Human leukocyte antigens (HLAs) are the most polymorphic genes in humans, and their allele's distributions and expressions vary by ethnic groups and geographical locations. The classical HLA locus is the class I (HLA-A, B, C, E, F and G) and class II

(HLA-DR, DQ, DM and DP) molecules, which provide the antigen presentation to CD8 and CD4 T-cells [49]. Therefore, identification of epitopes that can be recognized by multiple HLA alleles and cover most worldwide population are important for the development of successful vaccines. Population coverage by HLAs of all the epitopes from the immunogenic regions of SARS-CoV-2 was analyzed using the IEDB population coverage analysis tool [20]. Seven epitopes from five immunogenic regions, which cover more than 87% of the world population were identified (Table 3). Among these seven potential immunodominant epitopes, six are of 17 amino acids in length. It was found that the 891-918 regions of spike glycoprotein contain three potential immunodominant epitopes; of them, two have world population coverage of 97.46% and 92.52%. Similarly, 292-330 regions of nucleocapsid phosphoprotein contain three potential immunodominant epitopes; of them, two have 87.42% and 92.81% world population coverage. These results indicate that these seven immunodominant epitopes could be potential candidates for designing vaccines against SARS-CoV-2 that can cover almost the entire world population.

Epitopes	Epitope location	World population coverage (%)	Predicted HLA locus	SEQ ID
PKEITVATSRTLSTYYKL	Membrane glycoprotein:165-181	95.82%	HLA-A, HLA-B, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1	1
QFAPSASAFFGMSRIGM	Nucleocapsid phosphoprotein:306-322	92.81%	HLA-A, HLA-B, HLA-DRB1, HLA-DRB5, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1	2
FFGMSRIGMEVTPSGTW	Nucleocapsid phosphoprotein:314-330	87.42%	HLA-A, HLA-B, HLA-DRB1, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1	3
FIEDLLFNKVTLADAGF	Spike glycoprotein:817-833	94.26%	HLA-A, HLA-B, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DPA1, HLA-DPB1	4

GAALQIPFAMQMAYRFN	Spike glycoprotein:891-907	97.46%	HLA-A, HLA-B, HLA-DRB1, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DPA1, HLA-DPB1	5
PFAMQMAYRFNGIGVTQ	Spike glycoprotein:897-913	92.52%	HLA-A, HLA-B, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DPA1	6
EIDRLNEVAKNLNESLIDL QELGKYEQY	Spike glycoprotein:1182-1209	88.57%	HLA-A, HLA-B, HLA-DRB1, HLA-DRB3, HLA-DQA1, HLA-DQB1	7

Table 3: Epitopes with more than 85% world population coverage.

Example 4: Analysis of allergenicity, toxicity and autoimmune reactivity

The epitopes allergenicity is a prominent obstacle for vaccine development. The allergenicity analysis results of the seven immunodominant epitopes (Table 3) showed that six of these epitopes were not predicted as allergens using both tools, AllerTOP [35] and AlgPred [36]. Only one epitope "FIEDLLFNKVTADAGF" (SEQ ID 4) was predicted as an allergen using only one tool, AllerTOP, whereas the AlgPred methods predicted it as a non-allergen.

The toxicity profiling of these predicted epitopes revealed that all these epitopes were safe and possibly were non-toxic. Epitopes spreading is a process where the diversification of the immune response induced by an antigen to both B-cell and T-cell specificities during a chronic autoimmune or infectious response [50, 51]. Thus, we analyzed the possibilities that the seven predicted immunodominant epitopes (Table 3) may generate any autoimmune reactions. For this purpose, we performed the BLAST search of the epitopes against the database of epitope sequences of human antigens for autoimmune diseases, which were validated by positive B-cell/T-cell/MHC ligand assays. As the result, we found that none of the human epitopes for autoimmune disease

have significant sequence identity to our predicted SARS-CoV-2 immunodominant epitopes (Table 3). This result indicates that these seven epitopes have a very low risk for generating any autoimmune reactions in humans.

5 Example 5: Structural analysis and modeling of epitope presentation by MHC class I and II systems

In the cell, epitopes are faced with extremely complex and competitive environments. This includes the multitude of HLA proteins that bind immunogenic peptides with different
10 affinities, and present selected epitopes to surface receptors on immune cells. Therefore, to understand the binding interactions of the identified immunodominant epitopes with human MHC complexes, a molecular docking analysis was performed. Structures of different HLA-peptide complexes from MHC class I
15 and II were collected and aligned. Structures of HLAs are fairly similar within each group (I and II) and share the same canonical fold. Epitopes were clustered in similar conformations in the HLA antigen binding grooves created by two helices in parallel orientation (Figures 4A, 4B). For the most part, backbone
20 "traces" of peptides were similar (Figure 4A). The N and C-termini occupied essentially the same positions in the pockets A and F in HLA binding sites (Figures 4C, 4D). This suggests that conformational flexibility was mostly concentrated in the middle part of epitope sequences, whereas motion of terminal
25 residues was restricted, in agreement with the possibility of "bulged" conformations. Based on these similarities and common canonical structural properties in HLA-peptide binary complexes, 3D structures of the epitopes listed in Table 1 in their bound conformations were generated. These epitope molecules were built
30 using ~150 backbone templates taken from epitope structures collected in SCEptRe and AutoPeptiDB, and available in PDB.

Six types of peptide-MHC structures were considered: (1) peptide-HLA (MHC I), (2) peptide-HLA (MHC II), (3) peptide-HLA-

TCR (MHC I), (4) peptide-HLA-TCR (MHC II), (5) peptide-HLA-BCR (MHC I), (6) peptide-HLA-BCR (MHC II), out of which types 1, 2 and 3 were chosen. Binding of the epitopes to different HLA proteins from MHC class I and II, and to the HLA-TCR (MHC I) was modelled. In the peptide-HLA-TCR type of binding, the docking scores were mostly higher (compared to the binary peptide-HLA complexes). This was due to the fact that epitope molecules were confined in the interface area between their cognate HLA//TCR proteins (Figure 4C). This mode of binding implies that a peptide's N/C termini are bound to the HLA surface, whereas middle residues interact with TCR.

Using crystal structure of the nonapeptide KTFPPTEPK bound to HLA-A*1101 (PDB ID 1x7q) as the reference state, an extensive conformational sampling and docking study for this complex were performed. It was demonstrated that the peptide's top-score docking conformations were clustered around the native one, with the estimated energy -9.97 kcal/mol (corresponding to the nanomolar affinity range). Moreover, we found similar binding energies (~-9.5 kcal/mol) in docking simulations of KTFPPTEPK binding with HLA-A*02:01 (epitopes from Table 1, Supplementary Table S6). Therefore, the computational protocol we elaborated (see the Methods) enabled: (1) the generation of a library of immunogenic sequences, and (2) structure-based selection of appropriate candidates using docking to multiple HLA structural templates. This approach was applied to all the epitopes listed in Table 1. Some of these immunogenic sequences constitute overlapping sites. For example, the sequence of the reference nonapeptide (KTFPPTEPK) was identical to the region Lys362-Lys370 in the SARS-CoV nucleocapsid protein. In the SARS-CoV-2 variant, this motif was predicted in the epitope sequences LNKHIDAYKTFPPTEPK, KHIDAYKTFPPTEPKDKKK, and YKTFPPTEPKDKKKK, corresponding to positions from Lys361 to Lys369 (Figure 4A, sky-blue area on the nucleocapsid protein surface). The nonapeptide KTFPPTEPK has demonstrated high-affinity binding to

the protein from MHC Class I, whereas its interaction with the HLA-DRB1 (from MHC Class II) is less pronounced (estimated binding energy is $\sim -6-7$ kcal/mol). And vice versa, extended peptides LNKHIDAYKTFPPTEPK (length 17), KHIDAYKTFPPTEPKDKKKK (length 20), and YKTFPPTEPKDKKKK (length 16) do not fit HLA binding sites in HLAs from MHC Class I. It was found that the core part (KTFPPTEPK) of the LNKHIDAYKTFPPTEPK can bind to the recognition site of HLA proteins from MHC Class I ($\sim -7-8$ kcal/mol), whereas the N-terminal part of this 17-residue peptide is arranged outside the A-pocket. The C-terminal part was found to occupy the F-pocket of the binding site (Figure 4D). In agreement with the well-known binding mode in the peptide-MHC class II system, the 17-residue peptide LNKHIDAYKTFPPTEPK has demonstrated high-affinity docking scores $\sim -9-10$ kcal/mol in the interaction with DRB1 proteins. The molecular docking studies imply that peptides consisting of 9-11 amino acids were mostly recognized by MHC Class I molecules, whereas longer sequences tend to target the MHC Class II system. MHC-I processing of identified immunodominant epitopes (Table 1) for all the available MHC alleles of HLA-A, HLA-B and HLA-C using the IEDB tool (<http://tools.iedb.org/processing/>) [20], were predicted and it was found that all of the immunodominant epitopes can undergo further proteolysis and recognition by MHC class I molecules (considering processing score >1). Therefore, core part of immunodominant epitopes with longer sequence length can be presented by MHC class I molecules, after proteasomal processing.

Example 6: Production of synthetic peptides

The immunogenic peptides of the invention are produced using state of the art liquid-phase and/or solid-phase synthesis technologies. In addition, longer peptides are synthesized using chemical ligation technique and/or native chemical ligation (NCL) method.

Example 7: Production of recombinant peptides

The immunogenic peptides of the invention are produced using state-of-the-art recombinant peptide production methods. For example, the peptides are produced in *Escherichia coli*, *Saccharomyces cerevisiae*, and *Pichia pastoris*, CHO, HEK and insect cell lines. The peptides of the invention are produced in protease deficient strains of *Escherichia coli* and/or in insoluble inclusion bodies and/or as concatemers, to avoid degradation by proteases.

10 In addition, to achieve better stability the various tags are used. Common tags include His-tag (for affinity purification), thioredoxin (TrxA), small ubiquitin-like modifier (SUMO) and self-cleavable intein tags. TrxA and SUMO are used to enhance the solubility of peptides and thus are used to increase the
15 production yields of insoluble peptides. Also, the self-cleavable intein-based tags are used to reduce the toxicity and enhance the purification efficiency of toxic peptides. These tags can self-cleave in the presence of reducing agents or as a response to temperature, salt, and pH changes, thus, reducing
20 the need for expensive tag removal processes.

Example 8: Testing in animal model

The immunogenic peptides are synthesized and tested on the mouse model. The peptide is mixed 1:1 with the adjuvant ISA™51 (ISA 51) (a mixture of oil and water, an adjuvant for peptide vaccine)
25 by thorough vigorous shaking in the presence of a ceramic bead. Peptide/adjuvant mixes are injected subcutaneously into the mouse. Immunizations are given on days 0 and 14. Negative controls (mock-immunized) are also included in this study. On day 28, blood is sampled, and 5 mice of each experimental group are
30 euthanized to harvest spleen cells and serum. Average IgG titers are determined by ELISA to understand the extent of humoral response. IFN γ -producing T cells specific to each of the

peptides are detected by ELISPOT assay using a murine IFN γ ELISPOT kit.

Discussion

Immunoinformatics-based approaches were exploited to identify potential immunodominant epitopes from SARS-CoV-2, which could be useful for developing vaccines for the COVID-19 disease. The vaccines should be capable of activating both at the humoral and cellular immune responses in humans. Our approach to defining immunodominant epitopes entails identification of overlapping regions of B-cell and T-cell epitopes (MHC-I and MHC-II) from proteins of SARS-CoV-2. We identified 15 potential immunogenic regions from three proteins of SARS-CoV-2, and mapped 25 epitopes. Among 25 potential immunodominant epitopes identified from 9-28 amino acid residues, the lengths of most of the epitopes were 16-18 residues. To understand the binding patterns of epitopes with MHC-I and MHC-II, we performed structural and molecular docking analysis. We found that in the library of immunogenic sequences, epitopes of 9-11 length were mostly recognized by HLA proteins from MHC Class I, whereas longer epitopes tended to bind to MHC Class II proteins with higher affinities. This finding was in an agreement with the known canonical preferences. Further analysis of MHC class I processing reveals that epitopes of longer sequences can undergo proteasomal processing and the core part for MHC class I recognition region within the epitope may be presented on the cell surface for surveillance by the CD8 T-cells. An analysis of the population coverage by HLAs revealed seven epitopes among the predicted 25 immunodominant epitopes that cover more than 87% of the global worldwide population individually, and have a high binding affinity to MHC-I and MHC-II, as evidenced from structural and docking analysis. Furthermore, these seven epitopes were predicted as non-allergen, non-toxic and having a low risk to trigger the autoimmune responses, which highlight

their potentiality as successful vaccine targets. To develop an effective vaccine, the viral epitopes that are least likely to mutate should be selected. Thus, all the available SARS-CoV-2 genomes from various geographic locations were analyzed to identify the percentage of mutations in suggested epitope regions. We found evidences of point mutations in a few epitopes of SARS-CoV-2 isolates from USA. This suggests that human immune pressure-induced genetic drifts play a central role in the genetic adaptation of SARS-CoV-2. No point mutations in the mentioned seven potentially immunodominant epitopes were found. This result indicates that these seven epitopes are potentially effective vaccine candidates. Hence, the development of vaccines using these seven immunodominant epitopes could activate both humoral and cellular immune responses in humans, and these epitopes could cover almost of the worldwide population, our results have important insights in the peptide vaccine development for COVID-19.

Immunodominant epitopes from SARS-CoV-2 that could induce both humoral and cell-mediated immune response in humans were identified. Molecular docking of the immunodominant epitopes with HLA alleles support their higher binding affinities within different HLA alleles. Further, seven potential immunodominant epitopes were shortlisted based on their higher conservancy, higher global population coverage, and a significant interaction to MHC class I and class II alleles with a high affinity. These epitopes have a low risk for being allergen, toxic with and to generate autoimmune reactions.

The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used herein, the singular forms "a," "an" and "the" are intended to include plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms "comprises" or "comprising," when used

in this specification, specify the presence of stated features, integers, steps, operations, elements components and/or groups or combinations thereof, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components and/or groups or combinations thereof. As used herein the terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to". The term "consisting of" means "including and limited to".

10 As used herein, the term "and/or" includes any and all possible combinations or one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

15 Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the specification and claims and should not be interpreted in an idealized or overly formal sense unless expressly so defined herein. Well-known functions or constructions may not be described in detail for brevity and/or clarity.

25 It will be understood that, although the terms first, second, etc., may be used herein to describe various elements, components, regions, layers and/or sections, these elements, components, regions, layers and/or sections should not be limited by these terms. Rather, these terms are only used to distinguish one element, component, region, layer and/or section, from another element, component, region, layer and/or section.

Certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

Whenever the term "about" is used, it is meant to refer to a measurable value such as an amount, a temporal duration, and the like, and is meant to encompass variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, or $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

As used herein the term "patient" or "subject" is meant to include any mammal. A "mammal," as used herein, refers to any animal classified as a mammal, including but not limited to, humans, experimental animals including monkeys, rats, mice, and guinea pigs, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, and the like.

As used herein, a "pharmaceutically acceptable" carrier or excipient is one that is suitable for use with humans and/or animals without undue adverse side effects (such as toxicity, irritation, and allergic response) commensurate with a reasonable benefit/risk ratio.

"Treating" or "treatment" of a disease as used herein includes: preventing the disease, i.e. causing the clinical symptoms of the disease not to develop in a mammal that may be exposed to or predisposed to the disease but does not yet experience or display symptoms of the disease; inhibiting the disease, i.e., arresting or reducing the development of the disease or its clinical symptoms, or relieving the disease, i.e., causing regression of the disease or its clinical symptoms.

A "therapeutically-effective amount" or an "effective amount" means the amount of a compound or a dosage form that, when

administered to a subject for treating a disease, is sufficient to effect such treatment for the disease. The "therapeutically-effective amount" will vary depending on the compound, the disease, and its severity and the age, weight, etc., of the subject to be treated.

As used herein the term "Pharmaceutically-acceptable salt" refers to salts which retain the biological effectiveness and properties of compounds which are not biologically or otherwise undesirable. Pharmaceutically acceptable salts refer to pharmaceutically acceptable salts of the compounds, which salts are derived from a variety of organic and inorganic counter ions well known in the art.

The pharmaceutical dosage forms may be prepared as medicaments to be administered orally. Suitable forms for oral administration include, without limitation, tablets, capsules, solutions, syrups and suspensions, such as ready-to-use syrups and suspensions, or reconstituted from solid dosage form such as, without limitation, dry powder. The dosage form may contain suitable binders, lubricants, coloring agents, flavoring agents, flow-inducing agents, stabilizing agents, solubilizing agents, antioxidants, buffering agent, chelating agents, and fillers, all collectively or individually fall under the definition of the term "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient".

In the pharmaceutical composition, the active drug component can be combined with an oral, non-toxic, pharmaceutically acceptable, inert filler such as gelatin, agar, starch, methyl cellulose, mannitol, xylitol, sorbitol, maltodextrin and the like. Suitable binders include starch, gelatin, natural sugars such as corn starch, natural and synthetic gums such as acacia, tragacanth, or sodium alginate, povidone, cellulose based soluble polymers such as but not limited to hydroxypropylomethylcellulose, hydroxypropylcellulose,

polyethylene glycol, and the like. Glidants used in these dosage forms include sodium benzoate, sodium acetate, polyethylene glycole, and the like. Stabilizing (antimicrobial) agents include benzoic acid, and salts thereof, parahydroxybenzoate and salts thereof, sorbic acid and salts thereof and the like. Stabilizing (physical) agents include viscosity enhancing polymers such as hydroxyethyl cellulose, xanthan gum and the like.

All publications, patent applications, patents, and other references mentioned in the disclosures of these publications in their entirety are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains. In case of conflict, the patent specification, including definitions, will prevail.

In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. Throughout this application various publications, published patent applications and published patents are referenced.

It will be appreciated by persons skilled in the art that the present invention is not limited to what has been particularly shown and described hereinabove. Rather the scope of the present invention is defined by the appended claims and includes both combinations and sub-combinations of the various features described hereinabove as well as variations and modifications thereof, which would occur to persons skilled in the art upon reading the foregoing description. While certain features of the invention have been illustrated and described herein, many modifications, substitutions, changes, and equivalents may occur to those skilled in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the invention. Various embodiments have been presented. Each of these embodiments may of course include features from other

embodiments presented, and embodiments not specifically described may include various features described herein.

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CLAIMS:

1. A pharmaceutical composition comprising an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No.25, or a fragment, or a homologue thereof and at least one pharmaceutically acceptable carrier.
2. A pharmaceutical composition comprising an isolated immunogenic peptide having amino acid sequence set forth as SEQ ID No. 33 to SEQ ID No.45, or a fragment, or homologue thereof and at least one pharmaceutically acceptable carrier.
3. The pharmaceutical composition of claim 1, wherein the isolated immunogenic peptide comprises amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No.7.
4. The pharmaceutical composition of any one of claims 1 to 3, wherein the isolated immunogenic peptide has at least 90% homology to at least one epitope of SARS-CoV-2 proteome.
5. The pharmaceutical composition of any one of claims 1 to 4, wherein the isolated immunogenic peptide is selected from a synthetic peptide, a semi-synthetic peptide or a recombinant peptide.
6. The pharmaceutical composition of claim 5, wherein the immunogenic peptide comprises at least one unnatural amino acid.
7. The pharmaceutical composition of any one of claims 1 to 6, wherein the immunogenic peptide has amino acid sequence

- set forth as SEQ ID No. 1 to SEQ ID No.25 and SEQ ID No. 33 to SEQ ID No.45.
8. The pharmaceutical composition of any one of claims 1 to 6, wherein the immunogenic peptide has amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No.7.
 9. The pharmaceutical composition of any one of claims 1 to 8, further comprising at least one biopolymer.
 10. The pharmaceutical composition of claim 9, wherein the immunogenic peptide and the at least one biopolymer form a peptide-loaded nanoparticle.
 11. The pharmaceutical composition of claim 9, wherein the immunogenic peptide and the at least one biopolymer form a peptide-biopolymer conjugate complex.
 12. The pharmaceutical composition of any one of claims 9 to 11, wherein the at least one biopolymer is selected from the group consisting of liposome, nano-particles, lipids, poly-nucleotides, poly amino acids, and minerals.
 13. The pharmaceutical composition of any one of claims 1 to 12, for use as an immunomodulator.
 14. The pharmaceutical composition of any one of claims 1 to 12, for use as a medicament.
 15. The pharmaceutical composition of any one of claims 1 to 12 for use in the treatment of a condition associated with an infection by SARS-CoV-2.

16. The pharmaceutical composition of any one of claims 1 to 12 for use as a vaccine against an infection by SARS-CoV-2.
17. The pharmaceutical composition of any one of claims 1 to 12 for use in the treatment of a condition associated with an infection by SARS-CoV-2.
18. The pharmaceutical composition of any one of claims 1 to 17, suitable for a subcutaneous, intravenous, oral, transmucosal, topical or nasal administration.
19. The pharmaceutical composition of claim 18, in the form of solution, syrup, suspension, cream, gel, foam, transdermal patch, dispersion, or powder.
20. An isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 25, or a fragment, or a homologue thereof, wherein the immunogenic peptide is selected from a synthetic, a semi-synthetic or a recombinant peptide, and wherein immunogenic peptide has at least 85% homology to at least one epitope of SARS-CoV-2 proteome.
21. An isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 33 to SEQ ID No. 45, or a fragment, or a homologue thereof, wherein the immunogenic peptide is selected from a synthetic, a semi-synthetic or a recombinant peptide, and wherein immunogenic peptide has at least 85% homology to at least one epitope of SARS-CoV-2 proteome.

22. The isolated immunogenic peptide of claim 20, comprising amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 7, or a homologue thereof.
23. The isolated immunogenic peptide of any one of claims 20 to 22, comprising at least one unnatural amino acid.
24. The isolated immunogenic peptide of claim 20, having amino acid sequence set forth SEQ ID No. 1 to SEQ ID No. 25.
25. The isolated immunogenic peptide of claim 20, having amino acid sequence set forth SEQ ID No. 1 to SEQ ID No. 7.
26. An oligonucleotide having a nucleotide sequence encoding the isolated immunogenic peptide of any one of claims 20 to 25.
27. The oligonucleotide of claim 26, comprising at least one unnatural nucleotide.
28. An oligonucleotide having a nucleotide sequence set forth SEQ ID No. 26 to SEQ ID No. 32, or a fragment, or a homologue thereof.
29. An expression vector comprising the oligonucleotide of any one of claims 26 to 28 operably coupled to a promoter.
30. A host cell comprising the expression vector of any one of claims 26 to 29.
31. A method of treating a condition associated with an infection by SARS-Cov-2 in a subject in need of such treatment, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising an isolated immunogenic peptide

- comprising amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 25, or a fragment, or a homologue thereof, to thereby effectively treat the condition associated with an infection by SARS-Cov-2.
32. A method of preventing infection with SARS-Cov-2 in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition comprising an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 25, or a homologue thereof, to thereby effectively prevent infection with SARS-Cov-2.
33. A method of immunomodulation comprising administering to a subject an amount of a pharmaceutical composition comprising an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 25, or homologue thereof.
34. The method of any one of claims 31 to 33, wherein the immunogenic peptide has amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 25.
35. The method of any one of claims 31 to 33, wherein the immunogenic peptide has amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 7.
36. The method of any one of claims 31 to 35, wherein the immunogenic peptide comprises at least one unnatural amino acid.
37. The method of any one of claims 31 to 36, wherein the immunogenic peptide has at least 85% homology to at least one epitope of SARS-CoV-2 proteome.

38. The method of any one of claims 31 to 37, wherein the pharmaceutical composition further comprises a biopolymer.
39. The method of any one of claims 31 to 38, wherein the pharmaceutical composition is administered subcutaneously, intravenously, orally, transmucosally, transdermally, topically, or nasally.
40. A method of vaccinating a subject against infection by SARS-CoV-2, the method comprising:
- a. providing a pharmaceutical composition comprising the of any one of claims 1 to 19,
 - b. administering the pharmaceutical composition to the subject,
 - c. testing the subject for a presence of antibodies that bind to a peptide having amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 25.
41. A method of vaccinating a subject against infection by SARS-CoV-2, the method comprising:
- a. providing a pharmaceutical composition comprising the of any one of claims 1 to 19,
 - b. administering the pharmaceutical composition to the subject,
 - c. testing the subject for a presence of antibodies that bind to a peptide having amino acid sequence set forth as SEQ ID No. 1 to SEQ ID No. 7.

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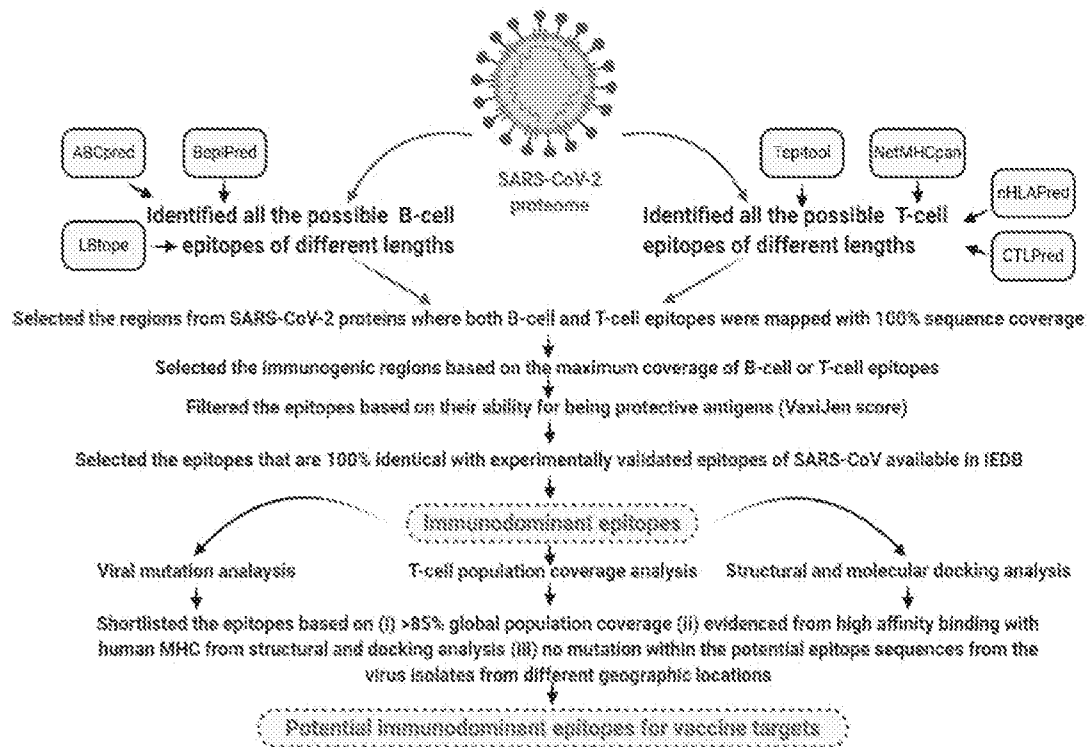


Figure 1

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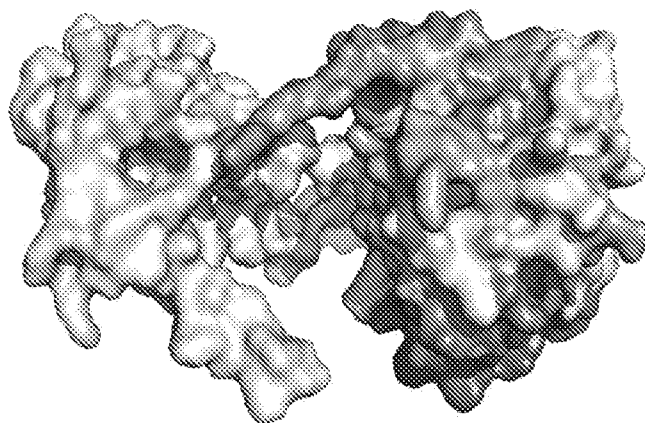


Figure 2A

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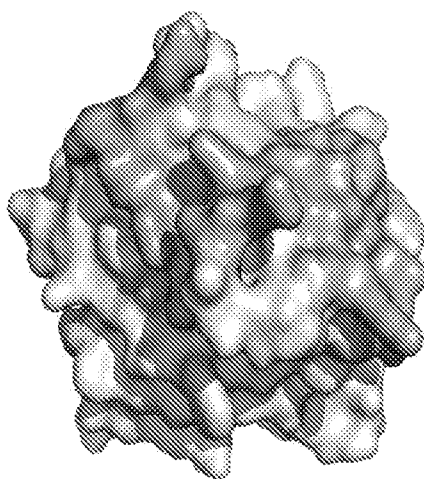


Figure 2B

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Figure 2C

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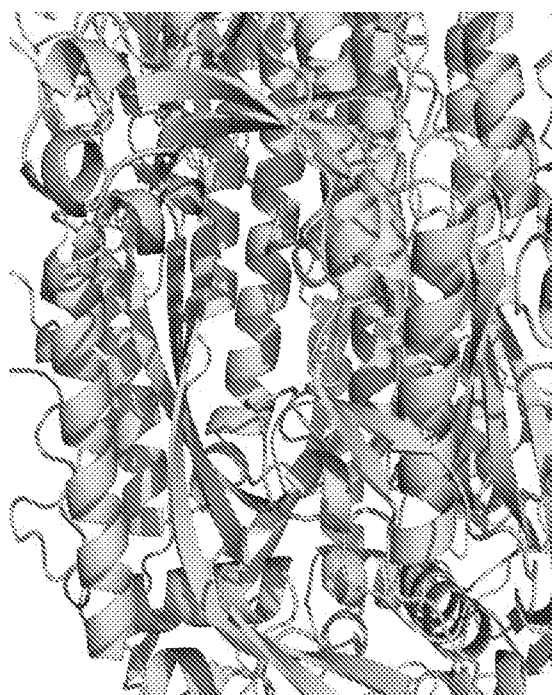


Figure 2D

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Figure 2E

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Membrane Glycoprotein:172-187	TSRTLSYYKLGASQRV	Membrane Glycoprotein:61-70	TLACFVLAAY
Q1Q49785, SARS-CoV2, USA	TSRNLISYYKLGASQRV	Q1B7843, SARS-CoV2, USA	TLACFVLAAY
Spike Glycoprotein:1254-1273	CKFDENDSEPVKGVKLHYT	Spike Glycoprotein:1157-1173	KNHTSPDVDLGNISGIN
Q1S60582, SARS-CoV2, USA	CKFDENDSEPVKGVKLHYT	Q1S60978, SARS-CoV2, USA	KNHTSPDVDLGNISGIN
Spike Glycoprotein:1165-1181	DLGDISGINASVVNIQK	Spike Glycoprotein:1165-1181	DLGDISGINASVVNIQK
Q1Q49922, SARS-CoV2, USA	DLGDISGINASVVNIQK	Q1S60978, SARS-CoV2, USA	DLGNISGINASVVNIQK
Nucleocapsid Phosphoprotein:268-286	YNVTQAFGRRGPEQTQGNF		
Q1S61010, SARS-CoV2, USA	YNVTQAFGRRGPEQTQGNF		

Figure 3

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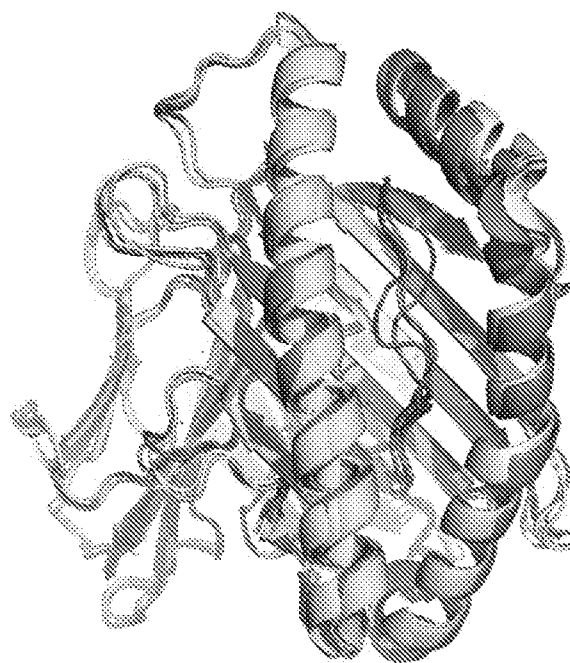


Figure 4A

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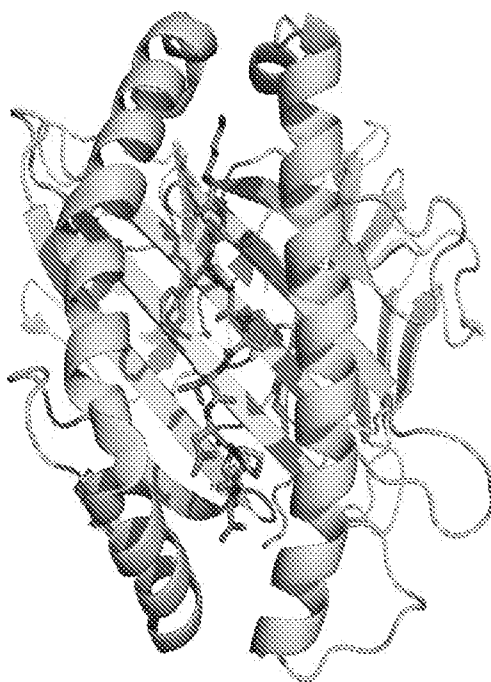


Figure 4B

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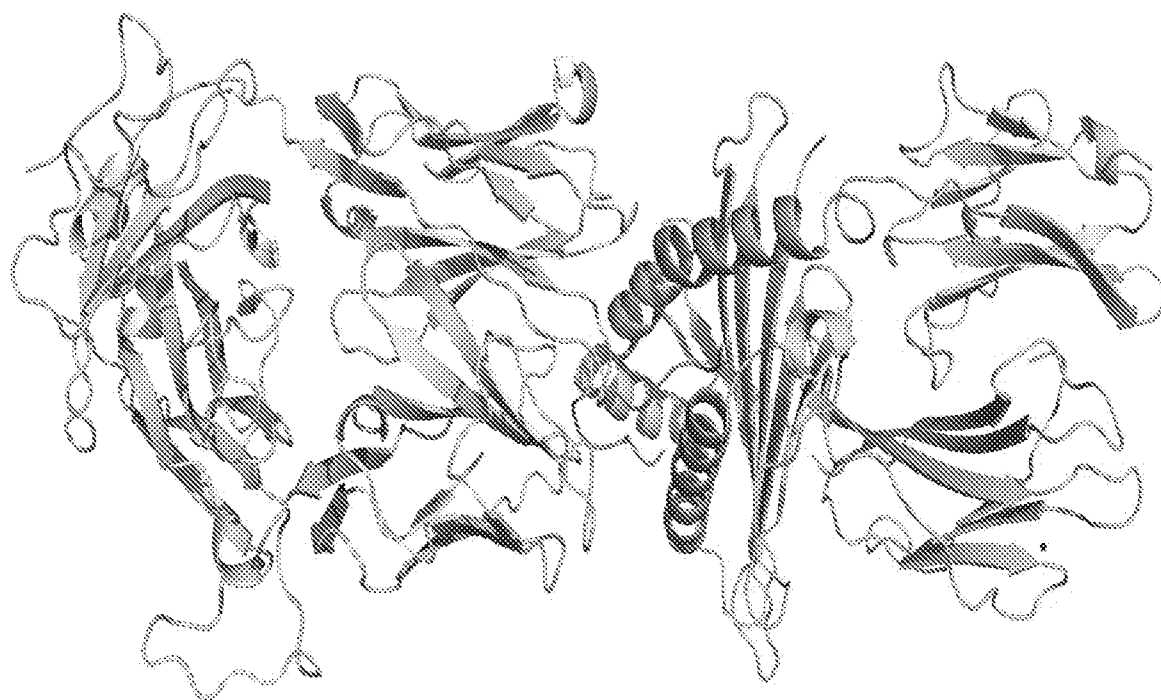


Figure 4C

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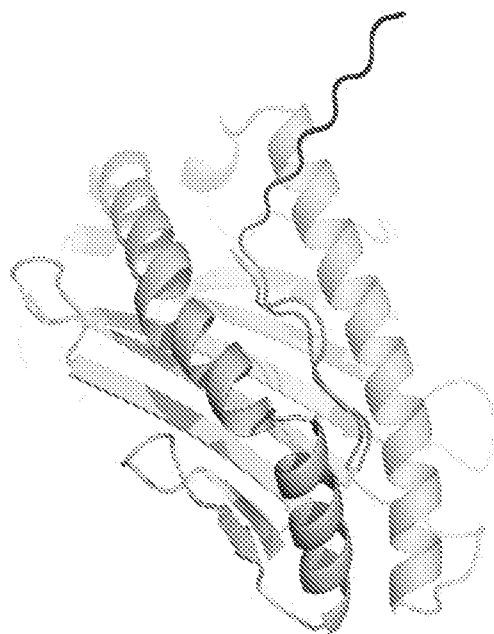


Figure 4D

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2021/050677

A. CLASSIFICATION OF SUBJECT MATTER

See extra sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC (20210101) A61K 39/215, C07K 7/04, C07K 14/165, C12N 15/09

CPC (20170801) A61K 39/215, C07K 7/04, C07K 14/165, C12N 15/09

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases consulted: BLAST, Esp@cenet, Google Patents, CAPLUS, BIOSIS, MEDLINE, REGISTRY, PubMed, Google Scholar, DWPI

Search terms used: Sequence search, peptide vaccine, membrane glycoprotein, spike glycoprotein, nucleocapsid phosphoprotein, epitope, immunogenic, Coronavirus, covid-19 or SARS-CoV-2, population coverage, non-allergen, applicant

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Ahmed, Syed Faraz, Ahmed A. Quadeer, and Matthew R. McKay. "Preliminary identification of potential vaccine targets for the COVID-19 coronavirus (SARS-CoV-2) based on SARS-CoV immunological studies." <i>Viruses</i> 12.3 (2020): 254. [online] [retrieved on 2021-08-10]. Retrieved from the Internet: <URL: https://www.mdpi.com/1999-4915/12/3/254 ><DOI: 10.3390/v12030254> 25 Feb 2020 (2020/02/25) abstract, page 6 second para, table 4, page 12 fourth para.	20,22,24,32-35,37
Y	page 9 last para, page 11 third para.	40,41
X	Liu, Ge, et al. "Robust computational design and evaluation of peptide vaccines for cellular immunity with application to SARS-CoV-2." <i>bioRxiv</i> (2020). [online] [retrieved on 2021-08-10]. Retrieved from the Internet: <URL: https://www.biorxiv.org/content/10.1101/2020.05.16.088989v1.abstract ><doi: 10.1016/j.cels.2020.06.009> 17 May 2020 (2020/05/17) abstract, figure 1, page 12 lines 388-391, page 2 lines 45-49	1-13,16,18-30,32-35,37,39

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

16 Aug 2021

Date of mailing of the international search report

18 Aug 2021

Name and mailing address of the ISA:

Israel Patent Office

Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel

Email address: pctoffice@justice.gov.il

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2021/050677

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Albagi, Sahar Obi Abd, et al. "A Multiple Peptides Vaccine against nCOVID-19 Designed from the Nucleocapsid phosphoprotein (N) and Spike Glycoprotein (S) via the Immunoinformatics Approach." (2020). [online] [retrieved on 2021-08-10]. Retrieved from the Internet: <URL: https://www.biorxiv.org/content/10.1101/2020.05.20.106351v1 <<doi: 10.1101/2020.05.20.106351> 20 May 2020 (2020/05/20) abstract, table 6, page 5, third and fourth para, page 12 second para., table 3,	2,20-22,24,32-34,37
X	CN 1634978 A UNIV PLA 2ND MILITARY MEDICAL? [CN]; CAO XUETAO [CN] ET AL; machine translated 06 Jul 2005 (2005/07/06) parapara. 002, 008, 0021, 0027, 0030-0031, 0051, 0055, claim 6	1,4,5,7,14,15,17-20, 26,31-37,39
Y	example 4	40,41
X	US 2006128628 A1 CHEN SHOW-LI [TW] et al; 15 Jun 2006 (2006/06/15) SEQ ID NO:2, para 009, table 1, claims, claim 16, example 4, para 0021	1,4,5,14,15,17,20, 31-37,39
P,X	KHAIRKHAH, Niloofar, et al. Design of novel multiepitope constructs-based peptide vaccine against the structural S, N and M proteins of human COVID-19 using immunoinformatics analysis. Plos one, 2020, 15.10: e0240577. [online] [retrieved on 2021-08-10]. Retrieved from the Internet: <URL: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0240577 ><doi: 10.1371/journal.pone.0240577> 15 Oct 2020 (2020/10/15) The whole document, table 1-4	1-5,7,8,13,16,20-22, 32-35,37

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet):

* This International Searching Authority found multiple inventions in this international application, as follows:

Invention/s 1 Claims 1-41 (partially) relate to a pharmaceutical Claim/s 1-41

composition comprising an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID Nos 1, 8, 9,10, 33-37, 39, 44-45 (from membrane glycoprotein) or a fragment, or a homologue thereof and at least one pharmaceutically acceptable carrier; An isolated immunogenic peptide comprising the same, wherein the immunogenic peptide is selected from a synthetic, a semi-synthetic or a recombinant peptide, and wherein immunogenic peptide has at least 85% homology to at least one epitope of SARS-CoV-2 proteome; An oligonucleotide encoding the same, an expression vector, an host cell comprising the oligonucleotide encoding the same; A method of treating a condition associated with an infection by SARS-Cov-2, A method of preventing infection with SARS-Cov-2, A method of immunomodulation, A method of vaccinating against infection by SARS-CoV-2 comprising the same.

Invention/s 2 Claims 1-41 (partially) relate to a pharmaceutical Claim/s 1-41

composition comprising an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID Nos: 2,3, 11,12,13,14,15,25, 37,39,40,42,44,45 (from nucleocapsid phosphoprotein) or a fragment, or a homologue thereof and at least one pharmaceutically acceptable carrier; An isolated immunogenic peptide comprising the same, wherein the immunogenic peptide is selected from a synthetic, a semi-synthetic or a recombinant peptide, and wherein immunogenic peptide has at least 85% homology to at least one epitope of SARS-CoV-2 proteome; An oligonucleotide encoding the same, an expression vector, an host cell comprising the oligonucleotide encoding the same; A method of treating a condition associated with an infection by SARS-Cov-2, A method of preventing infection with SARS-Cov-2, A method of immunomodulation, A method of vaccinating against infection by SARS-CoV-2 comprising the same.

Invention/s 3 Claims 1-41 (partially) relate to a pharmaceutical Claim/s 1-41

composition comprising an isolated immunogenic peptide comprising amino acid sequence set forth as SEQ ID Nos: 4- 7, 16-24 , 33-45 (from spike glycoprotein) or a fragment, or a homologue thereof and at least one pharmaceutically acceptable carrier; An isolated immunogenic peptide comprising the same, wherein the immunogenic peptide is selected from a synthetic, a semi-synthetic or a recombinant peptide, and wherein immunogenic peptide has at least 85% homology to at least one epitope of SARS-CoV-2 proteome; An oligonucleotide encoding the same, an expression vector, an host cell comprising the oligonucleotide encoding the same; A method of

treating a condition associated with an infection by SARS-Cov-2, A method of preventing infection with SARS-Cov-2, A method of immunomodulation, A method of vaccinating against infection by SARS-CoV-2 comprising the same.

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (20210101) A61K 39/215, C07K 7/08, C07K 7/06, C07K 7/02, C07K 14/165, A61P 31/14, A61K 39/00, C12N 15/09
CPC (20170801) A61K 39/215, C12N 2770/20034, C07K 7/08, C07K 7/06, C07K 7/02, C07K 14/165, A61P 31/14, A61K 2039/575, A61K 2039/55555, C12N 15/09

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/IL2021/050677

Patent document cited search report			Publication date	Patent family member(s)			Publication Date
CN	1634978	A	06 Jul 2005	CN	1634978	A	06 Jul 2005
				CN	100408595	C	06 Aug 2008
US	2006128628	A1	15 Jun 2006	US	2006128628	A1	15 Jun 2006