Peptides Based Vaccine Against SARS-nCoV-2 Antigenic Fragmented Synthetic Epitopes Recognized by T-Cell and β-Cell initiate Specific Antibody to Fight the Infection

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Abstract

The World Health Organization has declared the rapidly spreading coronavirus to be a global pandemic. The FDA till yet approved a vaccine for human novel coronavirus. Here we developed a peptide-based vaccine and used high throughput screening by molecular dynamics simulation to identify T-cell and β-cell recognized epitopes to the production of specific antibodies against SARS-nCoV-2. We construct ~12 P` antigenic epitope peptides used to develop a more effective vaccine and identify specific antibody. These epitope peptides selectively best antigens presentation scores for both human pMHC- class I-II alleles to develop strong bindings affinity that were all antigens identified of SARS-nCoV-2 different proteins by each attached specific 1-7L linkers adaptor to construct a large single peripheral peptide vaccine. It is expected to be highly antigenic with a minimum allergic effect. Furthermore, considering the conservancy, this peptide vaccine promising to be highly utilized to a developed specific antibody against SARS-nCoV-2 by an initiate of T-cell and β-cell. An *in-vitro* study for the proposed peptide-based vaccine mostly recommended. Further clinical trials are required to check the efficacy of this vaccine.

Introduction

A novel coronaviruses are large enveloped a single-stranded RNA viruses (size ranging from 26 to 32kbs in length) in the coronaviridae family that cause the common cold, influenza-like illness, and more serious acute respiratory illnesses, including pneumonia, exacerbations of underlying lung disease, croup, and bronchiolitis (LIM *et al.,* 2016). The subgroups of coronaviruses family are alpha (α), beta (β), gamma (γ) and delta (δ) coronavirus. Particularly this virus was reported to be a member of the β group of coronaviruses. The novel virus was as Wuhan coronavirus or 2019 novel coronavirus (2019-nCov) by the Chinese. The International Committee on Taxonomy of Viruses (ICTV) named the virus as SARS-CoV-2 and the disease as COVID-19 (Adachi *et al.,* 2020; WH 2020). Globally, so far there has been 1 death every 1,166 people under 65 years old compared to 1 death every 358 people in the general population. And 89 % of the times, the person who died had one or more underlying medical conditions. A total coronavirus cases are ~8,256,725 in about 3.4 % of reported COVID-19 cases have died according to COVID-19 outbreak as of June 17, 2020, 02:16 GMT. By comparison, seasonal flu generally kills far fewer than 1 % of those infected" (WHO 2020). Estimated by simply looking at the value of current total deaths plus current total recovered and pair it with a case total in the past that has the same value i.e.~445,959 / (445,959 + 4,306,426) = 9 % CFR (crude fatality ratio) in worldwide (Battegay *et al.,* 2020).

After all, since the 1930s when the coronavirus family of viruses was first identified, till now commercially there has been no successful vaccines or antiviral drugs that has been able to prevent or treat infections for COVID19, past and current vaccine development efforts against this disease might be of high value for the development of an effective vaccine for COVID-19. According to the WHO, 41 candidate vaccines are being developed for COVID-19 from different countries as of March 13, 2020 (WHO 15 Mar 2020). Many research teams in the world have been working on monoclonal and polyclonal antibodies of novel coronavirus by produced in-vitro using tissue-culture techniques, but few of them have entered into trials. Monoclonal antibodies are generally very rarely used in the treatment of infectious diseases. As will polyclonal antibodies composed a mixture that represents the natural immune response to an antigen, they are prone to an also higher risk since in both even not all in success. Secondly, drugs and small molecules that can calm the immune system but patients become seriously ill when their immune system overreacts and starts causing collateral damage to the body. The U.S. Food and Drug Administration today dated June 04, 2020 announced the following actions taken in its ongoing response effort to the COVID-19 pandemic. The dexmedetomidine hydrochloride in 0.9% sodium chloride injection (ANDA-209307) and hydroxychloroquine and chloroquine to treat COVID-19, but a sideeffects are more such like an irregular heartbeats, dizziness, or fainting, seek medical attention needful immediately.

The inhaled virus SARS-CoV-2 likely binds to epithelial cells in the nasal cavity and starts replicating (Zhang *et al.,* 2017). The human Angiotensin-converting enzyme 2 (hACE2) is the main receptor for the cell entry in both SARS-CoV and SARS-CoV-2 outbreak (Hoffmann *et al.,* 2020, Bérubé *et al.,* 2010). The spike glycoprotein (S) on the surface of coronaviruses is a major essential for virus entry through binding to the hACE2 receptor and for viral fusion with the host cell (Wrapp *et al.,* 2020). Thus, one could target this interaction site between hACE2 and SARS-CoV-2 spike protein with antibodies or small drug molecules (Iwata *et al.,* 2019). *In-vitro* data with SARS-CoV2 indicate that the ciliated cells are primary cells infected in the conducting airways (Deng *et al.,* 2017). At this time, the disease COVID-19 is clinically manifest. Overexpression of hACE2 enhanced disease severity in a physiologically relevant model for investigating hACE2 as a therapeutic target for antiviral intervention against COVID-19 pandemic.

Without a vaccine, we should not think of herd immunity as a light at the end of the tunnel. A vaccine is the only lifetime way to move directly from susceptibility to immunity, bypassing the pain from becoming infected and possibly dying. Moreover, In this study is to focus on identify selected fragmented (protein subunit) genetic code of antigenic epitope peptides from each novel coronavirus protein domains were in spike protein (S), membrane glycoprotein (M), envelop protein (E) and nucleocapsid protein (N) of the virus and use that as our vaccine. When the vaccine is injected into the body, muscle cells naturally "amplify" it by producing copies of the each antigenic epitope peptides from each protein domains, which the immune system detects as a threat. This trains the body's immune system to defend against novel coronavirus to produce specific antibody either IgG, IgA, IgM, and IgM through being able to recognize the all-antigenic peptides if it encounters it again. Finally, only a solution is vaccine that is produced antibodies by our own body's β-cells to fight off infections by novel coronavirus other than any sideeffects.

Materials And Methods

Creation of Clinical Dataset

SARS-nCoV-2 infected patient complete gene sequences and metadata for all COVID19 were obtained from different database were in NCBI Virus SARS-nCoV-2 Data Hub, GenBank SARS-CoV-2 sequences, Broad Terra Cloud and Geo COVID Datasets. All nucleotide sequences were translated using by Biopython

(Cock *et al.,* 2009) package. Well set of ~281 complete genome sequences of SARS-CoV-2, each one approximately 30,000 nucleotides in length using CLC Bio (Qiagen, 2020). We selected mapped to the complete genome of SARS-nCoV-2 Wuhan-Hu-1 isolate (Genbank accession number: MN908947.3). The protein sequences of ~2666 coronaviruses were collected from 2019 Novel Coronavirus Resource (2019nCoVR) Database of China National Genomics Data Center NGDC, (https://bigd.big.ac.cn/ncov) on Jan 29, 2020 (Zhao *et al* 2020). The corresponding data file was contains information about the length of each sequence, geographical location, isolation source, collection date of the sample etc. As well clinical information were taken from UCSF COVID-19 Clinical Data, Vivli, ClinicalTrials.gov COVID-19 Trials, Coronavirus Clinical Trials Explorer, and STAT's COVID-19 Drugs and Vaccines Tracker. These strains had full-length genomes and were isolated between 1941 and 2020, and included SARS-CoV-2 strains.

Selection of Antigenic Epitope Peptides

The specificity of peptides was confirmed by Basic Local Alignment Search Tool (BLAST) search against the UniProtKB/Swiss-Prot database (https://blast.ncbi.nlm.nih.gov), which required that the peptide length should be not more than 10-13 amino acids according to the human both MHC-I and II epitope recognized compatibility. For setting search criteria on the specific was protein-protein algorithm and no organism was excluded. According to the hit number, all antigenic peptides results were based on into three grades: high (H, hit number =0), medium (M, 1≤ hit number ≤10), and low (L, hit number ≥11) and the grade "high" means the best peptide specificity. Antigenic epitope peptides regions predictions were based on the amino acid properties including antigenicity (Kolaskar *et al.,* 1999), secondary structure (Pellequer *et al.,* 1993), solvent accessibility (Emini *et al.,* 1985), flexibility (Karplus *et al.,* 1985), and hydrophilicity (Parker *et al.,* 1986). In further, based on the epitope refined to databases such as IEDB (Vita *et al.,* 2010), FIMM (Schonbach *et al.,* 2000), and Bcipep, there are also some methods using machine learning approaches, such as Support Vector Machine, Hidden Markov Model (HMM) (Larsen *et al.,* 2006) to locate linear epitopes, such as BEPITOPE (Odorico *et al.,* 2003), BayesB (El-Manzalawy *et al.,* 2008), BepiPred (Larsen *et al.,* 2006), ABCPred (Saha *et al.,* 2006), BEOracle/BROracle (Wang *et al.,* 2011), and BEST (Gao *et al.,* 2012).

Identification of T-cell and β-cell Recognized Epitopes

T-cell epitope predictions were identify the shortest peptides ~10-13 amino acids within an antigen that are able to stimulate either CD4 or CD8 T-cells (Ahmed *et al.,* 2009). This capacity to stimulate T-cells immunogenicity, and it was confirmed in assays requiring synthetic peptides derived from antigens (Ahmad *et al.,* 2016). There are many distinct peptides within antigens and T-cell prediction methods aim to identify those that are immunogenic. As for complete novel coronavirus of antigenic epitopes regions were specifically determined by BepiPred-2.0 (Jespersen et al., 2017) that highly immune recognized. T-cell epitope immunogenicity was contingent based on three basics 1), antigen processing, 2) peptide binding to pMHC molecules, and 3) recognition by a cognate TCR. Of these three bases, pMHC-peptide binding was the most selective one at determining T-cell epitopes (Lafuente et al., 2009). Therefore, prediction of peptide-pMHC binding was the main basis to anticipate T-cell epitopes. In-addition, β-cell epitope predictions also to be facilitates β-cell epitope resolute with the replacing the antigen for antibody production. Any solvent-exposed region in the antigen can be recognition by antibodies. β-cell epitopes consist of peptides amino acid rage ~10-13, whereas conformational β-cell epitopes consist of patches of solvent-exposed atoms from residues that are not necessarily sequential. Most β-cell epitopes was approximately a 90 % are conformational and, in fact, only a minority of native antigens contains linear β-cell epitopes.

Epitope-Based Vaccine Construct and Validation

Synthetic peptide vaccines candidate of antigenic epitope fragments peptides sequence were to alignment accuracy, fold recognition and structure prediction by using several benchmarks that were determined by SPARKS-X (Yuedong *et al.,* 2011): Protein fold recognition and ORION: Optimized protein fold Recognition (Ghouzam *et al.,* 2015; Ghouzam *et al.,* 2016). In addition, PAComplex server to predict a peptide antigens and search the template-based homologous specific peptide antigens of our query short vaccine candidate peptide amino acid sequence by the following methods (Liu *et al.,* 2011). The server was initially a divide the vaccine candidate peptides sequence into fix length ranging from ~8 to 13 peptides based on selected pMHC class I-II allele. In each selected epitope peptide was aligned to the bound peptide of TCR–pMHC templates gathered from PDB. Next, the peptide antigen was examined by utilizing the template-based scoring function to statistically evaluate the complex similarity (Jz ≥ 4.0) between TCR–pMHC and TCR-pMHC. The binding interacting residues based on hydrogen bonds and VDW forces of pMHC and antigenic peptide-TCR interface for each antigen. Finally, the hit homolog identified template vaccine candidate of antigenic conserved amino acid peptides with ratio of Jz ≥ 4.0, that more recognized and interaction between TCR-pMHC. A validation of peptide measures by ProSA-web (Markus *et al.,* 2007) that exploits interactive scores and energy plots that highlight potential problems spotted in peptide structures. In particular, the quality scores of peptides determined whether problematic parts of a structure are shown and highlighted in a structure model. Furthermore, construct vaccine candidate peptides was inability of highly polar and charged molecules to enter cells without causing irreversible lipid membrane damage that were novel approach determined in CELLPM (Lomize *et al.,* 2018) cell-penetrating peptides (CPPs) and Positioning of Proteins in Membrane (PPM) server (Lomize *et al.,* 2012) to understand mechanisms of direct peptide translocation across the lipid bilayer.

Peptides Molecular Dynamics (MD) Simulations

The program PEPstrMOD (Singh *et al.,* 2015) allows extended Molecular dynamics (MD) simulation of predicted peptides that enables all-atom simulations of biomolecules. The initial structure was treated to energy minimization and molecular dynamics by using both AMBER11 (Salomon *et al.,* 2013) and GROMACS (Sunhwan *et al.,* 2008) to generate the final peptide structure. The systems were minimized for 10,000 steps followed by 200 ps of equilibration. This was followed by MD production runs for 200 ns at a temperature of 300 K using a 2 fs time-step. The long-range ionic interactions were calculated using the particle mesh Ewald (PME) (Essmann *et al.,* 1995) method. After simulations, a short energy minimization of all peptides was performed and the final tertiary structure was predicted. Further analysis the molecular docking of vaccine peptide candidate interacted between the TCR and pMHC-II. The generated peptide-docking complex was visualized in Molecular Operating Environment (MOE) (Wilson *et al.,* 2011).

Results

Detection of β-cell and T-cell recognized epitopes and antigenic analysis

The antigenic epitope peptides selection from SARS-nCoV-2 based on the peptide quantification method for peptides selection, we challenged screen peptides via specificity, digestibility, recovery, and stability was to determine the prototypic peptides. Investigated the presence of predicted peptides from the UniProt SARS-nCoV-2 pre-release (Jun23, 2020). MHC-T-cell and β-cell was highly recognized antigens that were prioritized based on the consensus score, binding affinity, and antigenicity. Data dependent acquisition analysis reveled the presence of more than ~20 unique antigenic epitopes peptides was ranked at the top of the list would correlate with the highest antigenicity Frank score of 0, and a Frank score of 0.5 would correspond to a random prediction. It was suggested that the consensus approach would improve the specificity and accuracy of the epitope prediction as it can reduce the false positives (Figure 1A). That were predicted, but overall construct ~12 synthetic small antigenic peptides of rage among ~13 amino acids out of the ~12 different proteins (S, E, M, N and ~8 other ORFs) in each predicted by UniProt SARS-nCoV-2 (Figure 1B). That was more interrelating between histocompatibility complex (pMHC)-T-cell receptor (TCR). T-cells and β-cell recognize small peptide fragments of optimal amino acid residues for pMHC-binding well produce a more stable pMHC complex and improved recognition of antigenic epitope peptides. Small peptides can significantly alter TCR binding in ways that are difficult to predict and thereby prime T-cells with altered TCR repertoires. These repertoire effects have clinical relevance as it was found that develop vaccination with contact residue-altered peptides was less effective than vaccination with natural peptides at priming SARS-nCoV-2-specific T-cells in patients. The peptide backbone was fixed amino acid and all other positions are degenerate, with degenerate positions containing any one of photogenic amino acid with in rage (~1-13) was excluded to reduce disulfide bond formation within the compound mixture.

Vaccine construction and validation by MD Simulation

A different selected epitopes sequences were designed, as described in Materials and Methods. We further estimated the effect of linker, epitope location, and sequence continuity on peptides structural characteristics. The range of linkers vary from more than ~2 to ~30 amino acids, optimized for each condition so that the linker does not impose any constraints on the conformation or interactions of the linked partners. The analysis results by IBIVU showed that the linker between the epitopes, which was used flexible linker “AVKELF” lyase that found to connect of every single epitopes to form vaccine candidate structure for therapeutic index (Figure 2A). However, once linkers linked with peptides the structural parameters changed greatly, especially indexes of α, β, turn, and coil regions that are indications of protein secondary structure. In particular, “AVKELF” linkers reduced the number of β-, turn-, and coil-regions, but increased the number of α-regions. This provides a strategy of combinatorial biosynthesis, in which modules are the building blocks of genetic manipulation. Epitopes peptides connective with a linker inability of highly polar and charged molecules to enter cells without causing irreversible membrane damage to passively penetrate across the lipid bilayer.

The structure of each SARS-nCoV-2 proteins epitopes peptides (P1 - P13) was further refined with energy minimization and molecular dynamic simulations. The duration of MD simulations from 100 ps to 1 nanosecond (ns) and compared the performance that were determined by PEPstrMOD and ORION at both of these time steps (Figure 2B). The results it was slightly better in first time as compared each peptides quality based on abinitio method. The performance was further improved from 3.97Å to 3.82Å (CA-RMSD: PEPstrMOD) by extending the duration of MD runs from 100ps to 1ns in hydrophilic environment even in the case of very short peptides. The Potential energy minimization on the initial peptides to get the minimized structures in order to compute based on RMSD values. The results improved to average CA-RMSD: PEPstrMOD and B-RMSD: ORION of 4.12 Å and 3.85 Å. The initial and minimized structure achieved an average RMSD: ORION of 3.76 Å and 3.64 Å. Finally, the minimized structure of peptides and structure after 100 ps MD simulations improved to 4.78 Å and 4.31 Å. Overall, secondly observed no improvement in the results by extending MD simulation from 100 ps to 1 ns by ORION and PEPstrMOD. Performing MD simulations in hydrophilic environment also produced similar results with an average CA-RMSD of 4.35Å as compared to 4.31Å in vacuum.

Peptide across bilayer and membrane-peptide interactions

The molecular dynamics simulations were the direct translocation of cell penetrating (CPPs) constructed 1-12 P

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small peptides with connected by 1-7L linkers adaptor to construct large single peripheral peptide, especially conformations of membrane to interacting loops, where the uncertainty in tilt angle may reach 50°. Large differences in orientations may be observed for alternative conformations of peptide. Moreover, distinct conformations of Ca2+-ATPase, a TM α-helical protein, differ in protein tilt by 17° and membrane thickness was averagely 3 Å, which might be other functional importance.

Peptide bindings to MHC class I-II in an extended conformation with TCR

A crystal structure with resolution 1.61-Å of the TCR class I-II antigen (PDB ID: 2AK4) as Swiss- Model and PAComplex identified the best template. The sequence identity template was 92%. The best TCR complex model was then selected templates were evaluate based on the accuracies of scoring functions on variant conditions including single template, multiple templates, single and both sides, as for these validation methods the model selected TCR complex template with binding antigenic peptide score was ~1.77 to ~4.22, respectively which induced dataset were comprises ≥108 peptide candidates (JZ ≥ 1.645) derived from (IEDB) ~864 628 sequences of 389 pathogens, was used to evaluate the reliability of SARS-nCov-2 homologous peptide antigens. The homologous peptide antigens was tested on >10^{10} peptides derived from ~864 628 protein sequences of ~389 pathogens. Among these peptides, over 108 peptide candidates with JZ ≥ 1.645 were selected for analyzing the relationships between JZ values with both the numbers of positive homologous peptide antigens. When JZ ration was higher than 4.0, the precision >0.8 and the number of positive antigens exceeds ~1600 according to both positive and negative data sets. The JZ threshold was set to ~4.0, the total number of inferring possible peptide antigens ~4 000 000 that were finally derived from ~12 antigenic peptide recognized of TCR–pMHC complexes. The pMHC class II binding peptides was difficult as compared to pMHC class I binding peptides due to their variable size. Our results of pMHC class II binding peptides number of each 10-13 amino acids long with a binding core of ~4-6 amino acids which

containing primary anchor residues. The docked allele to each epitope out of 1-12 P` complexes showed the same residue to antigenic peptide interactions observed in the epitope bound crystal structures of TCR–pMHC. Further additional information we provided as shown in the figure 4 and table 2.

Peptide vaccine produced antibodies against a specific antigen

Vaccine-induced immune effectors are essentially antibodies produced by β-cell capable of binding especially to a pathogen. Other potential effectors are cytotoxic CD8+ T lymphocytes that may limit the spread of infectious agents by recognizing and killing infected cells or secreting specific antiviral cytokines and CD4+ T-helper lymphocytes. This T helper cells well contributes to protection through cytokine production and provides support to the generation and maintenance of β-cell and CD8+ T-cell responses. Effector CD4+ T-helper cells were depending on their main cytokine production of interferon-γ or interleukin [IL]-4), respectively. SARS-Cov-2 peptide based vaccine antigens reached penetrate in the lipid membrane and translocated into the B-cell via pMHC class I-II. The β-cells equipped with surface β-cell receptors capable of binding to the vaccine antigens were activated and migrate to the interface between the β-cell and the T-cell. There, β-cells engage T-cells and initiate their proliferation. The cumulative amount of stimulate signals received by β-cells determines their fate.

SARC-nCoV-2 protein antigens were taken up as small peptides on the surface of specific antigen- presenting cells (APCs) activate T-cells. This induces a highly efficient β-cell differentiation pathway, through specific structures in which antigen-specific β-cells proliferate and differentiate into antibody- secreting plasma cells or memory β-cells against SARS-nCov-2. They encounter a SARC-nCoV-2 protein antigen to which their specific surface IgM receptor binds. Antigen binding initiates β-cell activation and triggers the up regulation of chemokine receptor (CCR7), a chemokine receptor that drives antigen-specific β-cells toward the outer T-cell zone of lymph nodes. At this location, peptide vaccine antigen-specific β-cells are exposed to (<24 hours) activate dendritic cells (DCs) and T-cells that have unregulated specific surface molecules and, thus, provide B-cell activating signals (Figure 5). This T-cell help rapidly drives β-cell differentiation into Ig-secreting plasma cells that produce low- affinity germline antibodies to extra follicular reaction.

Moreover, the β-cell affinity results from an extensive somatic hypermutation process within the variable-region segments of Ig genes. In a small minority of β-cells, the introduction of mutations in their Ig genes increases the affinity of their surface IgG for antigen. This cause enables these β-cells to efficiently compete for binding to the small amounts of peptide vaccine antigens that are associated with the surface of DCs. β-cells process these peptide vaccine antigens into small peptides at surface through major MHC class I-II molecules. MHC- peptide complexes thus become available for binding by the specific subset of CD4+ T-cells. These T-cells, which express CXCR5, migrate toward CXCL13-expressing FDCs. Differing from T-helper 1 and 2 cells by their chemokine receptors, transcription factors, surface markers, and interleukins, they are uniquely equipped was to provide efficient β-cell help through a series of co-stimulation molecules, including CD40L, ICOS inducible T- cell co-stimulator, the IL-10 β-cell growth factor, and IL-21 were antigen-specific antibodies against SARS-nCov-2 appear in the serum that was another hallmark of secondary responses for the antibody production.

Discussion

The novel coronavirus disease 2019 (COVID-19) pandemic has created a worldwide crisis and inspired an urgent search for prevention and treatment of severe acute respiratory syndrome coronavirus 2 (SARS-nCoV-2) infection (Zhou *et al.,* 2020). The world around researchers is currently developing more than ~140 vaccines against the coronavirus by different target proteins by in each. An effective vaccine is only necessary solution to the COVID-19 pandemic (Corey *et al.,* 2020). Although the vaccines development process typically require years of research and testing before reaching the clinic, but scientists are racing to produce a safe and effective vaccine. The first vaccine safety trials in humans started in March 2019, but the road ahead remains uncertain. Some trials are fail, and others may end without clear results. But a few may succeed in stimulating the better immune system to produce effective antibodies against the virus. Here is the current status of all the vaccines that have reached trials in humans, along with a selection of promising vaccines still being in a pipeline tested in cells or animals as shown in the Figure 6 (Jonathan *et al.,* 2020).

The S-protein is considered an attractive therapeutic target given its location on figure 5, and it is therefore targetable using antibodies (Marovich *et al.,* 2020). Immunization of animals with S protein-based vaccines has been shown to induce the formation of neutralizing antibodies that are effective in preventing infection by homologous coronaviruses (Kun *et al.,* 2020), but the clinical trial phase-III was not success in human. Although S-protein may elicit an immune reaction, it is not yet known whether or not this would be sufficient to mount the sustained immune response needed to fight COVID-19 infection (Catanzaro *et al.,* 2020). As for recent report among all the SARS-nCoV antigens, the S protein stimulates the highest level of antibody production. The epitopes of this protein that bound to CD4 and CD8 T cells were identified. Two of these epitopes binding to CD8 cells were presented by the MHC II molecule HAL-A*02:01 and stimulated a specific response in patients who had recovered from SARS-nCoV-2 but not healthy individuals. Many other T-cell epitopes in the M and N proteins of the virus have also been identified. But we are not targeted vaccine based on a single antigen such like S-protein (Grifoni *et al.,* 2020). Here we are designed epitopes of peptides based vaccines based on the complete genome of SARS-CoV-2 Wuhan-Hu-1 isolate (Genbank accession number: MN908947.3) that were constructed ~12 small fragmented antigenic epitopes peptides by each ~13 amino acids in different proteins (S, E, M, and N) and other necessary proteins (ORF 1a, 1b, 3a, 6, 7a, 7b, 8, and 10) of SARS-nCoV-2. We converted the 1-12 P` antigenic epitopes into a single vaccine candidate, using “AVKELF” peptide linker adaptor were more effective, which was penetrating translocated to the cell or lipid membrane (Khan *et al.,* 2019). These epitopes can be used to make immunogenic multi epitope peptides vaccine against SARS-nCoV-2.

Antigen presentations by human MHC I-II molecules proceed by two different pathways. MHC II molecules present peptide antigens derived from endocyted antigens that are degraded and loaded onto the MHC II molecule in endosome compartments and another Class I MHC molecules present peptides derived mainly from antigens degraded in the cytosol (Bjum *et al.,* 2013). Our resulting all peptide antigens are then transported to the endoplasmic reticulum by TAP where they are loaded onto nascent MHC class I-II molecules. Vaccines most efficiently trigger the activation of the innate immune system through multiple pathogen- associated signals, allowing their recognition by pattern-recognition receptors (Querec *et al.,* 2006, PrabhuDas *et al.,* 2011). Most antigens and vaccines trigger β-cell and T- cell responses, such that there is no rationale in opposing vaccines favoring antibody production and T- cell responses. In addition, CD4+ T-cells are required for most antibody responses, whereas antibodies exert significant in fluencies on T-cell responses to intracellular pathogens

(Ishikawa *et al.,* 2008). Our vaccine candidate's of antigenic peptides that were recognized by MHC class both I-II antigens providing valuable insight into detect specific pMHC–peptide complexes relies on the string induction strong activation of T-cells bearing relevant CD8+ T-cell responses. Therefore our vaccine candidates of antigenic immune complexes are taken up by macrophages and DCs, dissociate into their acidic phagolysosome compartment, and are processed into fragmented small peptides. These peptides are exposed at the surface of APCs and are binding by CD4+ and CD8+ T cells. Overall, most protective antibody responses are dependent on CD4+ T-cell help. Therefore, we assessed SARS-nCoV-2-collotion of antigens from different proteins that all specific CD4+ T-cell responses were associated with production of higher specific antibody against SARS-nCoV-2.

Conclusion

We are addressing and challenged of antigenic immune response, the potential of structural peptide based vaccine against the coronaviruses and the observed phenomenon of long-term immunity to SARS-nCoV-2 likely stemming from T-cell and β-cell activity. The identified of such peptides would indeed help to better understand the immune responded to SARS-nCoV-2 and promote to develop of peptide based specific vaccine. This vaccine is produced specific antibody for specific antigens, also functionally non-allergenic, and antigenic and more effective which might be strongly against SARS- nCoV-2. Further clinical trials are required to check the efficacy of this vaccine.

Significance statement:

This study provides information on the COID19 of SARS-nCov-2. Developed peptides based vaccine elicitation epitope-trigger of specific antibodies against the novel coronavirus to prevent infection.

Abbreviations

SARS-nCOV-2: Severe Acute Respiratory Syndrome Coronavirus 2 MHC: Major Histo-Compatibility Complex

TCR: T-Cell Receptor

APC: Antigen Presenting Cell

ICTV: The International Committee on Taxonomy of Viruses HMM: Hidden Markov Model

TCR: T-Cell Receptor

APCs: Antigen Presenting Cells RMSD: Root Mean Square Deviations RMSE: Root Mean Square Error PDB: Protein Data Bank

CPPs: Cell-Penetrating Peptides MD: Molecular Dynamics

MOE: Molecular Operating Environment

IEDB: The Immune Epitope Database and Analysis Resource hACE: Human Angiotensin-Converting Enzyme 2

Declarations

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Compliance with ethics guidelines:

Author Z.A, and Author F.A; declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

Author contributions:

Z.A., and F.A. designed research contributed new reagents, analytic tools and data analysis; Z.A. Analyzed data; F.A., and Z.A. wrote the paper. Principle Investigator; Z.A.

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Tables

Table 1: Translational positions of transmembrane and peripheral peptide in membranes

P.No.	Peptide seq	Oriental of protein in Membrane			Membrane Embedded Amino acid Residues (in Hydrocarbon Core)	
		Depth/Hydrophobic Thickness	$\Delta G_{transfer}$	Tilt Angle	Tilt	Embedded residues
1	MDEFIERYKLEGY	4.5 ± 1.7 Å	-5.1 kcal/mol	78.± 18.°	78	1,4-5,8
2	PYEDFQENWNTKH	3.4 ± 2.1 Å	-4.1 kcal/mol	80.± 15.°	80	5,9,13
3	LQDVVNQNAQALN	1.7 ± 1.6 Å	-3.1 kcal/mol	52.± 11.°	52	1,4
4	YDYCIPYNSVTSS	2.3 ± 0.4 Å	-4.6 kcal/mol	88.± 7.°	88	1,5-7
5	YVYSRVKNLNSSR	2.6 ± 1.6 Å	-4.5 kcal/mol	75.± 8.°	75	2-3,6
6	NGTITVEELKKLL	3.6 ± 0.8 Å	-7.9 kcal/mol	85.± 7.°	85	1,4,6,9,12-13
7	TENKYSQLDEEQP	2.2 ± 1.1 Å	-3.4 kcal/mol	79.± 13.°	79	4,8
8	SPKLFIRQEEVQE	2.2 ± 1.8 Å	-2.1 kcal/mol	86.± 13.°	86	8
9	FSLELQDHNETCH	1.6 ± 1.7 Å	-3.6 kcal/mol	72.± 17.°	72	1-3,5
10	FYEDFLEYHDVRV	2.6 ± 12.5 Å	-3.9 kcal/mol	79.± 14.°	79	6,8,13
11	DQELIRQGTDYKH	2.0 ± 1.1 Å	-2.8 kcal/mol	76.± 10.°	76	5
12	SRNYIAQVDVVNF	2.7 ± 1.6 Å	-5.1 kcal/mol	85.± 11.°	85	4-8,12-13

Note: This table display of transmembrane of lipid interacted embedded amino acid residues in single line colored each shown in red and while rest of the residues in black color.

Table 2. Selection of peptide antigens and T-cell receptor-pMHC complex

P.No.	Peptide	Start Position	Allele selection	Joint Z-value	Best Template	Domain
1	MDEFIERYKLEGY	6670	HLA-DR4	4.03	2ak4	ORF1ab polyprotein
2	PYEDFQENWNTKH	153	HLA-DR4	4.22	2ak4	ORF1a polyprotein
3	LQDVVNQNAQALN	948	HLA-DR4	4.12	2ak4	Surface glycoprotein-SPIKE
4	YDYCIPYNSVTSS	154	HLA-DR9	2.67	2ak4	ORF3a protein
5	YVYSRVKNLNSSR	1	HLA-DR4	0	2ak4	E protein
6	NGTITVEELKKLL	5	HLA-DR4	0	2ak4	Membrane glycoprotein
7	TENKYSQLDEEQP	45	HLA-DR4	0	2ak4	ORF6 protein
8	SPKLFIRQEEVQE	83	HLA-DR4	2.4	2ak4	ORF7a
9	FSLELQDHNETCH	30	HLA-DR4	1.97	2ak4	ORF7b
10	FYEDFLEYHDVRV	104	HLA-DR9	2.65	2ak4	ORF8
11	DQELIRQGTDYKH	288	HLA-DR4	3.42	2ak4	Nucleocapsid phosphoprotein
12	SRNYIAQVDVVNF	23	HLA-DR9	2.29	2ak4	ORF10

Note: This table displays all predicted binder for specific pMHC allele in the single line just by coloring the predicted binders. The starting residue of each predicted binder is shown in red and while rest of the residues in blue color.

Figures

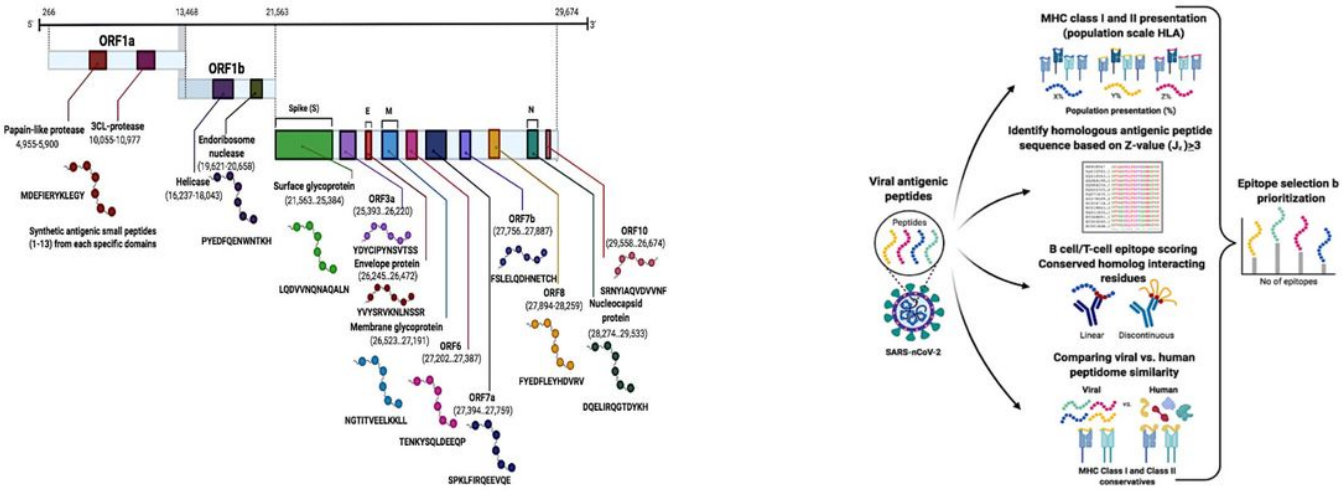

Figure 1

(Left) A. Systematic structures of SARS-nCov-2 genome organization and identified antigenic epitopes SARS-nCoV-2 genome is flanked by a 72 nucleotide-lengthy sequence at the 5' end and a Poly(A) tail at the 3' end. Several open reading frames (ORFs) have been identified corresponding to viral structural elements (S, E, M, and N proteins) and accessory genes (ORF 1a, 1b, 3a, 6, 7a, 7b, 8, and 10) both are represent different colors. The Antigen present epitope peptides sequence regions and ranges were identified from each the protein in different colors. (Right) B. Library design of antigenic epitopes peptide for β-cell / T-cell recognize SARS-nCov-2 antigenic epitope template selection based on scoring function to infer the peptide antigens and homologous peptide antigens through structural templates based on score Z-value (Jz)

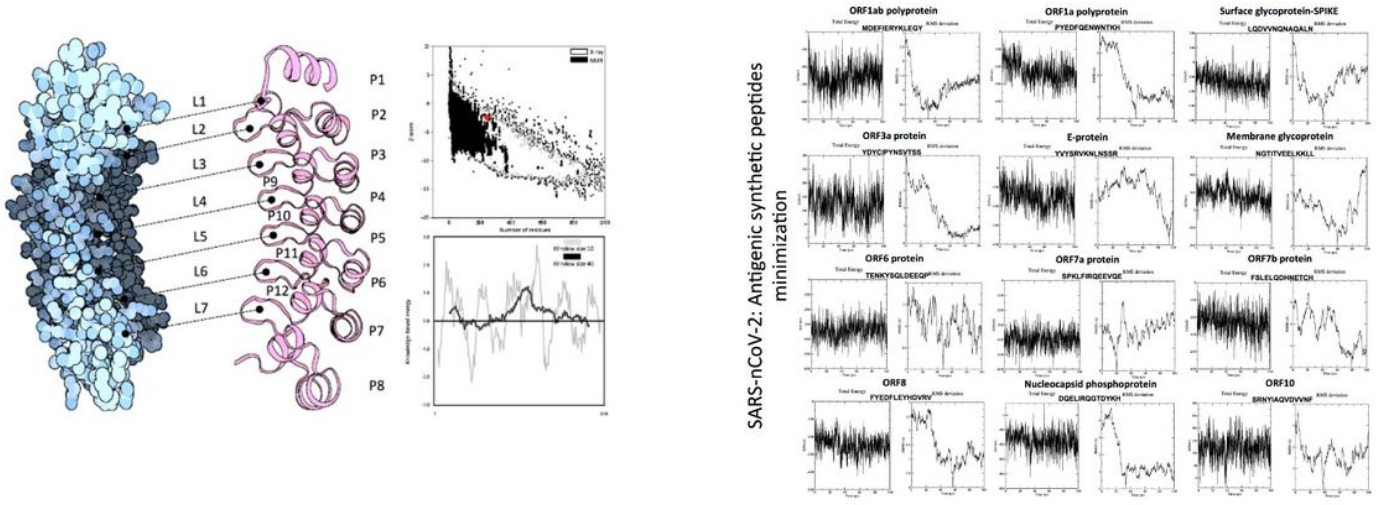

Figure 2

(Left) A. Constructed of vaccine candidates by linker and quality assessment A) Tertiary structural construct of 1-12 P small peptides in each connect to 1-7(L) linkers of vaccine model were determined by (ORION) peptide fold recognition. B) The structural quality estimation of vaccine model was DOPE Energy: -181.9 and Z-score of Energy: -0.961, and C) Overall vaccine model quality was Z-score: -1.99 determined by (ProSA) and arranged all residues shown knowledge-based energy plot. (Right) B. Energy minimizations of SARS-nCov-2 proteins construct peptides. Energy graph of SARS-nCoV-2 peptide based vaccine region range 1-12 P and simulation of RMSD graph.

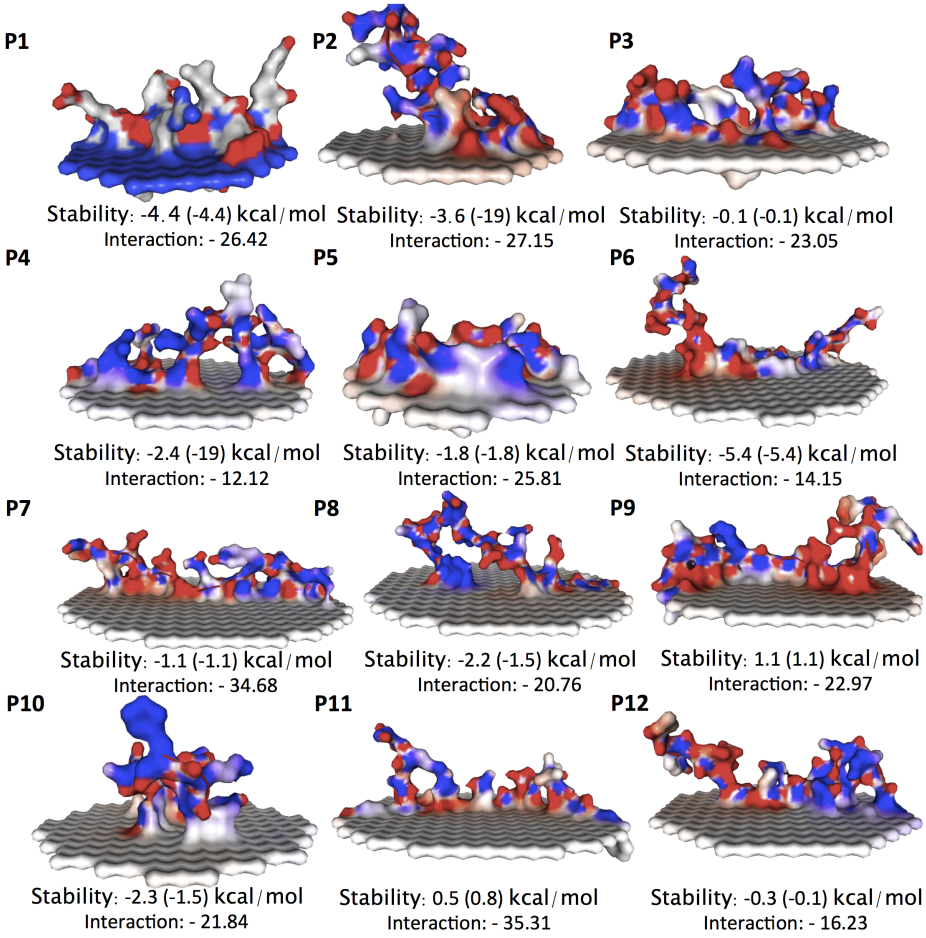

Figure 3

Short peptides of SARS-nCov-2 and penetrate cellular lipid bilayers. The fragmented of 1-12 P` peptides interact with lipid membrane is shown in electrostatic potential color in molecular surface representation, the lipid tails spears are shown in white, the stability and energy minimization were calculated in each peptide.

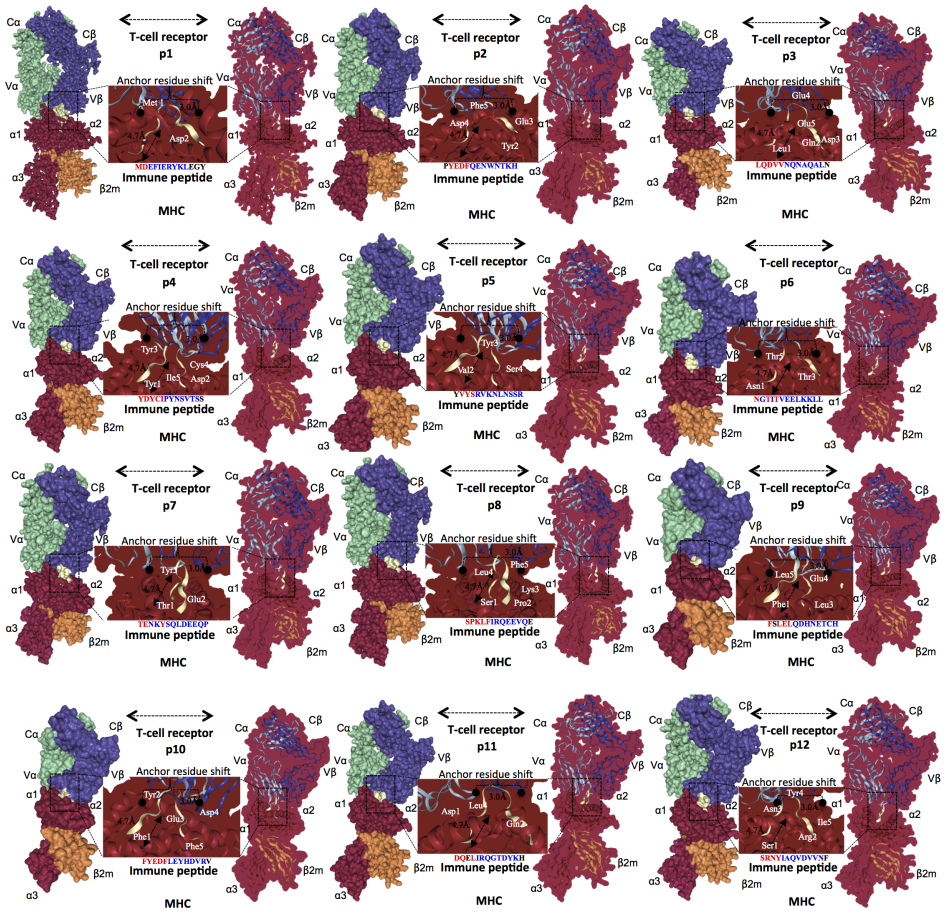

Figure 4

MHC class I-and II-T-cell Allele and epitope modeling and docking T-cell receptor consists of four binding domains in different colors (Cα; green Cβ; violet, and Vα; green, Vβ; violet). MHC class II and I consists of four binding domains in different colors (α1, α2, α3, and β2). Vaccine candidate of SARS-nCoV-2 antigenic peptides is consists of 1-12 P` binding complex between pMHC-TCR. MHC-TCR epitope complex minimized structure at the end of simulations t=0 and t=200ns antigenic peptide as shown in-between the middle of complex (yellow).

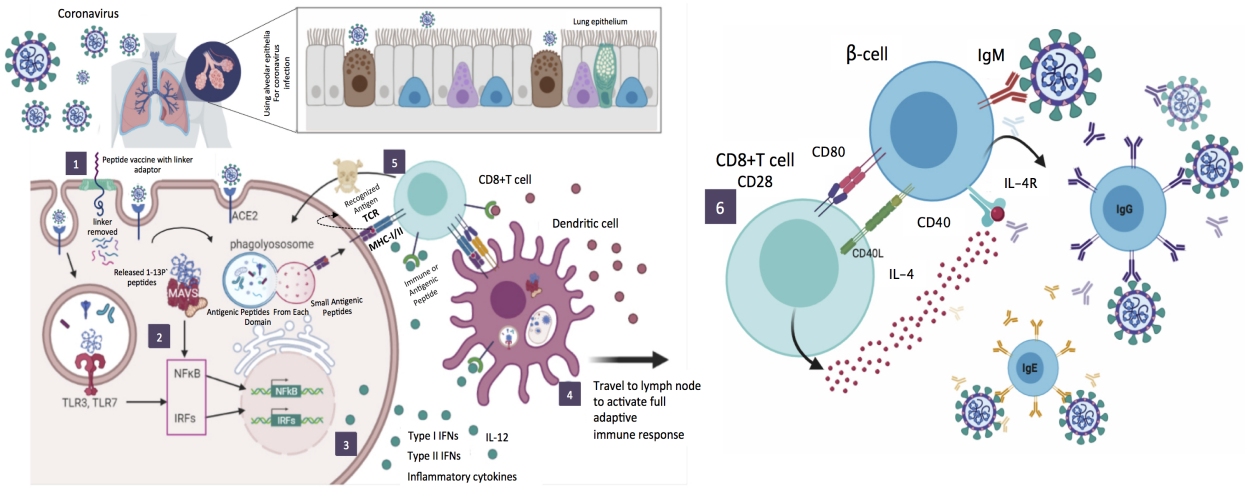

Figure 5

Systematic overview of antibody developed from specific epitopes of SARS-nCov-2. A severe acute respiratory syndrome (SARS-nCoV-2) entry into pulmonary and bronchial epithelial cells, in the lungs, connective tissue will replace the epithelial tissue on the damaged alveoli walls, thickening and hardening the lungs. Thicker walls mean it is more difficult for oxygen to diffuse out of the alveoli and into the bloodstream to reach other organs. These may cause to makes it breath harder and connective tissue can also replace damage on other organs. Since there is no way to remove this connective tissue, the damage is permanent. These CoVs enter host cells by first binding to their respective cellular receptors angiotensin-converting enzyme 2 (ACE-2) for SARS-nCoV-2. This figure is consists have followed by six steps. 1) The linker adaptor attached with each small antigenic peptide of SARS-Cov-2 ordered by each proteins (ORF 1a and ORF1b)-structural proteins [Spike-S, Envelope-E, Membrane-M, Nucleocapsid-N)-3'and some of addition accessory proteins, such as ORF, 3a, 6, 7a, 7b, 8, and 10, are inserted in genes of structural proteins. Full vaccine candidate of whole peptide with linker adaptors to inclusion through cell penetrate inside the membrane, and the exit of the peptide from the bilayer. 2 and 3) Transduce signals to the downstream kinase complexes which activate IFN regulatory factor-3 (IRF-3), nuclear factor kB (NF-kB) and ATF-2/c-jun. These transcription factors coordinately regulate the expression of type I Interferon's (IFN- α and β). Type I IFNs induce the activation of STAT transcription factors that induce the expression of hundreds of IFN-stimulated genes that establish an antiviral state in surrounding cells, thereby limiting viral replication and spread. Further, T-helper-2 subsets depending on their main cytokine production (interferon-γ or interleukin [IL]-4 and 12). 4) Lymphocytes that were apply to both β-cells and T-cells. We shall see that each lymphocyte is committed to respond to a specific antigen and that its response during its first encounter with an antigen ensures that a more rapid and effective response occurs on subsequent encounters with the same antigen. 5) β-cells process of our peptide vaccine antigens into small peptides 1-13 amino acids that they display at their surface through major MHC class I-II molecules. MHC- peptide complexes thus become available for binding by the specific subset of CD4+ T-helper cells. 6). This peptide vaccine is based on SARS-nCoV-2 proteins antigens as well-targeted β-cells and the types of T cells that were support specific antibody either IgM, IgD, IgG, IgA, and IgE production against SARS-nCoV-2. In such cases, this peptide vaccine can also be developed to promote cytotoxic T-cell activity, or perhaps a combination of both antibody and cytotoxic T-cell immune responses.

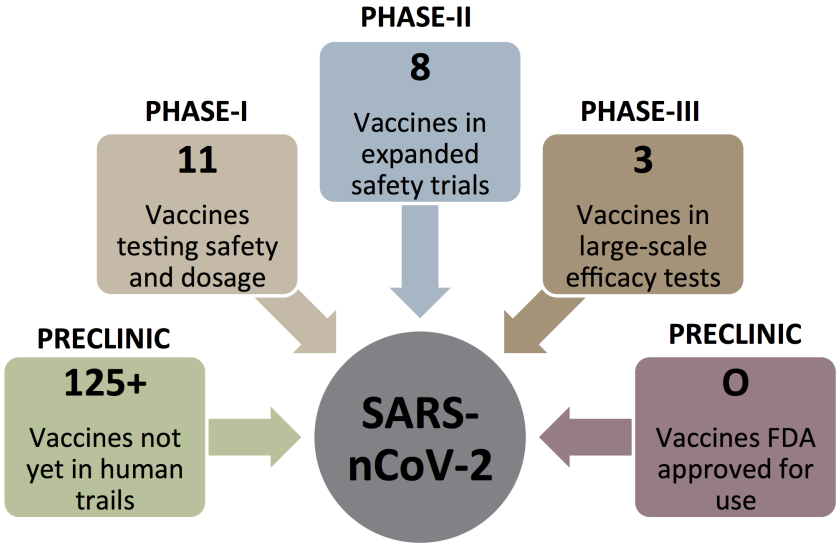

Figure 6

Coronavirus vaccine tracker tested in cells or animals.