Original Article

Design of multi epitope-based peptide vaccine against E protein of human 2019-nCoV: An immunoinformatics approach

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Abstract

Background: on the late December 2019, a new endemic has been spread across Wuhan City, China; within a few weeks, a novel coronavirus designated as 2019 novel coronavirus (2019-nCoV) was announced by the World Health Organization (WHO). In late January 2020, WHO declared the outbreak a "public-health emergency of international concern" due to the rapid spreading and increasing worldwide. There is no vaccine or approved treatment for this emerging infection; therefore, the objective of this paper is to design a multi epitope peptide vaccine against 2019-nCoV using immunoinformatics approach. **Method:** We will highlight a technique facilitating the combination of immunoinformatics approach with comparative genomic approach to determine our potential target for designing the T cell epitopes-based peptide vaccine using the envelope protein of 2019-nCoV as a target. **Results:** Extensive mutations, insertion and deletion were discovered with comparative sequencing in 2019-nCoV strain; in addition, 10 MHC1 and MHC2 related peptides were promising candidates for vaccine design with adequate world population coverage of 88.5% and 99.99% respectively. **Conclusion:** T cell epitopes-based peptide vaccine was designed for 2019-nCoV using envelope protein as an immunogenic target; nevertheless, the proposed T cell epitopes-based peptide vaccine rapidly needs to validates clinically to ensure its safety and immunogenic profile to assist on stopping this epidemic before it leads to devastating global outbreaks.

Keywords: novel coronavirus (2019-nCoV); Envelope protein; emerging infection; peptide vaccine; immunoinformatics approach.

1. Introduction:

In the last few decades, there were six strains of coronaviruses, but in December 2019, a new strain has been spread across Wuhan City, China.[1, 2] Within a few weeks, a novel coronavirus designated as 2019 novel coronavirus (2019-nCoV) was announced by the World Health Organization (WHO).[3] In late January 2020, WHO declared the outbreak a "public-health emergency of international concern" due to the rapid spreading and increasing worldwide with over 671 dead and 11,800 cases confirmed at 9:14 am 1 February 2020 (Khartoum time); the progressive of the virus is not yet determined, and that is why finding a Patient zero is very essential.

Phylogenetic analysis indicates a bat origin of 2019-nCoV,[4] it is airborne transmission (human-to-human) , the infected person characterized with fever, upper or lower respiratory tract symptoms, or diarrhoea, lymphopenia, thrombocytopenia, and increased C-reactive protein and lactate dehydrogenase levels or combination of these 3-6 days after exposure; further molecular diagnosis can be made by Real Time-PCR for genes encoding the internal RNA-dependent RNA polymerase and Spike's receptor binding domain, which can be confirmed by Sanger sequencing and full genome analysis by NGS, multiplex nucleic acid amplification and microarray-based assays.[5-9]

The sequence of 2019-nCoV RBD, together with its RBM that contacts receptor angiotensin-converting enzyme 2 (ACE2) , is similar to that of SARS coronavirus, strongly suggesting that 2019-nCoV uses ACE2 as its receptor; 2019-nCoV is more intelligence than other pervious strains by having several critical residues in 2019-nCoV receptor-binding motif (particularly Gln493) provide advantageous interactions with human ACE2.[4]

At present, there is no vaccine or approved treatment for humans, but Chinese traditional medicine, such ShuFengJieDu Capsules and Lianhuaqingwen Capsule, could be the treatment possibilities for 2019-nCoV. However, there are no clinical trials approve the safety and efficacy for these drugs.[10]

The main concept within all the immunizations is the ability of the vaccine to initiate an immune response in a faster mode than the pathogen itself. In traditional vaccines design that depends on biochemical trials can be costly, time consuming and they require the need to culture pathogenic viruses in vitro culture, beside, it causes allergenic or reactogenic responses; [11, 12] on the other hand, peptide -based vaccines don't need in vitro culture making them biologically safe, and their selectivity allows accurate activation of immune responses; [13, 14] therefore, In this study we aim to design a peptide vaccine using immuo-informatics analysis.[15-20] The corona envelope (E) protein is a small, integral membrane protein involved in several aspects of the virus' life cycle, such as: pathogenesis, envelope formation, assembly and budding; alongside with

its interactions with both other CoVs proteins (M, N & S) and host cell proteins (release of infectious particles after budding).[21-25] From this aspect, an epitope-based peptide vaccine has been raised.

The core mechanism of the peptide vaccines is built on the chemical method to synthesize the recognized B-cell and T-cell epitopes that are immunodominant and can induce specific immune responses. B-cell epitope of a target molecule can be linked with a T-cell epitope to make it immunogenic. The T-cell epitopes are short peptide fragments (8-20 amino acids), whereas the B-cell epitopes can be proteins.[26, 27] As far we know, this is the first study to design a multi epitope-based peptide vaccine against using an immunoinformatics approach. Rapid further studies are recommended to prove the efficiency of the predicted epitopes as a peptide vaccine against this emerging infection.

2. Materials and Methods:

2.1 Data retrieval:

The full genebank files of complete genomes and annotation of 2019-nCoV (NC_04551) SARS-CoV(FJ211859), MESA-CoV(NC_019843), HCoV-HKU1(AY884001), (HCoV-OC43 (KF923903), HCoV-NL63 (NC_005831) and HCoV-229E (KY983587) were retrieved from the National Center of Biotechnology Information (NCBI); while The FASTA format of envelope (E) protein (YP_009724392.1) , spike (S) protein (YP_009724390.1), nucleocapsid (N) protein (YP_009724397.2) and membrane (M) protein (YP_009724393.1) of 2019-nCoV and the envelope (E) protein of two Chinese and two American sequences (YP009724392.1, QHQ71975.1, QHO60596.1 and QHN73797.1) were obtained from the NCBI. It's available at: (https://www.ncbi.nlm.nih.gov/)

2.2 The Artemis Comparison Tool (ACT)

In silico analysis software for visualization of comparisons between complete genome sequences and associated annotations.[28] To identify regions of similarity, rearrangements, insertions and at any level from base-pair differences to the whole genome. It's available at (https://www.sanger.ac.uk/science/tools/artemis-comparison-tool-act).

2.3 VaxiJen server:

The first server for alignment-independent prediction of protective antigens. It allows antigen classification solely based on the physicochemical properties of proteins without recourse to sequence alignment. It predicts the probability of the antigenicity one or multiple of protein based on auto cross covariance (ACC) transformation of protein sequence. Structural CoV-2019 protein (N,S,E and M) was analyzed by VaxiJen with threshold of 0.4[29] It's available at (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html)

2.4 BioEdit:

It is a software package proposed to stream a distinct program that can run nearly any sequence operation as well as a few basic alignment investigations. The sequences of E protein were retrieved from UniProt run it by BioEdit to determine if the conserved sites through ClustalW in the application settings.[30]

2.5 The Molecular Evolutionary Genetics Analysis (MEGA):

MEGA (version 10.1.6) is software for comparative analysis of molecular sequences. It is used for pairwise and multiple sequences alignment alongside construction and analysis of phylogenetic trees and evolutionary relationships. The gap penalty was 15 for opening and 6.66 for extending the gap for both pairwise and multiple sequences alignment. Bootstrapping of 300 was used in construction of maximum like hood phylogenetic tree.[31, 32] It's available at: (https://www.megasoftware.net).

2.6 Prediction of T-cell epitopes:

IEDB tools were used to predict the conserved sequences (10-mer sequence) from HLA class I and class II T-cell epitopes by using artificial neural network (ANN) approach.[33-35] while HLA-II T-cell epitopes were recognized by using NN-align approach.[36] For the binding analysis, all the alleles were carefully chosen, and the length was set at 10 before prediction was done. Analysis of epitopes binding to MHC-I molecules was assessed by the IEDB MHC-I prediction server at (http://tools.iedb.org/mhci/). Artificial Neural Network (ANN) version 2.2 was chosen as Prediction method as it depends on the median inhibitory concentration (IC50)[33, 37-39] the selected candidates were evaluated by the IEDB MHC-II prediction tool at (http://tools.iedb.org/mhcii/). All Conserved Immunodominant peptides at score equal or less than 100 median inhibitory concentrations (IC50) were selected for additional analysis while epitopes with IC50 greater than 100 were eliminated.[40]

2.7 Population coverage analysis:

Population coverage for each epitope was carefully chosen by the IEDB population coverage calculation tool. The epitopes have diverse binding sites with different HLA alleles. Therefore, all the most promising epitope candidates were calculated for population coverage against the whole world, China and Europe population to get and ensure a universal vaccine.[41, 42] It's available at (http://tools.iedb.org/population/)

2.8 Tertiary structure (3D) Modeling:

3D Modeling By using the reference sequence of E protein that has been obtained from gene bank and use it as an input in RaptorX to predict the 3D structure of E protein;[43, 44] then the visualization of the obtained 3D protein structure was done by UCSF Chimera (version1.8).[45]

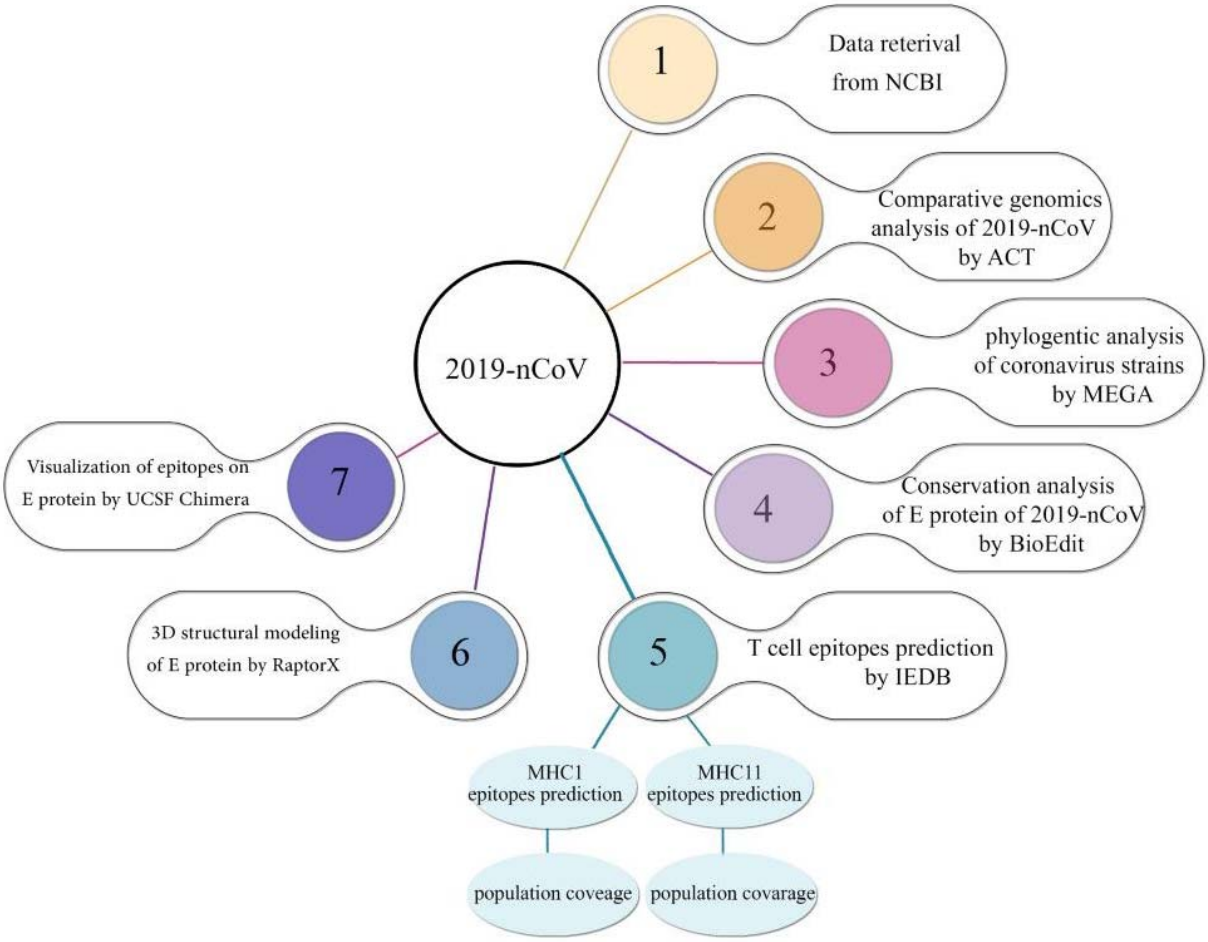

Figure (1): *work Flow summarizing the procedures for the epitope-based peptide vaccine prediction.*

3. Results:

The reference sequence of envelope protein was aligned with HCov-HKU1 reference protein using artemis comparison tool as illustrated in **(Figure 1)**; then the mutated protein were tested for antigenicity where the envelope protein founded as the best immunogenic target by Vaxigen software. **(Table 1)**

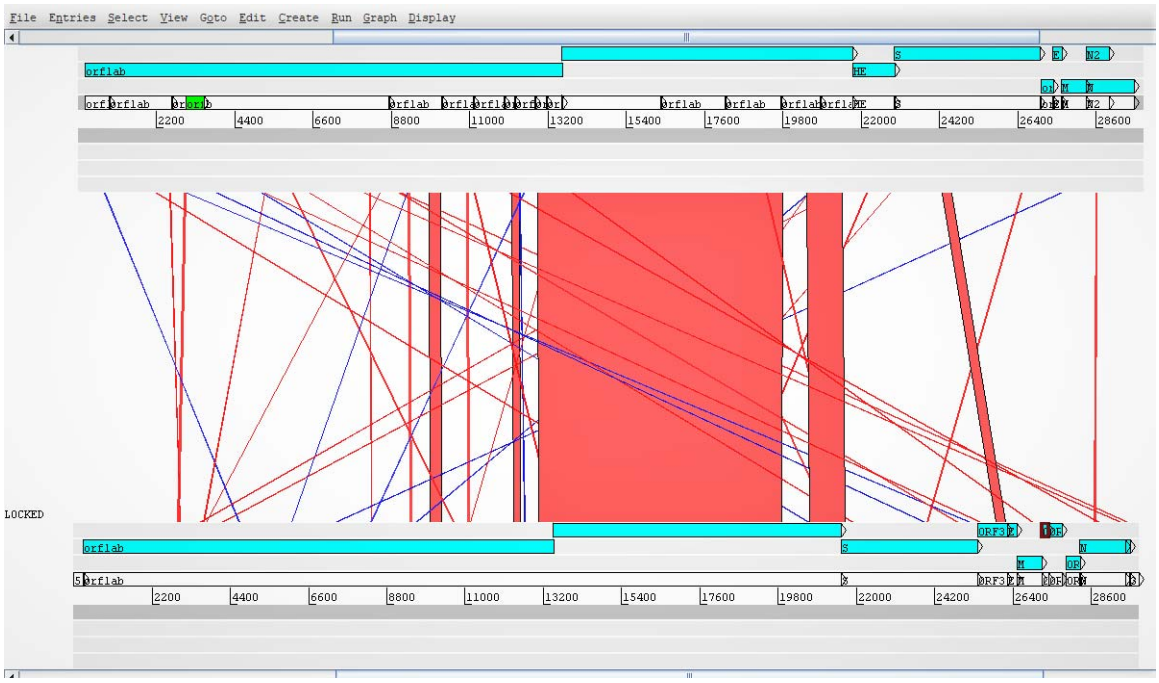

Figure (2): *Artemis analysis of envelope protein displaying 3 windows, the upper window represents HCov- HKU1 reference sequence and its genes are highlighted in blue starting from orflab gene and ending with N gene. The middle window describes the similarities and the difference between the two genomes. Red lines indicate match between genes from the two genomes blue lines indicates inversion which represents same sequences in the two genomes but they are organized in the opposite direction, and the lower windows represents 2019-nCoV and its genes started from orflab and ends with N genes .*

Table (1): VaxiJen overall prediction of probable 2019-nCoV antigen:

Protein	Result	VaxiJen prediction
Protein E	0.6025	Probable antigen
Protein M	0.5102	Probable antigen
Protein S	0.4646	Probable antigen
Protein N	0.5059	Probable antigen

Sequence alignment of envelope protein of 2019-nCoV was done using BioEdit software which shows total conservation across 4 sequences , two sequences retrieved from china and two sequence from USA. (figure 2) the IEDB website was used to analyzed T cell related peptide of envelope protein proposed vaccine, which shows 10 MHC1 and MHC2 associated peptides with the highest population coverage **(Table 2&3; Figures 5&6)** while the most promised peptide was visualized using UCSF Chimera software. **(Figure 3&4)**.

Figure (3): *sequence alignment of 4 strains of envelope protein of 2019-nCoV (2 form China and 2 from USA) showing total conservation through the 4 strains. The alignment was done by BioEdit software.*

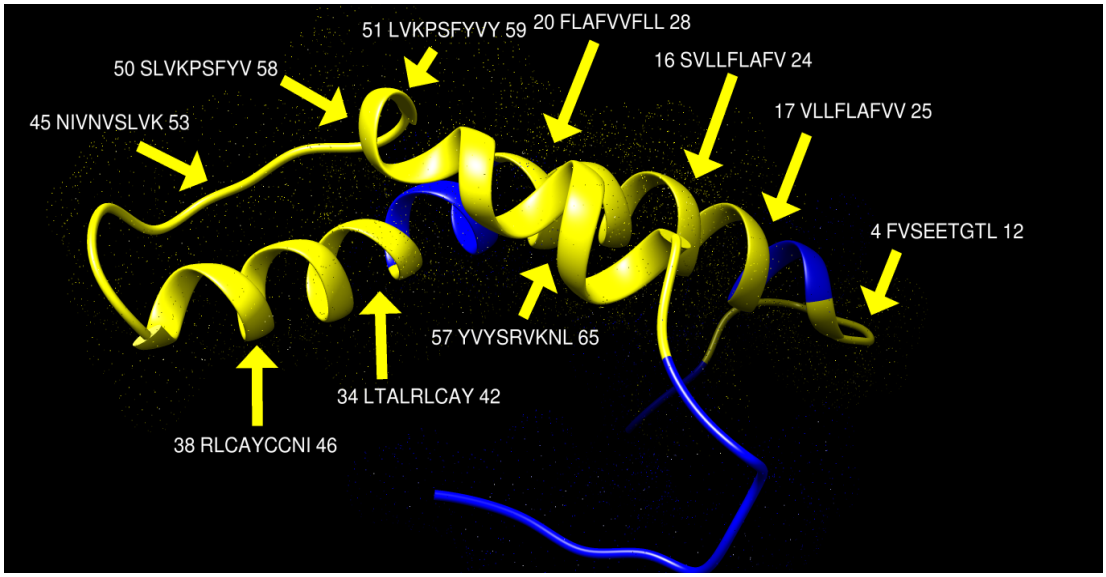

Figure (4): *visualization of most promising peptide in MHC1 in envelope protein of 2019-nCoV illustrated by chimera software. The yellow color refers to the promising peptides, while the blue color refers to the rest of protein structure.*

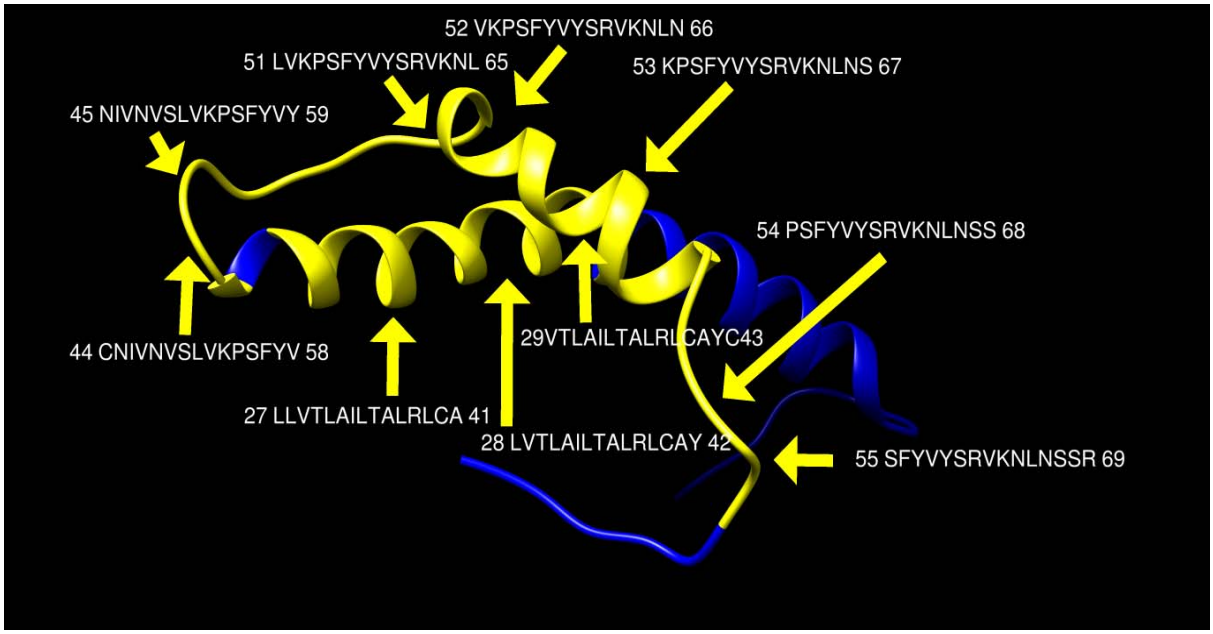

Figure (5): *Visualization of MHC2 related peptides in envelope protein of 2019-nCoV as visualized by chimera software, the yellow color refers to the promising peptides, while the blue color refers to the rest of protein structure.*

Population: World

MHC class	Coverage	Average hit	PC90
I	88.5%	3.67	0.87

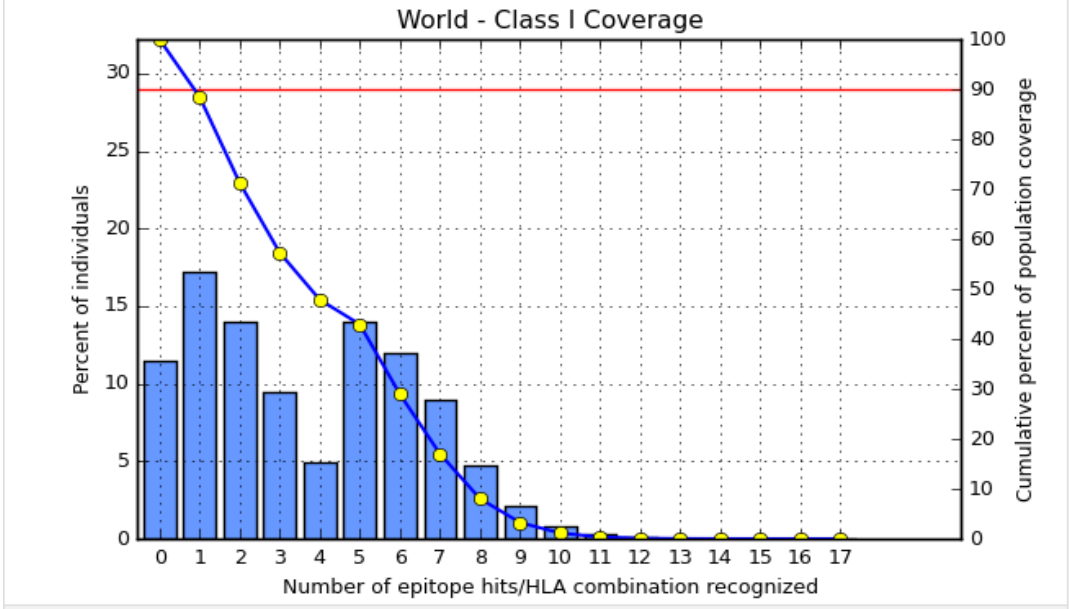

Figure (6): *Showing world coverage of 88.5%.for MHC1 predicted world Population coverage of the vaccine based on envelope protein of 2019-nCoV based on MHC1 data, as predicted in The Immune Epitope Database (IEDB) website.*

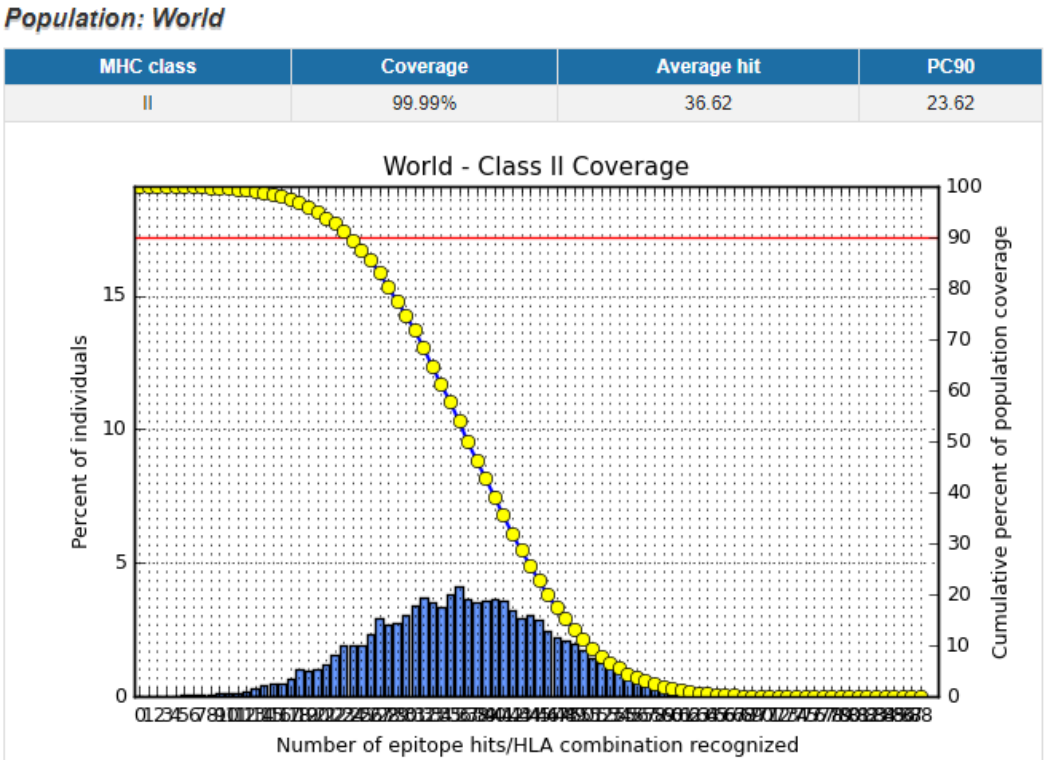

Figure (7) : *Showing world coverage of 99.99% for MHC2 predicted world Population coverage of the vaccine based on envelope protein of 2019-nCoV based on MHC2 data, as predicted in The Immune Epitope Database (IEDB) website.*

Table (2): the most promised 10 MHC1 related peptide in envelope protein based vaccine of 2019-nCoV along with the predicted world, China, Europe and East Asia:

Peptide	Alleles	coverage	COMBINED coverage of 10 peptide
YVYSRVKNL	HLA-C*14:02,HLA-C*12:03,HLA-C*07:01,HLA-C*03:03,HLA-C*06:02	50.02%	world: 88.5%
SLVKPSFYV	HLA-A*02:06,HLA-A*02:01,HLA-A*68:02	42.53%	china: 78.17%
SVLLFLAFV	HLA-A*02:06,HLA-A*68:02,HLA-A*02:01	42.53%	Europe: 92.94%
FLAFVVFLL	HLA-A*02:01,HLA-A*02:06	40.60%	East Asia: 80.78%
VLLFLAFVV	HLA-A*02:01	39.08%	
RLCAYCCNI	HLA-A*02:01	39.08%	
FVSEETGTL	HLA-C*03:03,HLA-C*12:03,HLA-A*02:06,HLA-A*68:02,HLA-B*35:01	28.22%	
LTALRLCAY	HLA-A*01:01,HLA-A*30:02,HLA-B*15:01	26.34%	
LVKPSFYVY	HLA-B*15:01,HLA-A*29:02,HLA-A*30:02,HLA-B*35:01	21.72%	
NIVNVSLVK	HLA-A*68:01,HLA-A*11:01	20.88%	

Table (3): the most promised 10 MHC2 related peptide in envelope protein based vaccine of 2019-nCoV along with the predicted world, China, Europe and East Asia:

Peptide Sequence	Alleles	world coverage	Coverage/10 peptide
KPSFYVYSRVKNLNS	HLA-DPA1*01:03,HLA-DPB1*02:01,HLA-DPB1*03:01,HLA-DPB1*04:01,HLA-DPA1*02:01,HLA-DPB1*05:01,HLA-DPA1*03:01,HLA-DPB1*04:02,HLA-DPB1*06:01,HLA-DPB1*14:01,HLA-DPB1*01:01,HLA-DQA1*05:01,HLA-DQB1*04:02,HLA-DQA1*06:01,HLA-DQA1*01:02,HLA-DQB1*05:01,HLA-DQA1*02:01,HLA-DRB1*01:01,HLA-DRB1*07:01,HLA-DRB1*08:01,HLA-DRB1*09:01,HLA-DRB1*11:01,HLA-DRB4*01:03,HLA-DRB1*04:01,HLA-DRB1*10:01,HLA-DRB1*04:05,HLA-DRB1*13:01,HLA-DRB1*08:02,HLA-DRB1*16:02,HLA-DRB1*15:01,HLA-DRB3*03:01,HLA-DRB5*01:01,HLA-DRB3*02:02,HLA-DRB1*04:04,HLA-DRB1*13:02	99.93%	world : 99.99%
VKPSFYVYSRVKNLN	HLA-DPA1*01:03,HLA-DPB1*02:01,HLA-DPB1*04:01,HLA-DPB1*03:01,HLA-DPA1*02:01,HLA-DPB1*05:01,HLA-DPA1*03:01,HLA-DPB1*04:02,HLA-DPB1*06:01,HLA-DPB1*01:01,HLA-DQA1*05:01,HLA-DQB1*04:02,HLA-DQA1*06:01,HLA-DQA1*01:02,HLA-DQB1*05:01,HLA-DQA1*02:01,HLA-DRB1*07:01,HLA-DRB1*08:01,HLA-DRB1*01:01,HLA-DRB1*09:01,HLA-DRB1*11:01,HLA-DRB4*01:03,HLA-DRB1*15:01,HLA-DRB1*13:01,HLA-DRB3*03:01,HLA-DRB1*10:01,HLA-DRB1*16:02,HLA-DRB1*08:02,HLA-DRB1*04:05,HLA-DRB5*01:01,HLA-DRB1*13:02,HLA-DRB3*02:02,HLA-DRB1*04:01,HLA-DRB1*04:04	99.92%	China: 99.96%
LVKPSFYVYSRVKNL	HLA-DPA1*01:03,HLA-DPB1*02:01,HLA-DPB1*04:01,HLA-DPA1*02:01,HLA-DPB1*05:01,HLA-DPB1*06:01,HLA-DPA1*03:01,HLA-DPB1*04:02,HLA-DPA1*02:01,HLA-DPB1*01:01,HLA-DQA1*06:01,HLA-DQB1*04:02,HLA-	99.90%	Europe : 100.0%

	DQA1*05:01,HLA-DQA1*02:01,HLA-DQA1*01:04,HLA-DQB1*05:03,HLA-DQA1*01:02,HLA-DQB1*05:01,HLA-DRB1*07:01,HLA-DRB1*08:01,HLA-DRB1*09:01,HLA-DRB1*11:01,HLA-DRB4*01:03,HLA-DRB3*03:01,HLA-DRB1*01:01,HLA-DRB1*15:01,HLA-DRB1*16:02,HLA-DRB1*13:01,HLA-DRB1*10:01,HLA-DRB1*08:02,HLA-DRB5*01:01,HLA-DRB1*13:02,HLA-DRB1*04:05,HLA-DRB3*02:02,HLA-DRB1*04:01		
PSFYVYSRVKNLNSS	HLA-DPA1*01:03,HLA-DPB1*02:01,HLA-DPB1*03:01,HLA-DPB1*04:01,HLA-DPA1*03:01,HLA-DPB1*04:02,HLA-DPA1*02:01,HLA-DPB1*05:01,HLA-DPB1*06:01,HLA-DQA1*05:01,HLA-DQB1*04:02,HLA-DQA1*01:02,HLA-DQB1*05:01,HLA-DQA1*06:01,HLA-DRB1*01:01,HLA-DRB1*08:01,HLA-DRB1*04:01,HLA-DRB1*11:01,HLA-DRB1*09:01,HLA-DRB1*07:01,HLA-DRB4*01:03,HLA-DRB1*04:05,HLA-DRB1*10:01,HLA-DRB1*13:01,HLA-DRB1*08:02,HLA-DRB1*16:02,HLA-DRB1*15:01,HLA-DRB3*03:01,HLA-DRB3*02:02,HLA-DRB1*04:04,HLA-DRB5*01:01,HLA-DRB1*13:02	99.86%	East Asia: 99.91%
NIVNVSLVKPSFYVY	HLA-DPA1*01:03,HLA-DPB1*02:01,HLA-DPB1*04:01,HLA-DPB1*06:01,HLA-DPA1*02:01,HLA-DPB1*01:01,HLA-DQA1*01:02,HLA-DQB1*05:01,HLA-DQA1*05:01,HLA-DQB1*04:02,HLA-DQA1*02:01,HLA-DQB1*03:01,HLA-DQB1*03:03,HLA-DQB1*03:03,HLA-DQB1*04:02,HLA-DRB1*12:01,HLA-DRB1*01:01,HLA-DRB5*01:01,HLA-DRB3*03:01,HLA-DRB1*13:01,HLA-DRB1*07:01,HLA-DRB1*15:01,HLA-DRB4*01:03,HLA-DRB1*04:04,HLA-DRB1*08:02,HLA-DRB1*09:01,HLA-DRB1*13:02,HLA-DRB1*11:01,HLA-DRB1*04:05,HLA-DRB1*10:01	99.77%	
LLVTLAILTALRLCA	HLA-DPA1*01:03,HLA-DPB1*02:01,HLA-DPB1*06:01,HLA-DPA1*03:01,HLA-DPB1*04:02,HLA-DQA1*01:02,HLA-DQB1*05:01,HLA-DQA1*02:01,HLA-DQB1*03:01,HLA-DQB1*03:03,HLA-DQA1*05:01,HLA-DQB1*04:02,HLA-	99.72%	

	DQA1*06:01,HLA-DQA1*01:03,HLA-DQB1*06:03,HLA-DRB4*01:03,HLA-DRB1*01:01,HLA-DRB1*13:01,HLA-DRB1*04:04,HLA-DRB5*01:01,HLA-DRB3*03:01,HLA-DRB1*10:01,HLA-DRB1*15:01,HLA-DRB1*07:01,HLA-DRB1*11:01,HLA-DRB1*08:01,HLA-DRB1*12:01,HLA-DRB1*03:01,HLA-DRB4*01:01,HLA-DRB1*16:02,HLA-DRB1*08:02	
SFYVYSRVKNLNSSR	HLA-DPA1*01:03,HLA-DPB1*03:01,HLA-DPB1*02:01,HLA-DPA1*03:01,HLA-DPB1*04:02,HLA-DPA1*02:01,HLA-DPB1*05:01,HLA-DPB1*06:01,HLA-DQA1*05:01,HLA-DQB1*04:02,HLA-DQA1*01:02,HLA-DQB1*05:01,HLA-DRB1*04:01,HLA-DRB1*01:01,HLA-DRB1*11:01,HLA-DRB1*08:01,HLA-DRB1*07:01,HLA-DRB1*09:01,HLA-DRB4*01:03,HLA-DRB1*10:01,HLA-DRB1*13:01,HLA-DRB1*04:05,HLA-DRB1*08:02,HLA-DRB1*16:02,HLA-DRB3*02:02,HLA-DRB3*03:01,HLA-DRB1*04:04,HLA-DRB1*15:01,HLA-DRB5*01:01,HLA-DRB1*13:02	99.72%
LVTLAILTALRLCAY	HLA-DPA1*01:03,HLA-DPB1*02:01,HLA-DPB1*06:01,HLA-DPA1*03:01,HLA-DPB1*04:02,HLA-DQA1*01:02,HLA-DQB1*05:01,HLA-DQA1*02:01,HLA-DQB1*03:01,HLA-DQB1*03:03,HLA-DQA1*05:01,HLA-DQB1*04:02,HLA-DQB1*06:02,HLA-DQA1*02:01,HLA-DQA1*06:01,HLA-DRB4*01:03,HLA-DRB1*01:01,HLA-DRB1*13:01,HLA-DRB1*04:04,HLA-DRB1*12:01,HLA-DRB1*10:01,HLA-DRB5*01:01,HLA-DRB1*15:01,HLA-DRB1*11:01,HLA-DRB3*03:01,HLA-DRB1*03:01,HLA-DRB1*08:01,HLA-DRB1*07:01,HLA-DRB4*01:01,HLA-DRB1*16:02,HLA-DRB1*04:02,HLA-DRB1*08:02	99.69%
VTLAILTALRLCAYC	HLA-DPA1*01:03,HLA-DPB1*06:01,HLA-DPA1*03:01,HLA-DPB1*04:02,HLA-DQA1*02:01,HLA-DQB1*03:01,HLA-DQA1*01:02,HLA-DQB1*05:01,HLA-DQB1*06:02,HLA-DQA1*05:01,HLA-DQB1*04:02,HLA-DQB1*03:03,HLA-DQA1*06:01,HLA-DQB1*04:02,HLA-	99.56%

	DRB1*01:01,HLA-DRB4*01:03,HLA-DRB1*13:01,HLA-DRB1*04:04,HLA-DRB1*12:01,HLA-DRB1*10:01,HLA-DRB5*01:01,HLA-DRB1*15:01,HLA-DRB1*11:01,HLA-DRB1*03:01,HLA-DRB3*03:01,HLA-DRB1*08:01,HLA-DRB1*07:01,HLA-DRB4*01:01,HLA-DRB1*04:02	
CNIVNVSLVKPSFYV	HLA-DPA1*01:03,HLA-DPB1*06:01,HLA-DPB1*04:02,HLA-DQA1*01:02,HLA-DQB1*05:01,HLA-DQA1*05:01,HLA-DQB1*04:02,HLA-DQA1*02:01,HLA-DQB1*03:01,HLA-DQB1*03:03,HLA-DQA1*01:03,HLA-DQB1*06:03,HLA-DRB3*03:01,HLA-DRB1*12:01,HLA-DRB5*01:01,HLA-DRB1*01:01,HLA-DRB1*07:01,HLA-DRB4*01:03,HLA-DRB1*13:01,HLA-DRB1*15:01,HLA-DRB1*08:02,HLA-DRB1*04:04,HLA-DRB1*09:01,HLA-DRB1*13:02,HLA-DRB1*11:01,HLA-DRB1*04:05,HLA-DRB4*01:01,HLA-DRB1*10:01	99.53%

To study the evolutionary relationship between all the 7 strains of coronavirus a multiple sequence alignment (MSA) was performed using ClustalW by MEGA software, this alignment was used to construct maximum likelihood phylogenetic tree as seen in **figure (3)**.

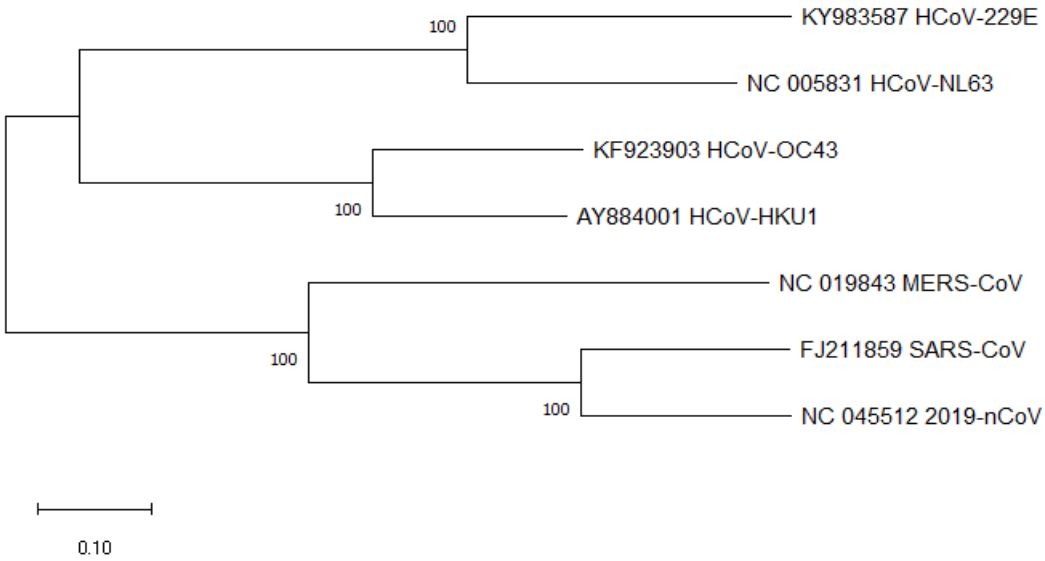

Figure (8): *Maximum like hood phylogenetic tree which describes the evolutionary relationship between the seven strains of coronavirus.*

4. Discussion:

Designing of a novel vaccine is very crucial to defending the rapid endless of global burden of disease.[46-49] in the last few decades, biotechnology has been advances rapidly, alongside with the understanding of immunology have assisted the rise of new approaches towards rational vaccines design.[50] With the advancement various bioinformatics tools and databases, we can design peptide vaccine[51, 52]; peptides can act as ligands in the development of vaccine.[53] This approach had been used frequently in Saint Louis encephalitis virus,[54] dengue virus,[55] chikungunya virus,[56] etc. has already been proposed.

The 2019-nCoV is an RNA virus,[9] which tends to mutate more commonly than the DNA viruses.[57] These mutations lied on the surface of the protein, which make 2019-nCoV more superior than other previous strains by inducing its sustainability which leaves the immune system in blind spot.[58]

In our findings, IEDB didn't give us any result for B cell epitopes, this may be due to the length of the 2019-nCoV (75 amino acids), but that will not affect our aim to design T cell epitopes-based peptide vaccine; recently, T cell peptide vaccine has been stimulated as the host can create a strong immune response by CD8+ T cell against the infected cell.[59-63] With time, due to antigenic drift, any invader can escape the antibody memory response; while the T cell immune response enduring much longer.[64]

We choose the following 10 peptide in MHC1 with the highest world population coverage as a good candidate for our vaccine: (YVYSRVKNL, SLVKPSFYV, SVLLFLAFV, FLAFVVFLL, VLLFLAFVV, RLCAYCCNI, FVSEETGTL, LTALRLCAY, LVKPSFYVY and NIVNVSLVK). These peptides provided world population coverage of world: 88.5%. Furthermore we selected another 10 peptides in MHC2 as candidates for vaccine designed based on the world population percentage: (KPSFYVYSRVKNLNS, VKPSFYVYSRVKNLN, LVKPSFYVYSRVKNL, PSFYVYSRVKNLNSS, NIVNVSLVKPSFYVY, LLVTLAILTALRLCA, SFYVYSRVKNLNSSR, LVTLAILTALRLCAY, VTLAILTALRLCAYC, and CNIVNVSLVKPSFYV) which show better world coverage: 99.99% of MHC2 related alleles. we furthermore test the population coverage in China, Europe, and East Asia where MHC2 related peptide again showed more promising results (99.96%, 100.0%, and 99.91% respectively.) compared to lower results of MHC1 related peptides (78.17%, 92.94% and 80.78% respectively). **(Table 2&3) (Figure 6&7**) The candidate peptides for both MHC1 and MHC2 were visualized using UCSF Chimera which shows the predicted 3D structures of these epitopes. **(Figure 4&5)**

For the analysis of the whole genome of coronavirus 2109 (2019-nCoV) we use comparative genomic approach[65] to determine our potential target for designing the

vaccine. The analysis was performed using Artemis Comparative Tool (ACT).[66] Many interesting findings were revealed during the analysis. We use human Coronavirus (HCov- HKU1) reference sequence Vs Wuhan-Hu-1 2019-nCoV. From Artemis windows in **(figure 2)** extensive mutation were observed among the conserved genes and new genes were inserted in 2019-nCoV which were absent in HCov- HKU1as ORF 8 and ORF6 which might be acquired by horizontal gene transmission.[67] High rate of mutation between the two genomes were observed in region from 20000 bp to the end of the sequence. This region encodes the 4 major structural proteins in coronavirus which are envelope (E) protein, nucleocapsid (N) protein, membrane (M) protein, and spike (S) protein, all of which are required to produce a structurally complete virus.[68, 69] In previous studies these conserved antigenic sites were revealed through sequence alignment between MERS-CoV and Bat-coronavirus[70] and analyzed in SARS-CoV.[71] We aimed to find conserved coding genes but yet highly mutated to select them as targets for the vaccine, since conserved genes are essential for the survival of the virus, and extensive mutations in conserved genes are tricky mechanisms by which a microorganism can adapt new environment and resist antibiotics. Such extensive mutation can lead to lethal diseases and epidemics that appear with specific strain of microorganism but not the other. Thus such unique genes can distinguish certain strains and can be used as a potential drug and vaccine targets.

To filter the four candidates we use Vaxigen software to test the probability of antigenic proteins, so the translated protein were tested by the software which revealed protein E as the most antigenic gene with the highest probability as showed in **(Table 1)**. This result was confirmed from the literature in which protein E was investigated in severe acute respiratory syndrome (SARS) in 2003 and, more recently, Middle-East respiratory syndrome (MERS)[68] the conservation of this protein against the seven strains was tested and confirmed through the use of BioEdit package tool. **(Figure 3)**.

As phylogenetic analysis is very powerful tool for determining the evolutionary relationship between strains. Multiple sequence alignment (MSA) was performed using ClustalW for the 7 strains of coronavirus ,which are 2019-nCoV(NC_04551) , SARS-CoV(FJ211859) ,MESA-CoV(NC_019843), HCoV-HKU1(AY884001)(,HCoV-OC43(KF923903),HCoV-NL63(NC_005831) and HCoV-229E (KY983587) . The maximum like hood phylogenetic tree in **(figure 8)** revealed that 2019-nCov is found in the same clade of SARS-CoV thus the two strains are highly related to each other.

Certain alleles in MHC2 yield no result when analyzed in IEDB ;(HLA-DRB3*03:01, HLA-DRB3*02:02, HLA-DRB4*01:03, HLA-DRB3*01:01, HLA-DRB4*01:01, HLA-DRB5*01:01) this could be perhaps to technical problems like un availability of the alleles in the website database or other factors, these peptides could be further studied in the future research projects.

Both flu and anti-HIV drugs are used currently on china for treatment of 2019-nCoV but more studies are required to standardize this therapy. Also there has been some success in the development of mouse models of MERS-CoV and SARS-CoV infection, and candidate vaccines where the envelope (E) protein is mutated or deleted have been described.[72-78] yet we strongly believe this will be the first paper to identify certain peptides in envelope (E) protein as candidates for 2019-nCoV.

5. Conclusion:

Extensive mutations, insertion and deletion were discovered with comparative sequencing in 2019-nCoV strain; in addition, 10 MHC1 and MHC2 related peptides were promising candidates for vaccine design with adequate world population coverage of 88.5% and 99.99% respectively. T cell epitope-based peptide vaccine was designed for 2019-nCoV using envelope protein as an immunogenic target; nevertheless, the proposed T cell epitopes-based peptide vaccine rapidly needs to be validates clinically to ensure its safety and immunogenic profile to assist on stopping this epidemic before it leads to devastating global outbreaks.

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Data Availability:

All data underlying the results are available as part of the article and no additional source data are required.

References:

[1] H. Lu, C. W. Stratton, and Y. W. Tang, "Outbreak of Pneumonia of Unknown Etiology in Wuhan China: the Mystery and the Miracle," *J Med Virol,* Jan 16 2020.

[2] D. S. Hui, I. A. E, T. A. Madani, F. Ntoumi, R. Kock, O. Dar*, et al.*, "The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health - The latest 2019 novel coronavirus outbreak in Wuhan, China," *Int J Infect Dis,* vol. 91, pp. 264-266, Jan 14 2020.

[3] D. Benvenuto, M. Giovannetti, A. Ciccozzi, S. Spoto, S. Angeletti, and M. Ciccozzi, "The 2019-new coronavirus epidemic: evidence for virus evolution," *J Med Virol,* Jan 29 2020.

[4] Y. Wan, J. Shang, R. Graham, R. S. Baric, and F. Li, "Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS," *J Virol,* Jan 29 2020.

[5] J. F. Chan, S. Yuan, K. H. Kok, K. K. To, H. Chu, J. Yang, *et al.*, "A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster," *Lancet,* Jan 24 2020.

[6] N. Zhang, L. Wang, X. Deng, R. Liang, M. Su, C. He, *et al.*, "Recent advances in the detection of respiratory virus infection in humans," *J Med Virol,* Jan 15 2020.

[7] J. B. Mahony, A. Petrich, and M. Smieja, "Molecular diagnosis of respiratory virus infections," *Crit Rev Clin Lab Sci,* vol. 48, pp. 217-49, Sep-Dec 2011.

[8] V. M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D. K. Chu, *et al.*, "Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR," *Euro Surveill,* vol. 25, Jan 2020.

[9] Y. Chen, Q. Liu, and D. Guo, "Emerging coronaviruses: genome structure, replication, and pathogenesis," *J Med Virol,* Jan 22 2020.

[10] H. Lu, "Drug treatment options for the 2019-new coronavirus (2019-nCoV)," *Biosci Trends,* Jan 28 2020.

[11] Y. T. Lo, T. W. Pai, W. K. Wu, and H. T. Chang, "Prediction of conformational epitopes with the use of a knowledge-based energy function and geometrically related neighboring residue characteristics," *BMC Bioinformatics,* vol. 14 Suppl 4, p. S3, 2013.

[12] W. Li, M. D. Joshi, S. Singhania, K. H. Ramsey, and A. K. Murthy, "Peptide Vaccine: Progress and Challenges," *Vaccines (Basel),* vol. 2, pp. 515-36, Jul 2 2014.

[13] A. W. Purcell, J. McCluskey, and J. Rossjohn, "More than one reason to rethink the use of peptides in vaccine design," *Nat Rev Drug Discov,* vol. 6, pp. 404-14, May 2007.

[14] N. L. Dudek, P. Perlmutter, M. I. Aguilar, N. P. Croft, and A. W. Purcell, "Epitope discovery and their use in peptide based vaccines," *Curr Pharm Des,* vol. 16, pp. 3149-57, 2010.

[15] V. Brusic and N. Petrovsky, "Immunoinformatics and its relevance to understanding human immune disease," *Expert Rev Clin Immunol,* vol. 1, pp. 145-57, May 2005.

[16] N. Tomar and R. K. De, "Immunoinformatics: a brief review," *Methods Mol Biol,* vol. 1184, pp. 23-55, 2014.

[17] S. Khalili, A. Jahangiri, H. Borna, K. Ahmadi Zanoos, and J. Amani, "Computational vaccinology and epitope vaccine design by immunoinformatics," *Acta Microbiol Immunol Hung,* vol. 61, pp. 285-307, Sep 2014.

[18] N. Tomar and R. K. De, "Immunoinformatics: an integrated scenario," *Immunology,* vol. 131, pp. 153-68, Oct 2010.

[19] N. R. Hegde, S. Gauthami, H. M. Sampath Kumar, and J. Bayry, "The use of databases, data mining and immunoinformatics in vaccinology: where are we?," *Expert Opin Drug Discov,* vol. 13, pp. 117-130, Feb 2018.

[20] A. A. Bahrami, Z. Payandeh, S. Khalili, A. Zakeri, and M. Bandehpour, "Immunoinformatics: In Silico Approaches and Computational Design of a Multi-epitope, Immunogenic Protein," *Int Rev Immunol,* vol. 38, pp. 307-322, 2019.

[21] P. Venkatagopalan, S. M. Daskalova, L. A. Lopez, K. A. Dolezal, and B. G. Hogue, "Coronavirus envelope (E) protein remains at the site of assembly," *Virology,* vol. 478, pp. 75-85, Apr 2015.

[22] J. L. Nieto-Torres, M. L. Dediego, E. Alvarez, J. M. Jimenez-Guardeno, J. A. Regla-Nava, M. Llorente, *et al.*, "Subcellular location and topology of severe acute respiratory syndrome coronavirus envelope protein," *Virology,* vol. 415, pp. 69-82, Jul 5 2011.

[23] K. M. Curtis, B. Yount, and R. S. Baric, "Heterologous gene expression from transmissible gastroenteritis virus replicon particles," *J Virol,* vol. 76, pp. 1422-34, Feb 2002.

[24] J. Ortego, D. Escors, H. Laude, and L. Enjuanes, "Generation of a replication-competent, propagation-deficient virus vector based on the transmissible gastroenteritis coronavirus genome," *J Virol,* vol. 76, pp. 11518-29, Nov 2002.

[25] T. R. Ruch and C. E. Machamer, "A single polar residue and distinct membrane topologies impact the function of the infectious bronchitis coronavirus E protein," *PLoS Pathog,* vol. 8, p. e1002674, 2012.

[26] S. Dermime, D. E. Gilham, D. M. Shaw, E. J. Davidson, K. Meziane el, A. Armstrong, *et al.*, "Vaccine and antibody-directed T cell tumour immunotherapy," *Biochim Biophys Acta,* vol. 1704, pp. 11-35, Jul 6 2004.

[27] R. H. Meloen, J. P. Langeveld, W. M. Schaaper, and J. W. Slootstra, "Synthetic peptide vaccines: unexpected fulfillment of discarded hope?," *Biologicals,* vol. 29, pp. 233-6, Sep-Dec 2001.

[28] T. J. Carver, K. M. Rutherford, M. Berriman, M. A. Rajandream, B. G. Barrell, and J. Parkhill, "ACT: the Artemis Comparison Tool," *Bioinformatics,* vol. 21, pp. 3422-3, Aug 15 2005.

[29] I. A. Doytchinova and D. R. Flower, "VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines," *BMC Bioinformatics,* vol. 8, p. 4, Jan 5 2007.

[30] O. O. Oni, A. A. Owoade, and C. A. O. Adeyefa, "Design and evaluation of primer pairs for efficient detection of avian rotavirus," *Trop Anim Health Prod,* vol. 50, pp. 267-273, Feb 2018.

[31] G. Stecher, K. Tamura, and S. Kumar, "Molecular Evolutionary Genetics Analysis (MEGA) for macOS," *Mol Biol Evol,* Jan 6 2020.

[32] K. Tamura, J. Dudley, M. Nei, and S. Kumar, "MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0," *Mol Biol Evol,* vol. 24, pp. 1596-9, Aug 2007.

[33] M. Nielsen, C. Lundegaard, P. Worning, S. L. Lauemoller, K. Lamberth, S. Buus, *et al.*, "Reliable prediction of T-cell epitopes using neural networks with novel sequence representations," *Protein Sci,* vol. 12, pp. 1007-17, May 2003.

[34] C. Lundegaard, K. Lamberth, M. Harndahl, S. Buus, O. Lund, and M. Nielsen, "NetMHC-3.0: accurate web accessible predictions of human, mouse and monkey MHC class I affinities for peptides of length 8-11," *Nucleic Acids Res,* vol. 36, pp. W509-12, Jul 1 2008.

[35] M. Andreatta and M. Nielsen, "Gapped sequence alignment using artificial neural networks: application to the MHC class I system," *Bioinformatics,* vol. 32, pp. 511-7, Feb 15 2016.

[36] M. Nielsen and O. Lund, "NN-align. An artificial neural network-based alignment algorithm for MHC class II peptide binding prediction," *BMC Bioinformatics,* vol. 10, p. 296, Sep 18 2009.

[37] S. Buus, S. L. Lauemoller, P. Worning, C. Kesmir, T. Frimurer, S. Corbet, *et al.*, "Sensitive quantitative predictions of peptide-MHC binding by a 'Query by Committee' artificial neural network approach," *Tissue Antigens,* vol. 62, pp. 378-84, Nov 2003.

[38] C. Lundegaard, O. Lund, and M. Nielsen, "Accurate approximation method for prediction of class I MHC affinities for peptides of length 8, 10 and 11 using prediction tools trained on 9mers," *Bioinformatics,* vol. 24, pp. 1397-8, Jun 1 2008.

[39] C. Lundegaard, M. Nielsen, and O. Lund, "The validity of predicted T-cell epitopes," *Trends Biotechnol,* vol. 24, pp. 537-8, Dec 2006.

[40] S. Southwood, J. Sidney, A. Kondo, M. F. del Guercio, E. Appella, S. Hoffman, *et al.*, "Several common HLA-DR types share largely overlapping peptide binding repertoires," *J Immunol,* vol. 160, pp. 3363-73, Apr 1 1998.

[41] A. Patronov and I. Doytchinova, "T-cell epitope vaccine design by immunoinformatics," *Open Biol,* vol. 3, p. 120139, Jan 8 2013.

[42] H. H. Bui, J. Sidney, K. Dinh, S. Southwood, M. J. Newman, and A. Sette, "Predicting population coverage of T-cell epitope-based diagnostics and vaccines," *BMC Bioinformatics,* vol. 7, p. 153, Mar 17 2006.

[43] D. A. Benson, M. Cavanaugh, K. Clark, I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, *et al.*, "GenBank," *Nucleic Acids Res,* vol. 45, pp. D37-d42, Jan 4 2017.

[44] S. Wang, W. Li, S. Liu, and J. Xu, "RaptorX-Property: a web server for protein structure property prediction," *Nucleic Acids Res,* vol. 44, pp. W430-5, Jul 8 2016.

[45] E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, *et al.*, "UCSF Chimera--a visualization system for exploratory research and analysis," *J Comput Chem,* vol. 25, pp. 1605-12, Oct 2004.

[46] S. J. Marshall, "Developing countries face double burden of disease," *Bull World Health Organ,* vol. 82, p. 556, Jul 2004.

[47] A. S. De Groot and R. Rappuoli, "Genome-derived vaccines," *Expert Rev Vaccines,* vol. 3, pp. 59-76, Feb 2004.

[48] B. Korber, M. LaBute, and K. Yusim, "Immunoinformatics comes of age," *PLoS Comput Biol,* vol. 2, p. e71, Jun 30 2006.

[49] A. S. Fauci, "Emerging and re-emerging infectious diseases: influenza as a prototype of the host-pathogen balancing act," *Cell,* vol. 124, pp. 665-70, Feb 24 2006.

[50] S. Chaudhury, E. H. Duncan, T. Atre, S. Dutta, M. D. Spring, W. W. Leitner, *et al.*, "Combining immunoprofiling with machine learning to assess the effects of adjuvant formulation on human vaccine-induced immunity," *Hum Vaccin Immunother,* pp. 1-12, Oct 7 2019.

[51] S. S. Usmani, R. Kumar, S. Bhalla, V. Kumar, and G. P. S. Raghava, "In Silico Tools and Databases for Designing Peptide-Based Vaccine and Drugs," *Adv Protein Chem Struct Biol,* vol. 112, pp. 221-263, 2018.

[52] S. K. Dhanda, S. S. Usmani, P. Agrawal, G. Nagpal, A. Gautam, and G. P. S. Raghava, "Novel in silico tools for designing peptide-based subunit vaccines and immunotherapeutics," *Brief Bioinform,* vol. 18, pp. 467-478, May 1 2017.

[53] Y. Khazaei-Poul, S. Farhadi, S. Ghani, S. A. Ahmadizad, and J. Ranjbari, "Monocyclic Peptides: Types, Synthesis and applications," *Curr Pharm Biotechnol,* Jan 20 2020.

[54] M. A. Hasan, M. Hossain, and M. J. Alam, "A computational assay to design an epitope-based Peptide vaccine against saint louis encephalitis virus," *Bioinform Biol Insights,* vol. 7, pp. 347-55, 2013.

[55] S. Chakraborty, R. Chakravorty, M. Ahmed, A. Rahman, T. M. Waise, F. Hassan, *et al.*, "A computational approach for identification of epitopes in dengue virus envelope protein: a step towards designing a universal dengue vaccine targeting endemic regions," *In Silico Biol,* vol. 10, pp. 235-46, 2010.

[56] M. Tahir Ul Qamar, A. Bari, M. M. Adeel, A. Maryam, U. A. Ashfaq, X. Du, *et al.*, "Peptide vaccine against chikungunya virus: immuno-informatics combined with molecular docking approach," *J Transl Med,* vol. 16, p. 298, Oct 27 2018.

[57] S. S. Twiddy, E. C. Holmes, and A. Rambaut, "Inferring the rate and time-scale of dengue virus evolution," *Mol Biol Evol,* vol. 20, pp. 122-9, Jan 2003.

[58] J. M. Christie, H. Chapel, R. W. Chapman, and W. M. Rosenberg, "Immune selection and genetic sequence variation in core and envelope regions of hepatitis C virus," *Hepatology,* vol. 30, pp. 1037-44, Oct 1999.

[59] B. Shrestha and M. S. Diamond, "Role of CD8+ T cells in control of West Nile virus infection," *J Virol,* vol. 78, pp. 8312-21, Aug 2004.

[60] Y. Ma, K. Tang, Y. Zhang, C. Zhang, Y. Zhang, B. Jin*, et al.*, "Design and synthesis of HLA-A*02-restricted Hantaan virus multiple-antigenic peptide for CD8(+) T cells," *Virol J,* vol. 17, p. 15, Jan 31 2020.

[61] L. P. Zhao, A. Fiore-Gartland, L. N. Carpp, K. W. Cohen, N. Rouphael, L. Fleurs*, et al.*, "Landscapes of binding antibody and T-cell responses to pox-protein HIV vaccines in Thais and South Africans," *PLoS One,* vol. 15, p. e0226803, 2020.

[62] S. Benede, J. Ramos-Soriano, F. Palomares, J. Losada, A. Mascaraque, J. C. Lopez-Rodriguez*, et al.*, "Peptide-glycodendrimers As Potential Vaccines for Olive Pollen Allergy," *Mol Pharm,* Jan 28 2020.

[63] G. Tapia-Calle, P. A. Born, G. Koutsoumpli, M. I. Gonzalez-Rodriguez, W. L. J. Hinrichs, and A. L. W. Huckriede, "A PBMC-Based System to Assess Human T Cell Responses to Influenza Vaccine Candidates In Vitro," *Vaccines (Basel),* vol. 7, Nov 13 2019.

[64] R. Sharmin and A. B. Islam, "A highly conserved WDYPKCDRA epitope in the RNA directed RNA polymerase of human coronaviruses can be used as epitope-based universal vaccine design," *BMC Bioinformatics,* vol. 15, p. 161, May 29 2014.

[65] R. C. Hardison, "Comparative genomics," *PLoS Biol,* vol. 1, p. E58, Nov 2003.

[66] T. Carver, M. Berriman, A. Tivey, C. Patel, U. Bohme, B. G. Barrell*, et al.*, "Artemis and ACT: viewing, annotating and comparing sequences stored in a relational database," *Bioinformatics,* vol. 24, pp. 2672-6, Dec 1 2008.

[67] C. Gilbert, A. Chateigner, L. Ernenwein, V. Barbe, A. Bezier, E. A. Herniou*, et al.*, "Population genomics supports baculoviruses as vectors of horizontal transfer of insect transposons," *Nat Commun,* vol. 5, p. 3348, 2014.

[68] D. Schoeman and B. C. Fielding, "Coronavirus envelope protein: current knowledge," *Virol J,* vol. 16, p. 69, May 27 2019.

[69] E. Mortola and P. Roy, "Efficient assembly and release of SARS coronavirus-like particles by a heterologous expression system," *FEBS Lett,* vol. 576, pp. 174-8, Oct 8 2004.

[70] R. Sharmin and A. B. Islam, "Conserved antigenic sites between MERS-CoV and Bat-coronavirus are revealed through sequence analysis," *Source Code Biol Med,* vol. 11, p. 3, 2016.

[71] J. Xu, J. Hu, J. Wang, Y. Han, Y. Hu, J. Wen*, et al.*, "Genome organization of the SARS-CoV," *Genomics Proteomics Bioinformatics,* vol. 1, pp. 226-35, Aug 2003.

[72] J. W. Westerbeck and C. E. Machamer, "A Coronavirus E Protein Is Present in Two Distinct Pools with Different Effects on Assembly and the Secretory Pathway," *J Virol,* vol. 89, pp. 9313-23, Sep 2015.

[73] M. L. Dediego, L. Pewe, E. Alvarez, M. T. Rejas, S. Perlman, and L. Enjuanes, "Pathogenicity of severe acute respiratory coronavirus deletion mutants in hACE-2 transgenic mice," *Virology,* vol. 376, pp. 379-89, Jul 5 2008.

[74] J. Netland, M. L. DeDiego, J. Zhao, C. Fett, E. Alvarez, J. L. Nieto-Torres*, et al.*, "Immunization with an attenuated severe acute respiratory syndrome coronavirus deleted in E protein protects against lethal respiratory disease," *Virology,* vol. 399, pp. 120-128, Mar 30 2010.

[75] P. B. McCray, Jr., L. Pewe, C. Wohlford-Lenane, M. Hickey, L. Manzel, L. Shi, *et al.*, "Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus," *J Virol,* vol. 81, pp. 813-21, Jan 2007.

[76] J. A. Regla-Nava, J. L. Nieto-Torres, J. M. Jimenez-Guardeno, R. Fernandez-Delgado, C. Fett, C. Castano-Rodriguez, *et al.*, "Severe acute respiratory syndrome coronaviruses with mutations in the E protein are attenuated and promising vaccine candidates," *J Virol,* vol. 89, pp. 3870-87, Apr 2015.

[77] J. Zhao, K. Li, C. Wohlford-Lenane, S. S. Agnihothram, C. Fett, J. Zhao, *et al.*, "Rapid generation of a mouse model for Middle East respiratory syndrome," *Proc Natl Acad Sci U S A,* vol. 111, pp. 4970-5, Apr 1 2014.

[78] X. Guo, Y. Deng, H. Chen, J. Lan, W. Wang, X. Zou, *et al.*, "Systemic and mucosal immunity in mice elicited by a single immunization with human adenovirus type 5 or 41 vector-based vaccines carrying the spike protein of Middle East respiratory syndrome coronavirus," *Immunology,* vol. 145, pp. 476-84, Aug 2015.