Immunoinformatic Responses of Host MHC I/II and CD4⁺ Cells Against Conserved Spike Fragments of SARS CoV-2 of 186 Countries With In-Silico Mutations: Implications in Universal Vaccination

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Research Article

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Abstract

Different types of quickly-developed vaccines have been introduced against Covid-19 with largely inconclusive results. On the course of time, somewhere Covid-19 declines its infection/mortality rate and in some countries it revived with some new mutant-variants. Considering the large number of global variants, in the current study several conserved (186 countries) sequences (checked by ClustalX2) epitopic regions (by SVMTriP and IEDB) and in-silico mutants of SARS CoV-2 spike protein fragments (Cut 1-4) were screened for their stability against proteases, antigenicity (VaxiJen V2.0 and for glycosylation effects NetOGlyc-NetNGlyc), MHCI/II reactivity (IEDB TOOLS) and CD4+ cellular responses utilizing Haddock 2.4 and PatchDock programme. The cut4 mutant and its peptide SRLFRKSNLKPFERD showed highest combined-score 48.23548 and Immunogenicity-Score of 92.0887. The core-sequence SRLFRKSNL showed highest Median Percentile Rank (7 HLA-allele) of 19. CD4+ immunogenicity also confirms representation of CUT4TM2 epitope SRLFRKSNL by MHC Class II. The epitope YNYKYRLFR from CUT4 showed IC50 value of ~30 nM with allele HLA-DRB1*11:01 and HLA-DRB5*01:01. Binding affinity and RMSD score suggest that different epitopic regions of Cut4 mutants interacted MHC II with large number of H-bonds. Cut4 double mutants strongly interacted with exposed T-cell surface and facilitated by its receptors. According to the antigenicity analysis, epitopes 3, 4, and 5 were the potent antigens with antigenicity values of 0.6268, 1.2404, and 0.4639, respectively. Screening of conserved SARS CoV-2 spike fragments globally may help to find most stable antigenic determinant and further their mutation-engendered counterparts have better immunogenic responses. Further studies are necessary to develop global vaccination strategies against Covid-19.

Introduction

At the end of 2020, at least 30 vaccines are in the process of clinical trials. Some of those like Oxford's AZD1222/AstraZeneca/Oxford's AZD1222/Moderna's mRNA-1273 and Sinovac's CoronaVac vaccines reported with inconclusive results [1]. Some of the countries have introduced self-generated vaccines to their populations. Covishield (Oxford-AstraZeneca) and Covaxin (produced by Bharat biotech) from India origin have been introduced to a significant number of populations (ref https://www.bbc.com/news/world-asia-india-55748124). Those have some diverse outcome, criticism and no definite clinical-trial outcome. In natural way, the infection and the case fatality rate (CFR) are declining but ample evidence of multiple mutation and highly evolving SARS CoV-2 urges in the development of more effective vaccine effective against global variants. In some country with largest populations induction of herd immunity by mass vaccination has been effective in the prevention of these types of diseases [2]. The autoimmune suppressive effects of this virus prevent or delay in the immunotherapy processes. In multiple cases vaccination or other immmuno-therapy cannot effect at ease. Negative outcome was also noticed during drug-induced inhibition of some subsets of B-cell hinders the initiation of innate and CD8 + cell responses [3]. None of the COVID-19 vaccines is fully effective with safety to get priority at this point of time. Only rigorous clinical trial and post application follow-up can recognize serious health hazards [4].

Report suggests that ChAdOx1 nCoV-19 has an acceptable safety profile against symptomatic COVID-19 [5]. But that statistical outcome generated from a small sample size. The candidate vaccine mRNA-1273 encodes the stabilized prefusion SARS-CoV-2 spike protein and generated some immune responses [6]. Not only the generation of specific subsets of IgG but the MHC, T and B cell responses have also been important for the optimized immune responses.

Th1-targeted antibody, $CD8^+$ and $CD4^+$ T cell responses and neutralizing IgG antibody responses were noticed with S protein-specific viral neutralization [7]. This suggested T and B cell mediated acquired immunity is one of the main natural immune therapy against Covid-19. However very few study detected MHC class recognition of spike protein epitope. Our earlier study has indicated some spike epitope capable in the generation of MHC responses [8]. In contrary, it is also evident that the antibodies may suppress viral replication through neutralization but might also augment SARS CoV-2 pathogenesis through a process termed antibody-dependent enhancement [9]. Many of the studies elicited vaccine efficacy in polyfunctional S-specific (some time exposed amino acids of S proteins) CD4+ (mainly Th1) and CD8 + T-cell responses, with a T effector memory phenotype. In some other strategy, DNA/MVA immunizations elicited higher T-cell responses [10].

The current study was aimed to find the role of small cut fragment of the globally conserved SARS Cov-2 Spike RBD and its in-silico mutants in the generation of the higher antigenicity. Further, antigen presentation by HLA and T-cell responses were tested to confirm the antibody-mediated immunogenic responses. Present results are significant to develop a potent and universal vaccination strategy against the Covid-19. Further studies are necessary.

Materials And Methods

Structure retrieval, analysis and prediction

Structure of different CUT4 and mutated peptide sequences were taken from our previous analysis. The binding sites of these CUTs with ACE2 Receptor Binding Domain (RBD) were analyzed using PyMol [11]. For immunoinformatics study, T cell receptor, MHC I and MHC II structures were downloaded from RCSB PDB [12]. Different mutated CUTs were analyzed for T cell receptor interaction analysis using PDB ID 1kgc, 2ak4, 3kxf and 4jrx. For different mutated CUTs interaction with MHC class I, PDB ID 1i4f was used. Different epitopes from mutated CUTs were also analyzed for MHC class I binding using the PDB structures of 6iex, 3x13, 4lnr, 6j1w and 6vb3. Different mutated CUTs interaction with MHC class II was analyzed with PDB ID 4p5m and 3c5j. Different mutated CUTs epitopes were analyzed with 1jk8, 3pdo, 3lqz, 1i4f, 5ni9 and 1BX2 MHC class II structures.

Docking studies

Protein-Protein docking studies were performed using Haddock 2.4 [13] and PatchDock [14] server to check the binding affinity with T cell receptor, MHC class I and MHC class II molecules. The results were analyzed using Pymol [11] and the CUTs which had the best binding affinity were chosen. The best

mutated structures from the docking result were accepted. The interaction study of the accepted unmutated cut and the predicted epitopes with best interacting T cell receptor, MHC I and MHC II according to IEDB tools was done using HADDOCK 2.4 [13].

Epitope study

Epitope prediction using SVMTRIP [15] was done and the probable epitopes were taken for the finalized structure. All the probable epitopes were analyzed using all the IEDB Tools [16]. The T cell Epitope prediction and analysis were performed using IEDB tools [8]. Epitope analysis using SVMTRIP [15] and analyzing results using all IEDB tools were performed. Antigenicity was checked using VaxiJen V2.0 [17] keeping the threshold value as 0.4. Glycosylation study using NetOGlyc and NetNGlyc [18] was performed.

The half-life of the selected peptide in blood was predicted using PlifePred server (http://crdd.osdd.net/raghava/plifepred/).

Global mutation study on SARS CoV-2 spike protein

Initially, 186 gene sequences of nCOV2 spike glycoproteins from different countries were retrieved from GISAID database (https://www.gisaid.org/). Then all the sequences were subjected to multiple sequence alignment for the identification of unique sequences using ClustalX2. From all the sequences, 103 sequences were selected with unique sequences and converted to protein sequences using SMS Sequence Manipulation Suite (bioinformatics.org/sms2/translate.html) online server. Among them 24 unique protein sequences were selected for further analysis. Among them two sequences were selected having sequential difference at the Receptor Binding Domain (RBD) in comparison to CUT4. Finally, sequence of CUT4, Model 1 and Model 15 were selected for epitope analysis. Model 1 represented the RBD sequences of Estonia, Latvia, Hong Kong, Costarica, Iran, Mexico, Mongolia, Japan, Italy, Egypt, Ireland, Denmark, Germany, France, India, DRC, Serbia, Pakistan, England and Wuhan (wild type). Whereas, the sequences of Model 15 represented Finland. As per the Epitope analysis and antigenicity of epitope, structure of selected sequences (Model 1 and Model15) was individually aligned with CUT4 and the respective epitope sequences and locations were analyzed using Pymol [11].

Results And Discussions

Different peptide CUT analysis

According to our previous study a peptide with an amino acid length of 84 (cut 4), has been selected for mutation analysis (data submitted elsewhere). Among 11 different mutations (4 Single mutations, 5 double mutations and 2 triple mutations) three were selected for their antigenicity and other immune-reactivity testing. The CUT4 peptide was shown to competitively inhibit the nCOV2-ACE2 attachment at the RB domain (data submitted elsewhere). These peptide CUT4 mutations were further studied for T-Cell reorganization, MHC class I and II attachment, protease cutting-sites, glycosylation effects stability for stable antigenic responses and that was compared with the corresponding mutants.

Class I and Class II immunogenicity analysis

According to the results, CUT4, CUT4SM3 and CUT4DM2 showed similar result in Class I immunogenicity (S Table 1). Though the length of the peptide remain same, score value differed due to single, double and triple mutations. Highest score of 0.27813 was found for CUT4 unmutated, CUT4SM3 also showed positive value of 0.07923. Whereas, score − 0.07827 was found for both CUT4DM2 and CUT4TM2 fragments.

For Class II analysis, single and double mutation did not affect the CD4 immunogenicity property. Whereas, CUT4TM2 showed better result than others (S Table 2). The peptide sequence SRLFRKSNLKPFERD showed highest combined score of 48.23548, Immunogenicity Score of 92.0887. The peptide core sequence SRLFRKSNL of CUT4TM2 showed highest Median Percentile Rank (among 7-allele) of 19. For all of these 7 interactive alleles; HLA-DRB1:03:01, HLA-DRB1:07:01, HLA-DRB1:15:01, HLA-DRB3:01:01, HLA-DRB3:02:02, HLA-DRB4:01:01 and HLA-DRB5:01:01, CUT4TM2 showed highest scores of interactions with 6 except HLA-DRB5:01:01. The Class II molecules present epitopes to CD4 T cells. So, increase in CD4 immunogenicity confirms representation of CUT4TM2 epitope SRLFRKSNL by MHC Class II.

Interaction analysis of CUT4 and other mutants with MHC class I and II

Different segments were analyzed for stabilized interaction with different alleles of MHC class II. All the data were selected on the basis of percentile rank below 10% and IC50 value below 50nM. Here stabilization matrix alignment method or SMM-align method has been used for IC50 value calculation (S Table 3). Only the lower IC50 values were represented in S Table 3, rest of the data not shown. The lower IC50 value indicated the higher chance to form stabilized structures with MHC II alleles. Here, epitope YNYKYRLFR from CUT4 showed IC50 value of 30 nM and 28 nM with allele HLA-DRB1*11:01 and HLA-DRB5*01:01 respectively. Similar results were found with CUT4DM2. For the same epitope, CUT4SM3 showed IC50 value of 28 nM using only allele HLA-DRB5*01:01. But CUT4TM2 showed higher value at the range of 44–47 nM with the same allele HLA-DRB5*01:01. The unique epitope ATSTGNYNY in CUT4SM3 showed lower IC50 value of 30 nM with HLA-DRB5*01:01. Whereas neural network based IC50 analysis, alignment represented that CUT4 and CUT4DM2 had the lowest and similar IC50 values with epitope sequences YRLFRKSNL, YNYKYRLFR and YRLFRKSNL. Sturniolo method is one of the best methods to predict the best fit between epitope and MHC Class II alleles. Sturniolo score also represented CUT4 and CUT4DM2 with the similar values where CUT4SM3 showed lower values. According to as all of them were less than 50nM. So, from the above analysis it could be predicted that, just like CUT4 unmutated different epitopes were equally capable to form a stabilized structure with different alleles of MHC Class II. For MHC Class I analysis, no significant changes were observed (S Table 4). So, Class II type of interaction and representation was observed for our epitope efficiency analysis.

Molecular docking analysis, CUT4SM3 and CUT4DM2 were found to interact with MHC Class I allele 1i4f with the docking score of -258.83 and − 267.99, and ligand RMSD of 120.06 and 103.43 respectively (Fig. 1). At those positions CUTs were found to block the MHC Class I peptide presenting site, but no effective bond formation were observed. Whereas, CUT4TM2 was found to interact with 1i4f with the amino acids involvement in H-bond were, GLN498 of CUT4TM2 with GLY16 1i4f and GLN 493 of CUT4TM2 with GLU19 of 1i4f. The docking score and ligand RMSD of that interaction were − 254.36 and 169.46 respectively. Whenever, the CUTs were analyzed for epitope, 6 fragments were found to interact with different alleles of MHC Class I. Based on the IEDB results, all the fragments were docked with their respective alleles as represented in Fig. 1. Where, effective H-bonding was observed for epitope segment 4 with a sequence of TRNIDATSTG. It interacted with HLA-A*30:01 (PDB ID: 6J1W) with a score of 0.008784 and rank 9. SER445 of CUT4TM2 formed H-bond with TYR7 of 6J1W. Docking score was − 164.37 and Ligand RMSD was 193.75. Rest of the epitope segments were found exactly at the epitope presenting domain of respective MHC Class I alleles but no H-bonding observed.

Similarly, molecular docking with MHC class II were also performed CUT4SM3 and CUT4DM2 were found at the epitope presenting domain of MHC class II allele 4P5M without any H-bonding (Fig. 2). Whereas, CUT4TM2 formed H-bond through SER14 to ARG76 of MHCII (PDB ID: 3c5j). But more affinity was observed for different epitopes as presented in Fig. 1. THR470 of Epitope 1 formed H-bond with TYR9 (PDB ID: 1kj8) within 2.9 A distance, docking score − 185.41. PHE490 of Epitope 3 formed H-bond with TYR9 (PDB ID: 3lqz) within 2.0 A distance, docking score − 188.50 and ligand RMSD value of 125.53 and finally GLN506 of Epitope 5 formed H-bond with GLN9 (PDB ID: 5NI9) within 2.7 A distance, docking score − 179.94 and ligand RMSD value of 131.92.

T cell receptor binding analysis of CUT4 and other mutated CUTs.

All the CUTs, SM3, DM2 and TM2 showed remarkable affinity to T cell receptors. Several strong bonds as well as H-bonds were formed as presented in Fig. 3. SM3 showed interactions with two different T cell receptors like 1kgc and 4jrx. H-binding pattern and surface attachment pattern has been presented in Fig. 3a & b. Most of the surface area has been exposed for DM2 in the peptide presenting site of receptor 1kgc (Fig. 3c). TM2 showed highest representation by 4 different T cell receptors like 1kgc, 4jrx, 2ak4 and 3kxf. The representations of different CUTs were facilitated by T cell receptors.

Epitope pattern of Receptor Binding Domain (RBD) analysis within various nCOV2 spike proteins found in 186 countries worldwide (Fig. 4). According to CUT4, Model 1 represented the Estonia, Latvia, Hong Kong, Costarica, Iran, Mexico, Mongolia, Japan, Italy, Egypt, Ireland, Denmark, Germany, France, India, DRC, Serbia, Pakistan, England and Wuhan (wild type) and Model 15 represented the Finland. Though, sequential diversity was present among CUT4, Model1 and Model15, sequential similarity found among 4 epitopes out of 5. The structural alignment also showed similarity among all except epitope 4.

In silico analysis of CUT4 and other mutant stability at physiological level

The stability of CUT4 and its different mutants in physiological level has been studied through proteasomal cleavage site analysis, N terminal and C-terminal glycosylation analysis and half life prediction in blood. There was no proteasomal cleavage sites found in CUT4 before and after mutation according to IEDB analysis. Whereas, according to neural network based program, NetCTL, predicted that there were some one Proteasomal processing site but the score of 0.1984 stands insignificant. So, the peptides may remain stable in physiological condition. All the un-mutated and mutated CUT4 showed no glycosylation at both end (N and C terminal), except TM2 with C terminal glycosylation. These indicated that, these peptides were effectively bound with T cell receptors, and enhance further immunological reactions as they were not glycosylated.

Finally, the presence of CUT4 epitope was analyzed among different nCOV2 spike glycoproteins from 186 countries worldwide (Fig. 4). Though sequential diversity was present among different nCOV2 spike whole proteins, the Receptor Binding Domain (RBD) showed higher sequential similarity. As a result of which two sequences and their specific predicted tertiary Models were selected as representatives of all 186 countries. Model 1 was representative of all countries except Finland. Model 15 was the representative of Finland. The 5 epitopes present within the CUT4 were also found within Model 1 and Model15. Epitope 1, 2, 3, and 5 were sequentially and structurally the same. The epitope 4 was found to be different. This result was also alike in 103 previously selected protein sequences (S Fig. 1.) According to the antigenicity analysis, epitope 3, 4, and 5 were the potent antigens with antigenicity values of 0.6268, 1.2404, and 0.4639. This epitope-based analysis could be applicable for worldwide varieties of nCOV2. The effect of mutation was reflected in antigenicity analysis (S Table 5). Comparative study among CUT4, SM3, DM2 and TM2 represented that, TM2 has the highest antigenicity of 0.6501. Whereas, CUT4, SM3 and DM2 have scores 0.6478, 0.6373 and 0.6197 respectively. On the other hand epitope mutation showed significant differences (S Table 5). In this study, both the wild and mutant type epitopes were found to be reactive with the CD4 + cells expected that cell mediated higher order of immune responses. SARS CoV-2 develops immunosuppression in autoimmunity, which leads to terminated and/or delayed immunotherapy. Although CD4 + T cells are the key player but it is ineffective until the naive B-cells upregulations. This is noticed in some severe patients to recover who have CD20-depleted state can overcome immunosuppression [19]. Virus mediated couple of vaccination strategies have been tested. Recombinant adenovirus vector has been used to carry SARS-CoV-2) spike glycoprotein and used as a heterologous vaccine that induced strong humoral and cellular immune responses [20]. Vaccine hypersensitivity reactions (VAH) are one of the demerits of this procedure. In some earlier cases of respiratory syndrome (SARS, MERS or respiratory syncytial virus) cases this adverse effect was noticed [21].

It is speculated that two human adenovirus-vector vaccines, three attenuated vaccines, and one peptide vaccine are in limited use and some more are under clinical trial but having no satisfactory results till

date [22]. Reactivity against the global populations a vaccine is required which should comprise B-cell, CTL, HTL epitopes. The epitopes associated with maximum HLA alleles are the requirement of the time to ensure the viral neutralization. One report claims this outcome have been currently under trial [23]. CRISPR-mediated genome editing approach has also been used for anti-viral strategies but yet to take time to be decisive [24].

In the current study, the globally conserved spike fraction of SARS CoV-2 has been extensively studied for greater immunogenicity and further in silico several of its mutants were generated with more immunogenic epitopic fragments which can develop MHC multi HLA responses and T-cell CD4 + signals. This is hypothesized that multi-epitopic combinations or cocktail can be used for global vaccination strategies. Further studies are necessary in this regard.

Declarations

Conflict of interests: none

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Authors Contributions: SM- study design, analysis and final manuscript approval, AB- Experiment and data analysis, MK and DS- Experiment and procurement of data, figure ppreperations.

All Authors read and approved the manuscript

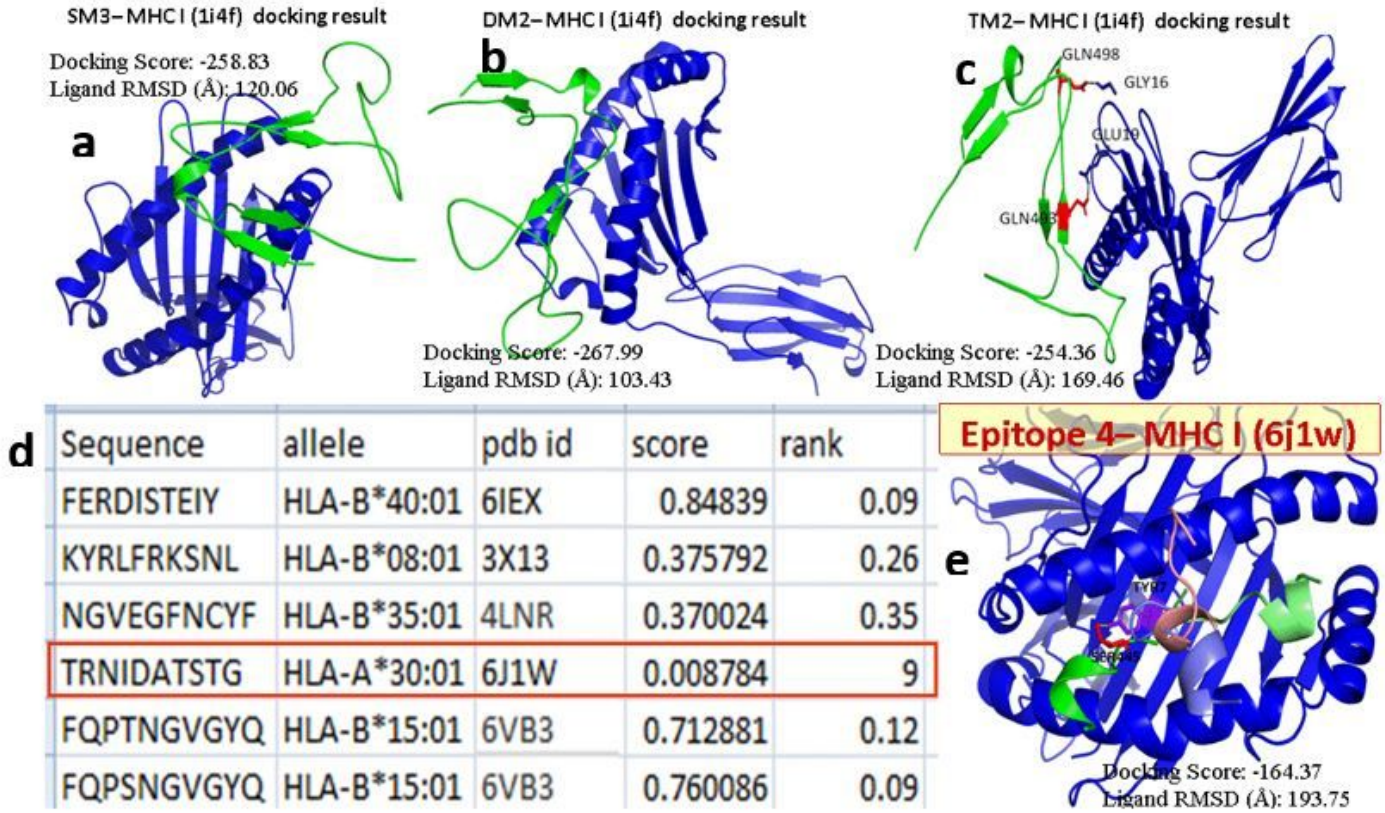
References

1. Sharma O, Sultan AA, Ding H, Triggle CR. A Review of the Progress and Challenges of Developing a Vaccine for COVID-19. Front Immunol. 2020 Oct 14;11:585354. doi: 10.3389/fimmu.2020.585354. PMID: 33163000; PMCID: PMC7591699.
2. Frederiksen LSF, Zhang Y, Foged C, Thakur A. The Long Road Toward COVID-19 Herd Immunity: Vaccine Platform Technologies and Mass Immunization Strategies. Front Immunol. 2020 Jul 21;11:1817. doi: 10.3389/fimmu.2020.01817. PMID: 32793245; PMCID: PMC7385234.
3. Baker D, Roberts CAK, Pryce G, Kang AS, Marta M, Reyes S, Schmierer K, Giovannoni G, Amor S. COVID-19 vaccine-readiness for anti-CD20-depleting therapy in autoimmune diseases. Clin Exp Immunol. 2020 Nov;202(2):149-161. doi: 10.1111/cei.13495. Epub 2020 Aug 1. PMID: 32671831; PMCID: PMC7405500.

4. Haynes BF, Corey L, Fernandes P, Gilbert PB, Hotez PJ, Rao S, Santos MR, Schuitemaker H, Watson M, Arvin A. Prospects for a safe COVID-19 vaccine. Sci Transl Med. 2020 Nov 4;12(568):eabe0948. doi: 10.1126/scitranslmed.abe0948. Epub 2020 Oct 19. PMID: 33077678.
5. Voysey M, Clemens SAC, Madhi SA et. al. Oxford COVID Vaccine Trial Group. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet. 2021 Jan 9;397(10269):99-111. doi: 10.1016/S0140-6736(20)32661-1. Epub 2020 Dec 8. Erratum in: Lancet. 2021 Jan 9;397(10269):98. PMID: 33306989; PMCID: PMC7723445.
6. Jackson LA, Anderson EJ, Rouphael NG et. al. mRNA-1273 Study Group. An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. N Engl J Med. 2020 Nov 12;383(20):1920-1931. doi: 10.1056/NEJMoa2022483. Epub 2020 Jul 14. PMID: 32663912; PMCID: PMC7377258.
7. Hörner C, Schürmann C, Auste A, Ebenig A, Muraleedharan S, Dinnon KH 3rd, Scholz T, Herrmann M, Schnierle BS, Baric RS, Mühlebach MD. A highly immunogenic and effective measles virus-based Th1-biased COVID-19 vaccine. Proc Natl Acad Sci U S A. 2020 Dec 22;117(51):32657-32666. doi: 10.1073/pnas.2014468117. Epub 2020 Nov 30. PMID: 33257540; PMCID: PMC7768780.
8. Banerjee A, Santra D, Maiti S. Energetics and IC50 based epitope screening in SARS CoV-2 (COVID 19) spike protein by immunoinformatic analysis implicating for a suitable vaccine development. J Transl Med. 2020 Jul 10;18(1):281. doi: 10.1186/s12967-020-02435-4. PMID: 32650788; PMCID: PMC7351549.
9. Lu L, Zhang H, Zhan M, Jiang J, Yin H, Dauphars DJ, Li SY, Li Y, He YW. Antibody response and therapy in COVID-19 patients: what can be learned for vaccine development? Sci China Life Sci. 2020 Dec;63(12):1833-1849. doi: 10.1007/s11427-020-1859-y. Epub 2020 Dec 1. PMID: 33355886; PMCID: PMC7756132.
10. García-Arriaza J, Garaigorta U, Pérez P, Lázaro-Frías A, Zamora C, Gastaminza P, Del Fresno C, Casasnovas JM, Sorzano CÓS, Sancho D, Esteban M. COVID-19 vaccine candidates based on modified vaccinia virus Ankara expressing the SARS-CoV-2 spike induce robust T- and B-cell immune responses and full efficacy in mice. J Virol. 2021 Jan 7:JVI.02260-20. doi: 10.1128/JVI.02260-20. Epub ahead of print. PMID: 33414159.
11. Janson G, Zhang C, Prado MG, Paiardini A. PyMod 2.0: improvements in protein sequence-structure analysis and homology modeling within PyMOL. Bioinformatics. 2017 Feb 1;33(3):444-446. doi: 10.1093/bioinformatics/btw638. PMID: 28158668.
12. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank. Nucleic Acids Res. 2000 Jan 1;28(1):235-42. doi: 10.1093/nar/28.1.235. PMID: 10592235; PMCID: PMC102472.
13. van Zundert GCP, Rodrigues JPGLM, Trellet M, Schmitz C, Kastritis PL, Karaca E, Melquiond ASJ, van Dijk M, de Vries SJ, Bonvin AMJJ. The HADDOCK2.2 Web Server: User-Friendly Integrative Modeling of Biomolecular Complexes. J Mol Biol. 2016 Feb 22;428(4):720-725. doi: 10.1016/j.jmb.2015.09.014. Epub 2015 Sep 26. PMID: 26410586.

14. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ. PatchDock and SymmDock: servers for rigid and symmetric docking. Nucleic Acids Res. 2005 Jul 1;33(Web Server issue):W363-7. doi: 10.1093/nar/gki481. PMID: 15980490; PMCID: PMC1160241.
15. Yao B, Zhang L, Liang S, Zhang C. SVMTriP: a method to predict antigenic epitopes using support vector machine to integrate tri-peptide similarity and propensity. PLoS One. 2012;7(9):e45152. doi: 10.1371/journal.pone.0045152. Epub 2012 Sep 12. PMID: 22984622; PMCID: PMC3440317.
16. Fleri W, Paul S, Dhanda SK, Mahajan S, Xu X, Peters B, Sette A. The Immune Epitope Database and Analysis Resource in Epitope Discovery and Synthetic Vaccine Design. Front Immunol. 2017 Mar 14;8:278. doi: 10.3389/fimmu.2017.00278. PMID: 28352270; PMCID: PMC5348633.
17. Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics. 2007 Jan 5;8:4. doi: 10.1186/1471-2105-8-4. PMID: 17207271; PMCID: PMC1780059.
18. Azevedo R, Silva AMN, Reis CA, Santos LL, Ferreira JA. In silico approaches for unveiling novel glycobiomarkers in cancer. J Proteomics. 2018 Jan 16;171:95-106. doi: 10.1016/j.jprot.2017.08.004. Epub 2017 Aug 4. PMID: 28782717.
19. Baker D, Roberts CAK, Pryce G, Kang AS, Marta M, Reyes S, Schmierer K, Giovannoni G, Amor S. COVID-19 vaccine-readiness for anti-CD20-depleting therapy in autoimmune diseases. Clin Exp Immunol. 2020 Nov;202(2):149-161. doi: 10.1111/cei.13495. Epub 2020 Aug 1. PMID: 32671831; PMCID: PMC7405500.
20. Logunov DY, Dolzhikova IV, Zubkova OV, et al. Safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-randomised phase 1/2 studies from Russia. Lancet. 2020 Sep 26;396(10255):887-897. doi: 10.1016/S0140-6736(20)31866-3. Epub 2020 Sep 4. Erratum in: Lancet. 2021 Jan 9;397(10269):98. PMID: 32896291; PMCID: PMC7471804.
21. Halstead SB, Katzelnick L. COVID-19 Vaccines: Should We Fear ADE? J Infect Dis. 2020 Nov 13;222(12):1946-1950. doi: 10.1093/infdis/jiaa518. PMID: 32785649; PMCID: PMC7454712.
22. Zhao J, Zhao S, Ou J, Zhang J, Lan W, Guan W, Wu X, Yan Y, Zhao W, Wu J, Chodosh J, Zhang Q. COVID-19: Coronavirus Vaccine Development Updates. Front Immunol. 2020 Dec 23;11:602256. doi: 10.3389/fimmu.2020.602256. PMID: 33424848; PMCID: PMC7785583.
23. Mahapatra SR, Sahoo S, Dehury B, Raina V, Patro S, Misra N, Suar M. Designing an efficient multi-epitope vaccine displaying interactions with diverse HLA molecules for an efficient humoral and cellular immune response to prevent COVID-19 infection. Expert Rev Vaccines. 2020 Sep;19(9):871-885. doi: 10.1080/14760584.2020.1811091. Epub 2020 Sep 24. PMID: 32869699; PMCID: PMC7544970.
24. Faiq MA. B-cell engineering: A promising approach towards vaccine development for COVID-19. Med Hypotheses. 2020 Nov;144:109948. doi: 10.1016/j.mehy.2020.109948. Epub 2020 Jun 2. PMID: 32516733; PMCID: PMC7264927.

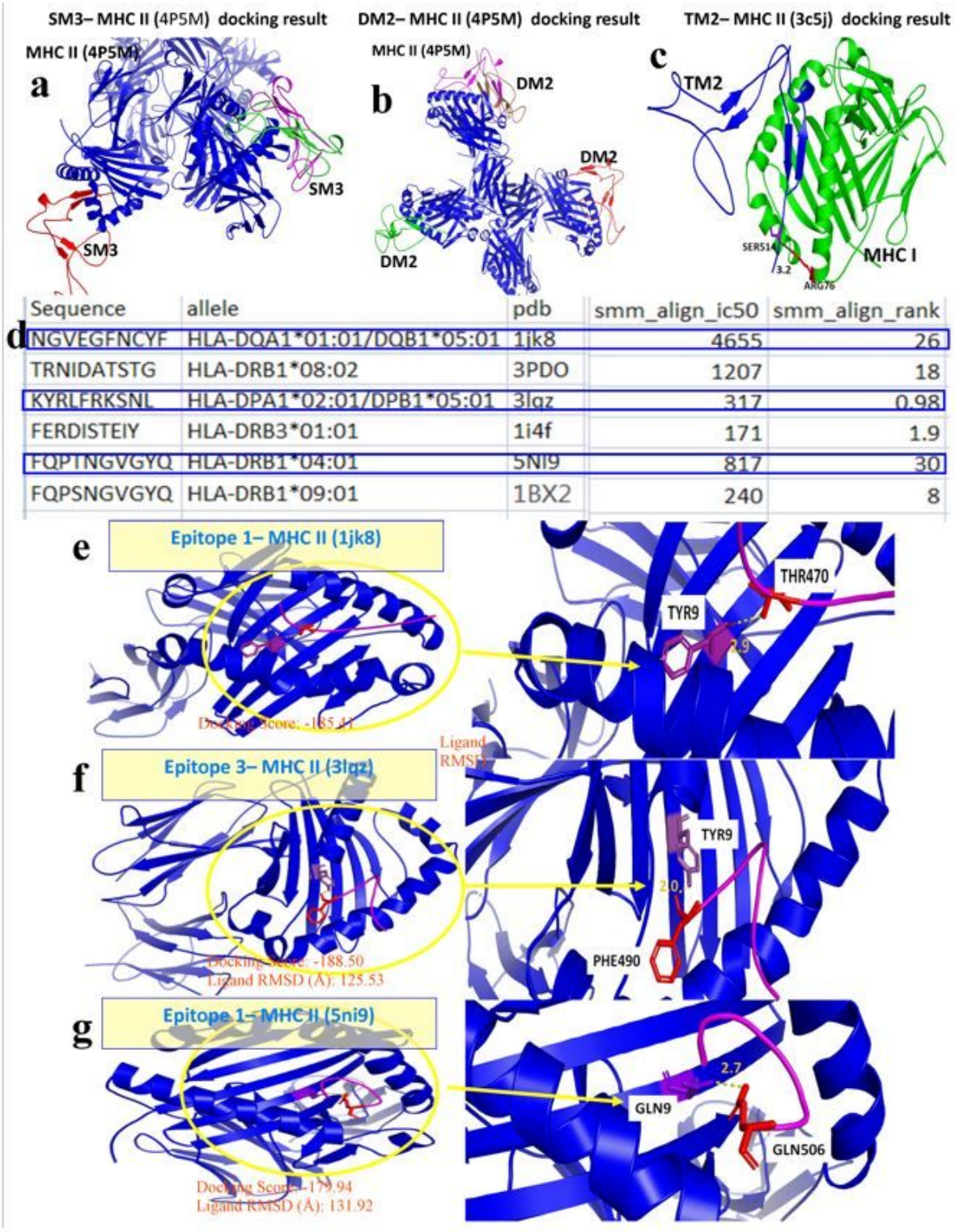
Figures



Sequence	allele	pdb id	score	rank
FERDISTEIY	HLA-B*40:01	6IEX	0.84839	0.09
KYRLFRKSNL	HLA-B*08:01	3X13	0.375792	0.26
NGVEGFNCYF	HLA-B*35:01	4LNR	0.370024	0.35
TRNIDATSTG	HLA-A*30:01	6J1W	0.008784	9
FQPTNGVGYQ	HLA-B*15:01	6VB3	0.712881	0.12
FQPSNGVGYQ	HLA-B*15:01	6VB3	0.760086	0.09

Figure 1

Interaction of different selected CUT4 mutant (SM3, DM2 and TM2) with MHC class I (1i4f). Interaction showed SM3 and DM2 at the nearby location of 1i4f Epitope presenting site but no interactions were observed (a, b). TM2 formed H-bond with 1i4f (c). Different Epitope from these selected mutants (d) showed that Epitope4 to interact with MHC class I (6j1w). It formed H-bond between TYR7 of Epitope4 and SER445 of MHC I.



d

Sequence	allele	pdb	smm_align_ic50	smm_align_rank
NGVEGFNCYF	HLA-DQA1*01:01/DQB1*05:01	1jk8	4655	26
TRNIDATSTG	HLA-DRB1*08:02	3PDO	1207	18
KYRLFRKSNL	HLA-DPA1*02:01/DPB1*05:01	3lqz	317	0.98
FERDISTEIY	HLA-DRB3*01:01	1i4f	171	1.9
FQPTNGVGYQ	HLA-DRB1*04:01	5NI9	817	30
FQPSNGVGYQ	HLA-DRB1*09:01	1BX2	240	8

Figure 2

Interaction of different selected CUT4 mutant (SM3, DM2 and TM2) with MHC class II (4P5M and 3C5J). Interaction showed SM3 and DM2 at the nearby location of 4P5M Epitope presenting site but no interactions were observed (a, b). TM2 formed H-bond with 3C5J (c). Different Epitope from these selected mutants (d) showed that Epitope1, 3 and 5 to interact with MHC class II (1jk8, 3lqz and 5ni9).

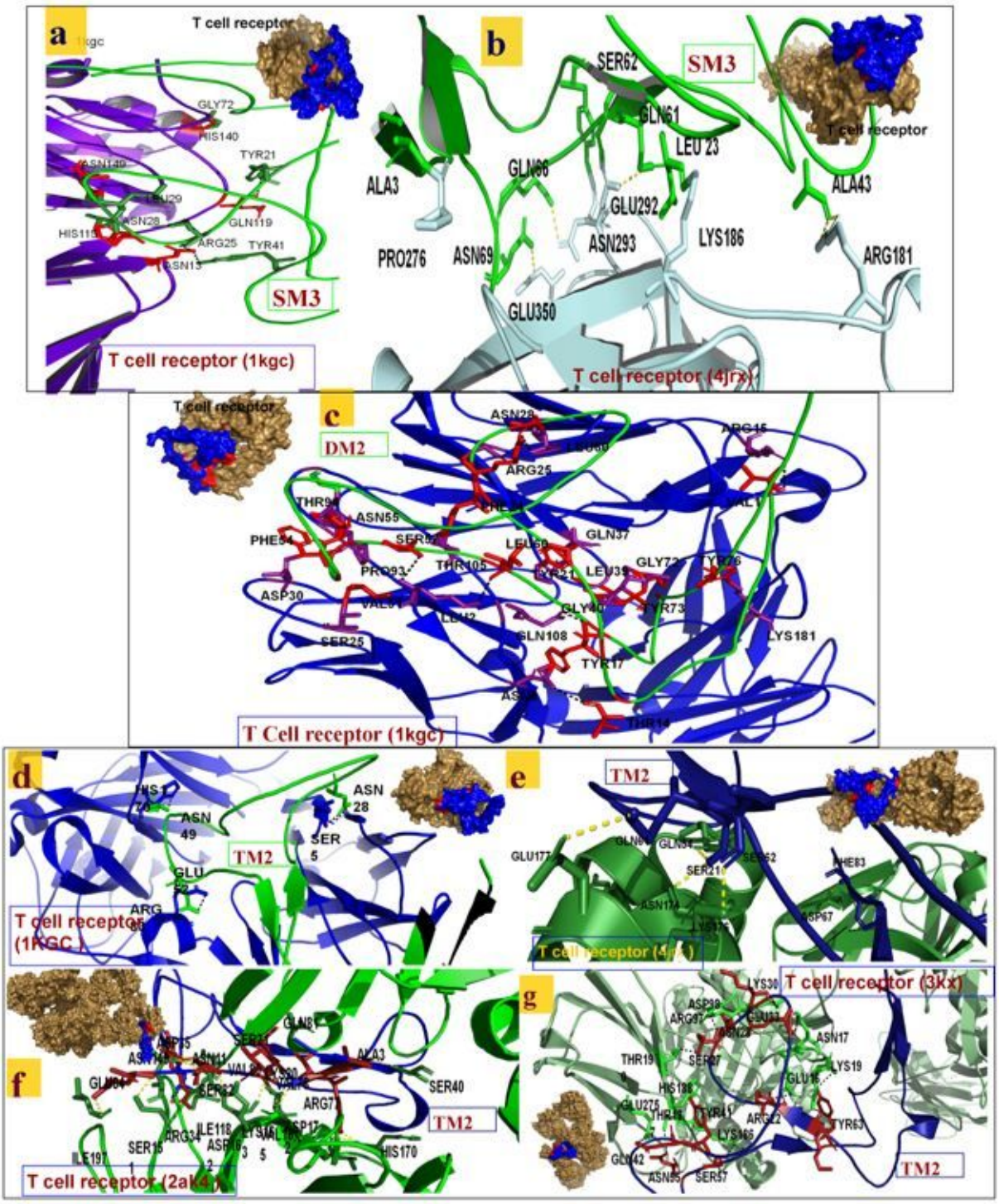
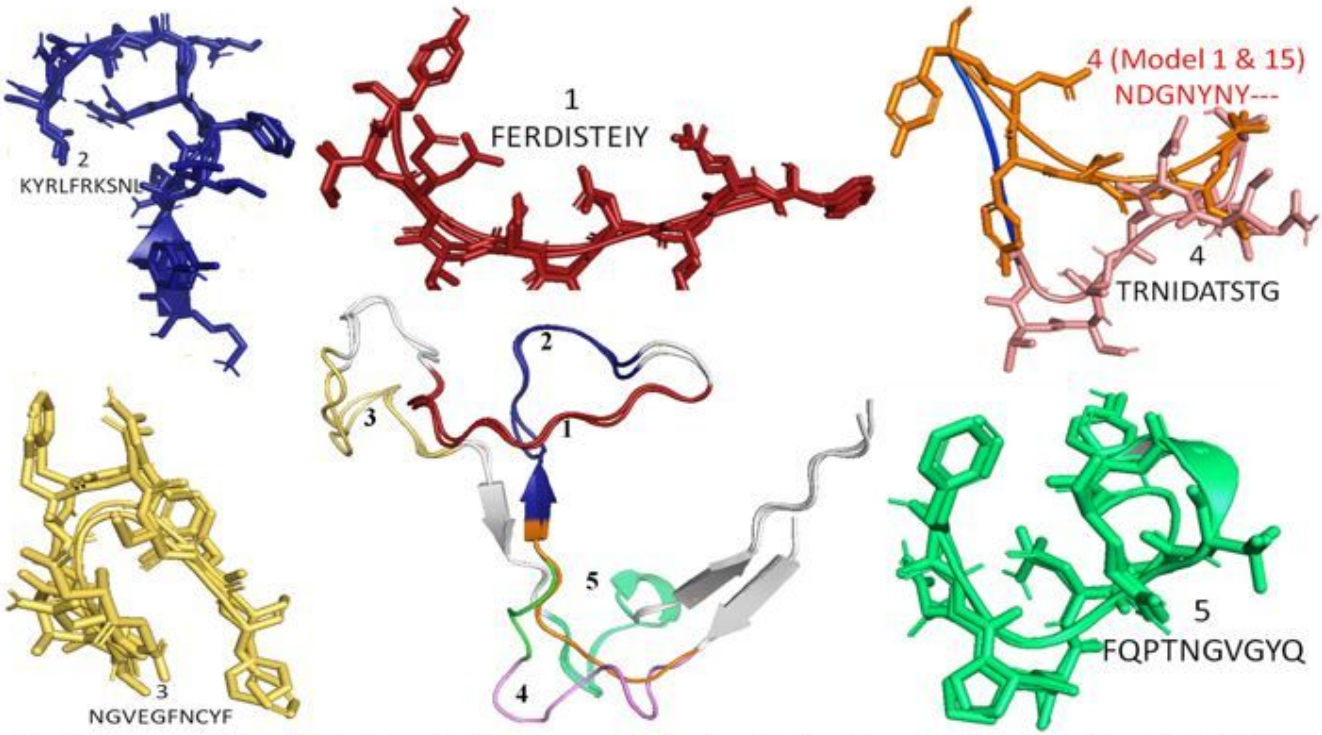


Figure 3

Interaction of different selected CUT4 mutant (SM3, DM2 and TM2) with T cell receptors. SM3 interacted with 1kgc and 4jrx both directly and through H-bond interaction (a, b). DM2 showed highest interactions with 1kgc (c). TM2 interacted with 4 different T cell receptors like 1kgc, 4jrx, 2ak4 and 3kxf. These results indicated that different mutant cuts could be represented by different T cell receptors.



Model 1: representative of Estonia, Latvia, Hong kong, Costarica, Iran, Mexico, Mongolia, Japan, Italy, Egypt, Ireland, Denmark, Germany, France, India, DRC, Serbia, Pakistan , England and Wuhan (wild type)

Model 15: representative of Finland

Rank	Location	Epitope	Score	Antigenicity	Remarks	Model 1 (Representative of most of the countries)	Model 15 (Finland)
1	32 - 41	FERDISTEIY	1.000	**-0.7585**	Probable Non antigen	FERDISTEIY	FERDISTEIY
2	20 - 29	KYRLFRKSNL	0.844	**-0.2129**	Probable Non antigen	-YRLFRKSNL	-YRLFRKSNL
3	49 - 58	NGVEGFNCYF	0.608	**0.6268**	Probable antigen	NGVEGFNCYF	NGVEGFNCYF
4	6 - 15	TRNIDATSTG	0.595	**1.2404**	Probable antigen	NDGNYNY---	NDGNYNY---
5	65 - 74	FQPTNGVGYQ	0.593	**0.4180**	Probable antigen	FQPTNGVGYQ	FQPTNGVGYQ

Figure 4

Epitope pattern of Receptor Binding Domain (RBD) analysis within various nCOV2 spike proteins found in 186 countries worldwide.

Supplementary Files

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- Supplimentarymaterials.docx