RESEARCH ARTICLE

REVISED *In silico* identification of vaccine targets for 2019-nCoV

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Abstract

Background: The newly identified coronavirus known as 2019-nCoV has posed a serious global health threat. According to the latest report (18-February-2020), it has infected more than 72,000 people globally and led to deaths of more than 1,016 people in China.

Methods: The 2019 novel coronavirus proteome was aligned to a curated database of viral immunogenic peptides. The immunogenicity of detected peptides and their binding potential to HLA alleles was predicted by immunogenicity predictive models and NetMHCpan 4.0.

Results: We report *in silico* identification of a comprehensive list of immunogenic peptides that can be used as potential targets for 2019 novel coronavirus (2019-nCoV) vaccine development. First, we found 28 nCoV peptides identical to Severe acute respiratory syndrome-related coronavirus (SARS CoV) that have previously been characterized immunogenic by T cell assays. Second, we identified 48 nCoV peptides having a high degree of similarity with immunogenic peptides deposited in The Immune Epitope Database (IEDB). Lastly, we conducted a *de novo* search of 2019-nCoV 9-mer peptides that i) bind to common HLA alleles in Chinese and European population and ii) have T Cell Receptor (TCR) recognition potential by positional weight matrices and a recently developed immunogenicity algorithm, iPred, and identified in total 63 peptides with a high immunogenicity potential.

Conclusions: Given the limited time and resources to develop vaccine and treatments for 2019-nCoV, our work provides a shortlist of candidates for experimental validation and thus can accelerate development pipeline.

Keywords

Coronavirus, adaptive immunity, immunogenicity, T cell cross-reactivity, vaccine development

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Any further responses from the reviewers can be found at the end of the article

Introduction

The emergence and rapid spread of the recent novel coronavirus known as 2019-nCoV has posed a serious global health threat¹ and has already caused a huge financial burden². It has further challenged the scientific and industrial community for quick control practices, and equally importantly to develop effective vaccines to prevent its recurrence. In facing a rapid epidemical outbreak to a novel and unknown pathogen, a key bottleneck for a proper and deep investigation, which is fundamental for vaccine development, is the limited -- to almost no -- access of the scientific community to samples from infected subjects. As such, *in silico* predictions of targets for vaccines are of high importance and can serve as a guidance to medical and experimental experts for the best and timely use of the limited resources.

In this regard, we report our recent effort to computationally identify immunogenic and/or cross-reactive peptides from 2019-nCoV. We provide a detailed screen of candidate peptides based on comparison with immunogenic peptides deposited in the Immune Epitope Database and Analysis Resource (IEDB) database including those derived from Severe acute respiratory syndrome-related coronavirus (SARS CoV) along with *de novo* prediction from 2019-nCoV 9-mer peptides. Here, we found i) 28 SARS-derived peptides having exact matches in 2019-nCoV proteome previously characterized to be immunogenic by *in vitro* T cell assays, ii) 22 nCoV peptides having a high sequence similarity with immunogenic peptides but with a greater predicted immunogenicity score, and iii) 44 + 19 nCoV peptides predicted to be immunogenic by the iPred algorithm and IG4 TCR positional weight matrices respectively.

Results

Identification of 28 exact matches to SARS CoV immunogenic peptides by screening all epitopes deposited in IEDB

We collected all peptides in IEDB (3, as of 13-02-2020) reported positive in T cell assays and have human as the host organism. We then conducted a local sequence alignment of 10 2019-nCoV open reading frames (ORFs) against 35,225 IEDB peptides, and found 28 exact matches. Surprisingly, all identical hits (against peptides having sequence length greater than 3) were from SARS-CoV (Table 1, Data Table 1⁴). These peptides have been shown to bind various HLA alleles, although with higher tendency towards HLA-A:02:01, from both class I and class II, and can be target for CD8+ and CD4+ T cells respectively.

Identification of 22 2019-nCoV peptides with high degree of similarity to previously reported immunogenic viral peptides

In addition to 28 identical hits against SARS CoV, we observed a long tail in distribution of normalized alignment scores between 10 2019-nCoV ORFs and 35,225 IEDB peptides (Figure 1A, Methods). We therefore set out to further investigate potential vaccine targets among highly similar sequences.

The peptides having an exact sequence alignment with epitopes in IEDB had normalized alignment scores ranging from 4 to 6. Taking the normalized alignment score of exact matches as a reference, we extracted 2019-nCoV peptides having score greater or equal to 4. As illustrated in Figure 1A, we observed 45 and 11 peptides having normalized alignment score ≥ 4 and ≥ 5 respectively (Figure 1A inset). The target peptides were originated from 10 different sources (Figure 1B) where a total 36 peptides were derived from strains associated to SARS CoV. Of interest, we also observed 7 hits having high sequence similarity to targets from *Homo sapiens*.

In order to investigate the extent to which the difference between the source (2019-nCoV) and target (IEDB) peptides influences the immunogenicity of the source peptides we used a recently published immunogenicity model⁵ to predict and compare the immunogenicity between the source and target peptides (Data Table 2⁴).

We could see a similar (close to identical) immunogenicity scores for a number of IEDB and 2019-nCoV peptides especially for those with high immunogenicity scores (Figure 2). While all 48 can be potential targets, of particular interest were those having higher immunogenicity score than IEDB peptides. Here, we list 22 out of 48 2019-nCoV peptides that scored higher compared to their targets that have been characterized to be immunogenic (Table 2). In this list 15 (68%) 2019-nCoV peptides have a score higher than 0.5 whereas only 11(50%) of IEDB get a score immunogenicity score greater than 0.5.

It is worth noting that in general predicting immunogenicity of given a peptide is challenging and not a fully solved problem, and therefore current models for predicting immunogenicity are suboptimal. iPred is also not an exception. In fact, we could see that a substantial number of IEDB immunogenic peptides were scored < 0.5 (the threshold score used to classify immunogenic vs non-immunogenic). This led us to ask whether we can gather any other evidence of either immunogenicity or cross-reactivity.

De novo search of immunogenic peptides in 2019-nCoV proteome

As a complementary reciprocal approach, we conducted a *de novo* search of immunogenic peptides against the 2019-nCoV proteome sequence. We scanned 9-mers from 2019-nCoV proteome with a window of 9 amino acids and step length of 1 amino acid (9613 in total). The immunogenicity of 9-mer peptides were predicted using iPred and MHC presenta-

Table 1. 28 2019-nCoV peptides having exact matches with immunogenic SARS-CoV peptides.

IEDB.peptide	2019-nCoV.pattern	Antigen.Name	Allele.Name
TLACFVLA AV	TLACFVLA AV	Membrane glycoprotein	HLA-A*02:01
AFFGMSRIGMEVTPSGTW	AFFGMSRIGMEVTPSGTW	N protein	
ALNTPKDHI	ALNTPKDHI	Nucleoprotein	HLA-A*02:01
AQFAPSASAFFGMSR	AQFAPSASAFFGMSR	nucleocapsid protein	HLA class II
AQFAPSASAFFGMSRIGM	AQFAPSASAFFGMSRIGM	N protein	
GMSRIGMEV	GMSRIGMEV	Nucleoprotein	HLA-A*02:01
ILLNKHIDA	ILLNKHIDA	Nucleoprotein	HLA-A*02:01
IRQGTDYKHW PQIAQFA	IRQGTDYKHW PQIAQFA	N protein	
KHW PQIAQFAPSASAFF	KHW PQIAQFAPSASAFF	N protein	
LALLLLDRL	LALLLLDRL	Nucleoprotein	HLA-A*02:01
LLLDRLNQL	LLLDRLNQL	Nucleoprotein	HLA-A*02:01
LLNKHIDAYKTFPPTEPK	LLNKHIDAYKTFPPTEPK	N protein	
LQLPQGTTL	LQLPQGTTL	Nucleoprotein	HLA-A*02:01
RRPQGLPNNTASWFT	RRPQGLPNNTASWFT	nucleocapsid protein	HLA class I
YKTFPPTEPKDKKKK	YKTFPPTEPKDKKKK	N protein	
ILLNKHID	ILLNKHID	Nucleoprotein	HLA-A*02:01
MEVTPSGTWL	MEVTPSGTWL	nucleocapsid protein	HLA-B*40:01
ALNTLVKQL	ALNTLVKQL	S protein	HLA-A*02:01
FIAGLIAIV	FIAGLIAIV	Spike glycoprotein precursor	HLA-A2
LITGRLQSL	LITGRLQSL	Spike glycoprotein precursor	HLA-A2
NLNESLIDL	NLNESLIDL	S protein	HLA-A*02:01
QALNTLVKQLSSNFGAI	QALNTLVKQLSSNFGAI	S protein	HLA-DRB1*04:01
RLNEVAKNL	RLNEVAKNL	Spike glycoprotein precursor	HLA-A*02:01
VLNDILSRL	VLNDILSRL	S protein	HLA-A*02:01
VVFLHVTYV	VVFLHVTYV	Spike glycoprotein precursor	HLA-A*02:01
GAALQIPFAMQMAYRF	GAALQIPFAMQMAYRF	S protein	HLA-DRA*01:01/DRB1*07:01
MAYRFNGIGVTQNVLY	MAYRFNGIGVTQNVLY	S protein	HLA-DRB1*04:01
QLIRAAEIRASANLAATK	QLIRAAEIRASANLAATK	S protein	HLA-DRB1*04:01

*SARS-CoV: Severe acute respiratory syndrome coronavirus

Figure 1. 2019-nCoV peptides with high sequence similarity to immunogenic peptides in IEDB. A. Comparison of normalized sequence alignment score for peptides with exact and non-exact matches. **B.** Number of target peptides grouped by their source organism.

Figure 2. Predicted immunogenicity for IEDB immunogenic vs. 2019-nCoV peptides. 2019-nCoV peptides having a high sequence similarity to immunogenic peptides and their targets were analysed for their immunogenicity potential by iPred algorithm.

Table 2. List of 22 2019-nCoV peptides having a higher predicted immunogenicity score than their target peptides.

IEDB.peptide	2019-nCoV.pattern	IEDB.prob	nCol.prob
WYMWLGARY	WYIWLK	0.999249	0.999441
GLMWLSYFV	GLMWLSYFI	0.995073	0.998216
GLVFLCLQY	GIVFMCVEY	0.98123	0.984127
TWLTTHGAIKLDDKDPQFKDNVILL	TWLTTHGAIKLDDKDPNFKDQVILL	0.925862	0.975242
IGMEVTPSGTWLTYH	IGMEVTPSGTWLTY	0.903518	0.919184
GETALALLLLDRLNQ	GDAALALLLLDRLNQ	0.853114	0.900655
TPSGTWLTYHGAIKL	TPSGTWLTYTGAIKL	0.620894	0.662417
SIVAYTMSL	SIIAYTMSL	0.589694	0.693763
RRPQGLPNNIASWFT	RRPQGLPNNIASWFT	0.533253	0.584355
YNLKW	YNL-WN	0.520244	0.765309
AGCLIGAHEVDTSYECDI	AGCLIGAHEVNNSYECDI	0.503905	0.56813
GFMKQYGECLGDINARDL	GFIKQYGDCLGDIAARDL	0.471939	0.506817
ANKEGIVWVATEGAL	ANKDGIWVATEGAL	0.367723	0.404796
WNPDDY	WNADLY	0.355018	0.584726
PDDYGG	PDDFTG	0.334887	0.527287
TWLTTHGAIKLDDKDPQF	TWLTTHGAIKLDDKDPNF	0.27017	0.529675
DEVNQI	DEVRI	0.18504	0.187797
SSKRFQPFQFGRDV	SNKKFLPFQFGRI	0.098384	0.119472
NHSDPAEL	NHTSPDVL	0.067808	0.17889
TKQYNVTQAF	TKAYNVTQAF	0.054818	0.171488
VKQMYKTPTLKYFGGFNF	VKQIYKTPPIKDFGGNF	0.018685	0.135681
QKRTATKQYNVTQAF	QKRTATKAYNVTQAF	0.004891	0.037776

tion scores were gauged using NetMHCpan 4.0⁶ for various HLA types. In this task, we focused on haplotypes common in Chinese and European populations, which include HLA-A*02:01, HLA-A*01:01, HLA-B*07:02, HLA-B*40:01 and HLA-C*07:02 alleles (Data Table 3⁴).

Based on MHC presentation and immunogenicity prediction, we detected 5 peptides predicted to bind 4 different HLA alleles of which 2 had strong immunogenicity scores (Figure 3). For those 65 strong binders to 3 different HLA types, 39 had immunogenicity scores ≥ 0.5 (Table 3). Collectively this analysis suggests a number of 9-mer immunogenic candidates for further experimental validation.

Immunogenicity of 2019-nCoV peptides to 1G4 CD8+ TCR molecule

While our *de novo* candidates are appealing shortlisted targets for experimental validation, it does not provide information about target T cell receptors (TCRs). We therefore set out to interrogate the possibility of cross reactivity with one well-studied TCR.

T cell cross-reactivity has been instrumental for the T cell immunity against both tumor antigens and external pathogens. In that regard, a number of T cells have been extensively characterized including 1G4 CD8+ TCR, which is known to recognize the 'SLLMWITQC' peptide presented by HLA-A*02:01. We therefore set out to leverage the data from a recently

published study⁷ and exploit the possibility of cross reactivity of this TCR to any 2019-nCoV peptide.

Here, we scanned all 9-mers from the 2019-nCoV proteome (9613 peptides) with Binding, Activating and Killing Position Weight Matrices (PWM, see the method section) and associated each peptide with the geometric mean of these three assays as a measure of immunogenicity (Data Table 4⁴). The distributions of binding, activation and killing scores along with their multiplicative score and geometric mean are illustrated in Figure 4. Based on geometric mean, we observed 20 2019-nCoV peptides with a score > 0.8 and 516 peptides > 0.7 . The 9-mer peptides with geometric mean > 0.7 and positive HLA-A*02:01 binding prediction by NetMHCpan 4.0 are listed in Table 4.

We further analysed the MHC binding propensities and gathered peptides not only predicted positive by NetMHCpan but also to have leucine (L) and valine (V) in anchor positions 2 (P2) and 9 (P9) respectively. Previous studies have shown that for MHC-I HLA-A02:01 specific peptides, 9-mer peptides with leucine at P2 and valine at P9 are preferably presented on the surface of HLA-A02:01⁸. Looking at the LV peptide, we identified 44 2019-nCoV peptides of which 2 peptides had immunogenicity score > 0.7 and 12 peptides > 0.6 (Table 5). Thus, here we provide the list of peptides that are potential targets for 1G4 TCR recognition for subjects with HLA-A02:01 haplotype.

Figure 3. De novo search of 9-mer 2019-nCoV peptides with MHC presentation and immunogenicity potential. The MHC binding was predicted for HLA-A*02:01, HLA-A*01:01, HLA-B*07:02, HLA-B*40:01 and HLA-C*07:02 alleles by NetMHCpan 4.0 and immunogenicity was predicted by iPred.

Table 3. 2019-nCoV 9-mer peptides predicted to bind 4 different HLA alleles by NetMHCpan 4.0, and those predicted to bind ≥ 3 HLA alleles and immunogenicity score ≥ 0.9 by iPred. For different alleles, 0 denotes non-binding and 1 denotes binding predicted for specific HLA allele.

Antigen.epitope	Imm.prob	A0101.NB	A0201.NB	B0702.NB	B4001.NB	C0702.NB	Total binding HLA
VQMAPISAM	0.893938	0	1	1	1	1	4
AMYPHTVL	0.862427	0	1	1	1	1	4
TLDSKTQSL	0.254998	1	1	1	0	1	4
KVDGVVQQL	0.191786	1	1	1	0	1	4
KVDGVDVEL	0.18632	1	1	1	0	1	4
MADQAMTQM	0.991227	1	0	1	0	1	3
LEAPFLYLY	0.983072	1	0	0	1	1	3
RTAPGHVM	0.972153	1	0	1	0	1	3
IPFAMQMAY	0.961569	1	0	1	0	1	3
FLTENLLLY	0.951715	1	1	0	0	1	3
YLQPRTFLL	0.947743	1	1	0	0	1	3
MMISAGFSL	0.941318	0	1	1	0	1	3
ATLPGKIMM	0.926603	1	0	1	0	1	3

Figure 4. Distribution of 1G4 TCR positional weight matrix scores for 2019-nCoV peptides. The positional weight matrices were obtained from 7 and 9613 9-mers generated from 10 2019-nCoV ORFs were computed for their TCR recognition potential.

Discussion

In this study we provide a profile of computationally predicted immunogenic peptides from 2019-nCoV for functional validation and potential vaccine developments. We are fully aware that an effective vaccine development will require a very thorough investigation of immune correlates to 2019-nCoV. However, due to the emergency and severity of the outbreak as well as the lack of access to samples from infected subjects,

such approaches would not serve the urgency. Therefore, computational prediction is instrumental for guiding biologists towards a quick and cost-effective solution to prevent the spread and ultimately help eliminate the infection from the individuals.

With a rising global concern of novel coronavirus outbreak, numerous research groups have started to investigate and

Table 4. 2019-nCoV 9-mer peptides with geometric mean ≥ 0.7 by 1G4 TCR positional weight matrix and predicted positive to bind HLA-A*02:01 by NetMHCpan 4.0 (Rank = NetMHCpan rank).

Peptide	Binding score	Activation score	Killing score	geoMean	Rank	Binder
RIMTWLDMV	0.866377428	0.853995	0.776303	0.831249	0.3481	SB
ALNTLVKQL	0.802453741	0.75073	0.785957	0.779413	0.6159	WB
LLLDRLNQL	0.809895414	0.7752	0.741096	0.774888	0.0423	SB
MIAQYTSAL	0.766262499	0.789511	0.749477	0.768242	0.9238	WB
VLSTFISAA	0.799672451	0.756117	0.687278	0.746239	0.536	WB
NVLAWLYAA	0.761297552	0.686117	0.739944	0.728423	1.4457	WB
RLANECQV	0.783161706	0.719705	0.680504	0.726572	0.2049	SB
KLLKSIAAT	0.748896679	0.708996	0.697463	0.718118	1.0923	WB
QLSLPVLQV	0.70128376	0.715259	0.708405	0.708293	0.4768	SB
VQMAPISAM	0.729320768	0.698514	0.689612	0.705612	1.4677	WB
LLLTILTSL	0.7131709	0.715194	0.680064	0.702623	0.2712	SB
SVLLFLAFV	0.736972762	0.690855	0.679534	0.70202	1.1449	WB
LMWLIINLV	0.727847374	0.681119	0.694007	0.700717	1.304	WB

Table 5. 2019-nCoV 9-mer peptides having leucine-valine in anchor positions. Peptides have geometric mean ≥ 0.6 and ≤ 0.7 (for those ≥ 0.7 , refer to Table 4) by 1G4 TCR positional weight matrix and predicted positive for HLA-A*02:01 binding by NetMHCpan 4.0 (Rank = NetMHCpan rank).

Peptide	Binding score	Activation score	Killing score	geoMean	Rank	Binder
TLMNVLTLV	0.723687	0.658986	0.652178	0.677534	0.0444	SB
QLEMELTPV	0.711291	0.651003	0.608605	0.655625	1.6769	WB
MLAKALRKV	0.668756	0.610664	0.65968	0.645854	0.3524	SB
GLFKDCSKV	0.675952	0.632375	0.594753	0.633494	0.2677	SB
ALSKGVHVV	0.652549	0.604952	0.586236	0.613954	0.0425	SB
YLNTLTLLAV	0.624147	0.610826	0.575445	0.603119	0.0453	SB

publish their findings. At the time of preparing this manuscript, we became aware of a similar study conducted in comparing 2019-nCoV proteome with SARS CoV immunogenic peptides⁹. Our *in silico* approach takes the search beyond presenting only common immunogenic peptide between SARS and 2019-nCoV and provides the experimental community with a more comprehensive list including *de novo* and cross reactive candidates. On the other hand, considering the fact that two studies have been accomplished independently with distinct approaches, this serves to demonstrate a high level of confidence in reproducing the results. Reproducibility of computational prediction is always of high importance and becomes even more significant under urgent scenarios as of this outbreak.

Our study also suggests the need for further efforts to develop accurate predictive models and algorithms for the characterization of immunogenic peptides.

In this study, we provide potential immunogenic peptides from 2019-nCoV for vaccine targets that i) have been characterized immunogenic by previous studies on SARS CoV, ii) have high degree of similarity with immunogenic SARS CoV peptides and iii) are predicted immunogenic by combination of NetMHCpan and iPred/1G4 TCR positional weight matrices. Given the limited time and resources, our work serves as a guide to save time and cost for further experimental validation.

Method

Data acquisition

2019-nCoV open reading frame sequences were downloaded from NCBI (MN908947.3). All sequences subjected for analysis are deposited in GitHub repository.

Data analysis

All subsequent analyses have been conducted in R 3.6.1.

Sequence similarity comparison

The sequence similarity between 2019-nCoV open reading frames and previously characterized immunogenic peptides in IEDB was analysed by local alignment using R 'pairwiseAlignment' function from [Biostrings](#) v2.40.2 package. The local alignment utilized BLOSUM62 matrix, gapOpening of 5 and gapExtension of 5. The alignment score was normalized by length of target peptides.

In extracting peptides with the exact sequence alignment with epitope sequences in IEDB, only peptides with more than 3 amino acids in length were shortlisted.

Immunogenicity prediction

We have used [iPred](#)⁵ to predict immunogenicity of each given peptide. Briefly, iPred employs peptides' length and physicochemical properties of amino acids modelled by sums of ten Kidera factors and associates a score to each peptide reflecting its likelihood of recognition by a T cell.

Predicting presentation by MHCs

In order to predict peptide binding to MHC we used [NetMHCpan](#) V4⁶. This version of NetMHCpan that comes with a number of improvements, incorporate both eluted ligand and peptide binding affinity data into a neural network model to predict MHC presentation of each given peptide.

Predicting cross reactivity to 1G4 TCR

To gauge the level of 1G4 TCR cross-reactivity to list of 2019-nCoV virus, we have leveraged the data from a recently published study⁷. 1G4 or NY-ESO-1-specific TCR is a very well-studied and clinically efficacious TCR which recognize the peptide 'SLLMWITQC' presented by HLA-A*02:01. Karapetyan *et al.* have recently provided data from three experimental assays reflecting Binding, Activating and Killing upon each mutation at each position of all possible 9-mers using these three datasets. In a similar way to the original paper, we trained three Position Weight Matrices named B, A and K respectively from Binding, Activating and Killing assay. We defined the cross-reactivity score of a given 9-mer sequence as the geometric mean of B, A and K.

We then scanned 2019-nCoV virus protein sequence with each of B, A and K PWMs and associated each of 9613 9-mers with a cross reactivity score. At the same we utilized NetMHCpan and associated each 9-mer with its presentation score. Our final list of cross-reactive candidate peptides were those with a cross-reactivity score ≥ 0.8 and reported as strong binders from NetMHCpan and have 'L' and 'V' amino acids at anchor positions. The custom R codes are accessible from GitHub repository (see software availability⁴).

Software availability

Replication code: <https://github.com/ChloeHJ/Vaccine-target-for-2019-nCoV.git>

Archived source code at time of publication: <http://doi.org/10.5281/zenodo.3676908>⁴

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Data availability

Source data

2019-nCoV open reading frame sequences were downloaded from NCBI ([MN908947.3](#)).

Underlying data

Zenodo: *In silico* identification of vaccine targets for 2019-nCoV (Data tables). <http://doi.org/10.5281/zenodo.3676886>¹⁰

This project contains the following underlying data:

- Table1 nCoV peptides having exact match with immunogenic SARS CoV peptides.xlsx (Table of nCoV peptides having exact match with immunogenic SARS CoV peptides)
- Table2 nCoV peptides with high sequence similarity with immunogenic IEDB peptides.csv (Table of peptides with high sequence similarity with immunogenic IEDB peptides)
- Table3 *de novo* search on 9-mer nCoV for immunogenic peptides by NetMHCpan and iPred.csv (Table of results of *de novo* search on 9-mer nCoV for immunogenic peptides by NetMHCpan and iPred)
- Table4 *de novo* search on 9-mer nCoV for immunogenic peptides by NetMHCpan and PWM.xlsx (Table of results of *de novo* search on 9-mer nCoV for immunogenic peptides by NetMHCpan and PWM)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

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[Publisher Full Text](#)
10. Lee CH, Koohy H: **In silico identification of vaccine targets for 2019-nCoV (Data tables).** [Data set]. *Zenodo.* 2020.
<http://www.doi.org/10.5281/zenodo.3676886>

Open Peer Review

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Version 2

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The authors present a list of potential 2019-nCoV vaccine candidates based on an *in silico* prediction approach. Overall, they present 28 2019-nCoV-derived peptides that exactly match epitopes of SARS-CoV, and additional 48 2019-nCoV peptides that overlap with already known immunogenic epitopes reported in IEDB. The latter have been evaluated using immunogenicity prediction resulting in 22 2019-nCoV peptides with higher immunogenicity scores than their IEDB counterpart. Additional analysis of encoded 2019-nCoV peptides using HLA binding likelihood and immunogenicity scores predicted by NetMHCpan 4.0 and iPred, respectively, showed 13 additional vaccine candidates regarding prevalent European and Asian HLA-types. Finally, the authors modeled possible cross-reactivity of a well described HLA-A*02:01-NY-ESO-1-peptide-reactive TCR to 2019-nCoV peptides with predicted HLA binding affinity and found 19 hits. With this, authors provide a shortlist of potential candidates for development of a 2019-nCoV vaccine applicable to a broad population.

The paper would benefit from clearer labelling of the figures and tables, and more extensive description of shown results in the figure legends. Column titles should be consistent among tables and figures, and ideally not consist of the internally used abbreviations but be self-explanatory.

In Figure 1A, the axis labeling (Counts and Alignment Score) seems to be switched. 1B: Adding a note that these are non-exact peptides with alignment score ≥ 4 would be helpful. In Figure 2, 45

peptides are shown, but 48 peptides are mentioned in the text. Please clarify.

General remarks: It would be better to use “encoded proteins” than the term “proteome”, as the data derives from 10 ORF sequences. The authors should indicate in the methods the employed thresholds of the used predictors to label a peptide a binder or immunogenic. These thresholds could also be shown in the figures where appropriate. As iPred only uses MHC binding likelihood and amino acid qualities to predict peptide presentation which is not very different from general NetMHCpan approach (which is also implemented in this tool), the authors should discuss why they used iPred instead of well described tools such as NetTepi or NetCTL and NetCTLpan. It does also not come across why an anti-NY-ESO-1 TCR should be cross-reactive to any 2019-nCoV peptide?

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Therapeutic cancer vaccination; T cell epitope prediction, identification and validation; Immunopeptidomics

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 31 March 2020

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Raffaele De Palma

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The Authors use an *in silico* approach to identify antigenic peptides derived from 2019-nCoV. First, they screened ten open reading frame of 2019-nCoV sequence used the IEDB database, finding a series of peptides potentially immunogenic. The first piece of data relies on the identification of 28 peptides that were exactly matching SARS CoV peptides. Moreover, using combinatory approaches, modelling HLA and TCR binding, they identified 13 peptides potentially able to bind a given TCR in HLA A2 restriction fashion, and cutting a list of peptides able to bind several HLA alleles and characterizing several peptides that may be de novo candidates or crossreactive peptides to be used either to study immune response to 2019-nCoV or to set a vaccine.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: T cell responses in Health and Diseases. Modulation of Immune Responses. Clinical Immunology. Vaccines

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 05 March 2020

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Katalin A. Wilkinson

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This is an important manuscript identifying potential vaccine targets for 2019-nCoV, using computational prediction. In the absence of patient samples, such approaches are instrumental to guide towards quick and efficient identification of vaccine candidates. The authors used 10 open reading frame sequences of 2019-nCoV deposited at NCBI and conducted sequence alignment against immunogenic peptides deposited in the Immune Epitope Database and Analysis Resource (IEDB) database. They identified 28 peptides with sequences matching exactly to severe acute respiratory syndrome-related coronavirus (SARS CoV), that have previously been characterised as immunogenic by T cell assays. These findings are very promising and have the added benefit of potentially developing a vaccine against both SARS and COVID-19.

Additional peptides were identified to most likely bind common Chinese and European HLA alleles and have high immunogenicity potential. The authors provide a shortlist of peptides as potential vaccine candidates. While this manuscript presents a good model for identifying such targets, a comment should be included in the discussion about the necessity of expanding the analysis to include wider HLA allele types, considering that the virus is likely to spread worldwide.

Minor comment: Please explain the methods and analysis in greater details, including the following terms:

1. 'Towards target peptide length > 3'.
2. 'Normalized alignment scores' (and their scale, such as the significance of 4 for the data presented in Figure 1).
3. The importance of leucine (L) and valine (V) in anchor positions for MHC binding (for data presented in Table 5).

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: T cell immunology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 07 Apr 2020

Hashem Koohy, Medical Research Council (MRC) Human Immunology Unit, MRC Weatherall Institute of Molecular Medicine (WIMM), John Radcliffe Hospital, University of Oxford, Oxford, UK, Oxford, UK

Dear Prof Wilkinson,
Thank you so much for your time and valuable suggestions. In our new and revised version we have taken your suggestions into consideration.

Best regards
Hashem Koohy

Competing Interests: No competing interests were disclosed.

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