Identification of SARS-CoV-2 T-cell Epitopes for Assessing T-cell Immunity

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1 Challenges for selecting T-cell epitopes against SARS-CoV-2

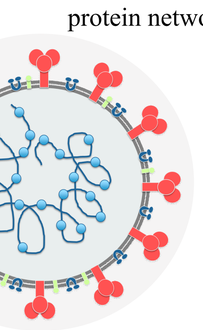
- Recombination between coronaviruses is common.
- The number of mutations within SARS-CoV-2 genome is increasing.
- Human Leukocyte Antigen (HLA) alleles are polymorphic.
- Cellular immunity against SARS-CoV-2 is cross-reactive to seasonal human coronaviruses that cause the common cold.

2 Aims

- Design a novel immunoinformatics analysis pipeline that selects for immunogenic peptides from non-variable and topologically important (i.e. highly networked) protein regions.
- Monitor genetic mutations within Spike and Nucleocapsid protein sequences and determine if the immunogenic peptides selected by our immunoinformatics analysis pipeline avoid these mutations.

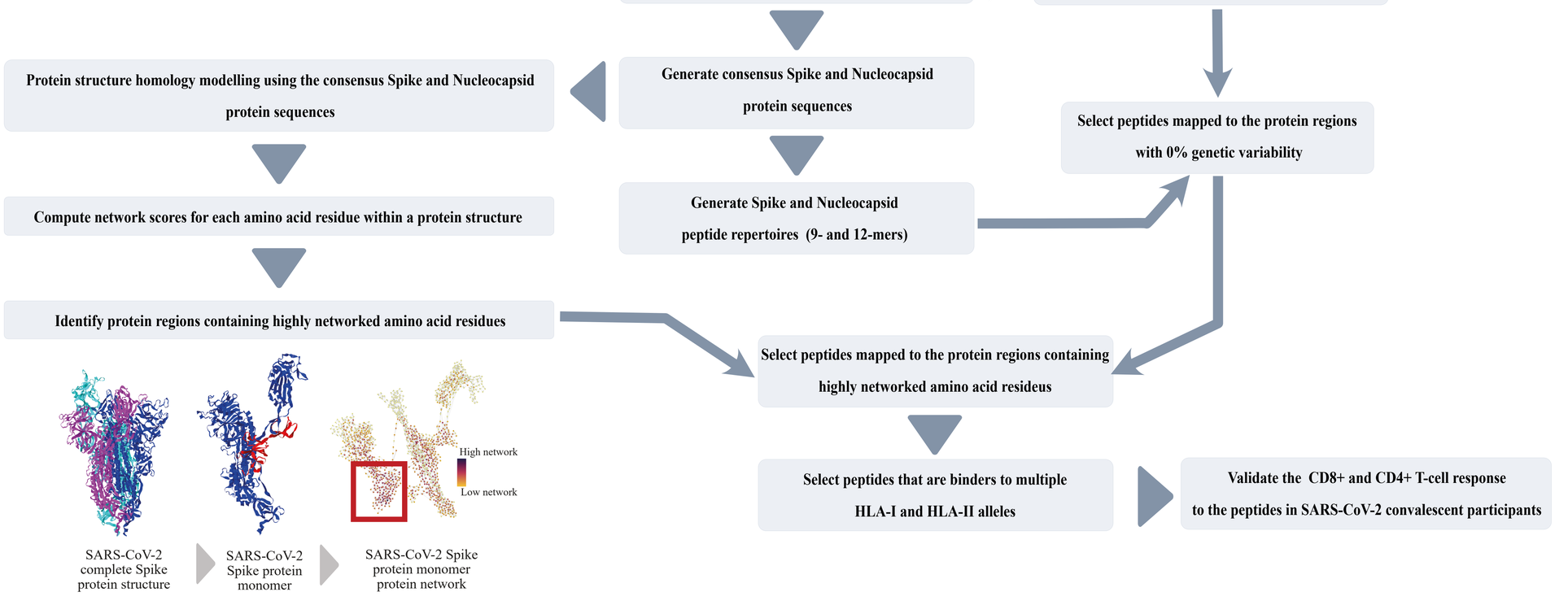
3 New immunoinformatics analysis pipeline for T-cell epitope selection

- The regions comprised of "highly networked" amino acid residues are topologically important for the maintenance of tertiary and quaternary viral protein structures.
- In human immunodeficiency virus (HIV)-infected individuals with diverse HLA class I alleles, targeting epitopes from these topologically important (i.e. highly networked) regions with cytotoxic T-cells provided virologic control (Gaiha et al., 2019).
- Therefore, out immunoinformatics analysis pipeline integrated open-access databases/tools with protein network analysis.



- Our target SARS-CoV-2 proteins for selecting highly networked T-cell epitope derived peptides are Spike and Nucleocapsid.

Spike
Nucleocapsid



4 Highly networked T-cell epitope derived peptides for SARS-CoV-2 specific T-cell immunity assays

- We have identified a total of 57 highly networked T-cell epitope derived peptides from Spike and Nucleocapsid proteins.
- The peptides identified from Spike protein avoid mutations that enhance viral infectivity (Lee et al., 2021).
- Of these, 18 peptides have limited genetic homology to seasonal human coronaviruses making them promising candidates for SARS-CoV-2 specific T-cell immunity assays.

Peptides derived from highly networked protein regions

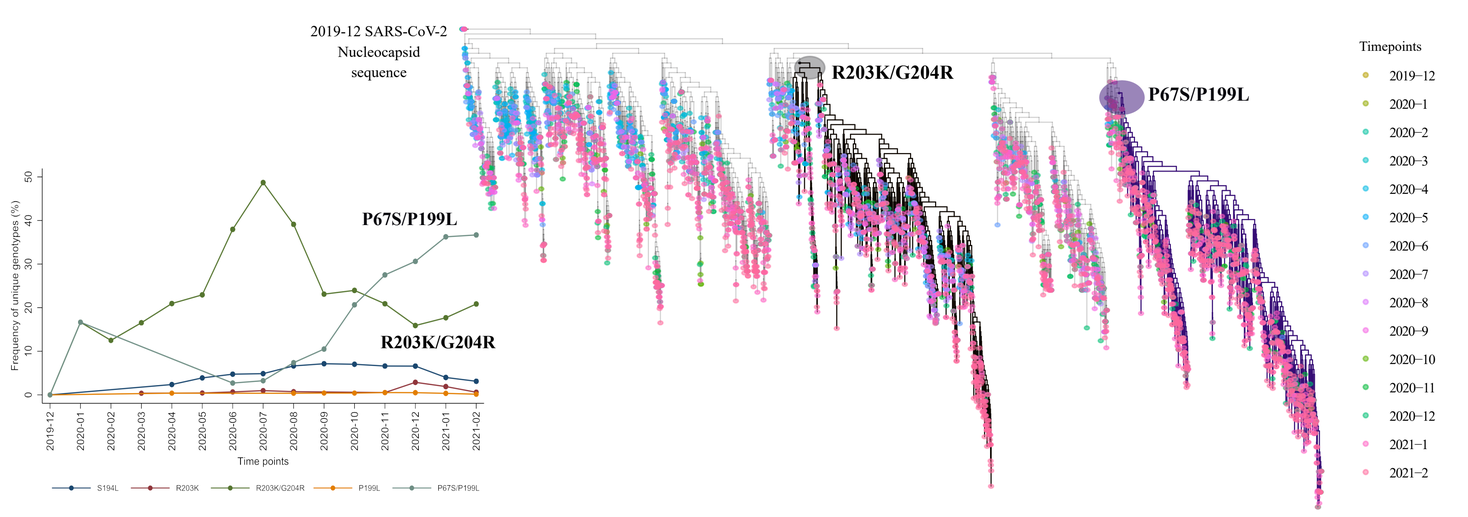
9-mers				12-mers			
NC N-terminal	NTASWFTAL			NC N-terminal	QIGYYRRATRRI	■	
NC N-terminal	TASWFTALT			NC N-terminal	IGYYRRATRRIR	■	
NC N-terminal	IIWVATEGA	■		NC N-terminal	GYYRRATRRIRG	■	
NC N-terminal	TTLPKGFYA			NC N-terminal	IIWVATEGALNT	■	
NC C-terminal	RTATKAYNV	■		NC N-terminal	GIIWVATEGALN	■	
NC C-terminal	ILLNKHIDA	■	■	S protein	LFLPFFSNVTWF	■	■
S protein	FLPFFSNVT			S protein	FLPFFSNVTWFH	■	■
S protein	VTWFHAIHV	■	■	S protein	LPFFSNVTWFHA	■	■
S protein	TLDSKTQSL			S protein	PFFSNVTWFHAI	■	■
S protein	FQFCNDPFL	■		S protein	FFSNVTWFHAIH	■	■
S protein	FCNDPFLGV			S protein	FSNVTWFHAIHV	■	■
S protein	PLVDLPIGI			S protein	SNVTWFHAIHVS	■	■
S protein	YLQPRTFLL	■		S protein	NVTWFHAIHVSG	■	■
S protein	AVDCALDPL	■		S protein	VTWFHAIHVSGT	■	■
S protein	FSTFKCYGV	■		S protein	CTFEYVSQPFLM	■	■
S protein	NVYADSFVI			S protein	YVGYLQPRTFLL	■	
S protein	RVVVLSFEL	■	■	S protein	EKGIYQTSNFRV	■	
S protein	SIIAYTMSL	■	■	S protein	KGIYQTSNFRVQ	■	
S protein	SVTTEILPV	■		S protein	FNFNGLTGTGVL	■	
S protein	LLQYGSFCT	■		S protein	TWRVYSTGSNVF	■	
S protein	QLNRALTGI	■		S protein	WRVYSTGSNVFQ	■	
S protein	KQIYKTPPI			S protein	SNVFQTRAGCLI	■	
S protein	LLFNKVTLA	■		S protein	NVFQTRAGCLIG	■	
S protein	GLTVLPPLL	■		S protein	QSIIAYTMSLGA	■	■
S protein	MIAQYTSAL	■		S protein	SIIAYTMSLGAE	■	■
S protein	ALLAGTITS			S protein	IIAYTMSLGAEN	■	■
S protein	ITSGWTFGA	■		S protein	IAYTMSLGAENS	■	■
S protein	WTFGAGAAL	■		S protein	EMIAQYTSALLA	■	
S protein	LQIPFAMQM	■		S protein	QIPFAMQMAYRF	■	
S protein	QMAYRFNGI	■		S protein	IPFAMQMAYRFN	■	
S protein	VLYENQKLI	■		S protein	PFAMQMAYRFNG	■	
S protein	ALNTLVKQL	■		S protein	FAMQMAYRFNGI	■	
S protein	KQLSSNFGA	■					
S protein	VLNDILSRL	■					
S protein	RLDKVEAEV	■					

■ T-cell epitope derived peptides with top 5% network scores, binding levels to HLA-I and HLA class I mediated antigen processing and immunogenicity scores

■ T-cell epitope derived peptides with top 5% network scores and binding levels to HLA-II

■ T-cell epitope derived peptides with <20% genetic homology to four seasonal human coronaviruses (229E, HKU1, NL63, OC43)

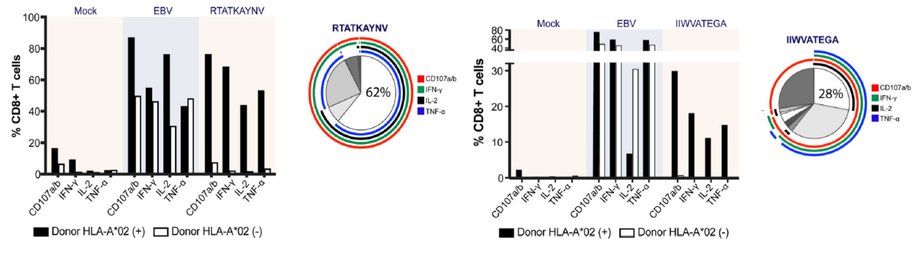
5 A predictive fitness model for Nucleocapsid protein identified two linked mutations which contribute to an increase in the frequency of SARS-CoV-2 variants



- Within Nucleocapsid protein sequences, the predicted fitness model identified P67S/P199L mutation within global SARS-CoV-2 variants sampled from January 2020 to February 2021. The number of Nucleocapsid sequences containing P67S/P199L mutation increased during this period, suggesting that this mutation can enhance viral fitness.
- We also found another mutation, R203K/G204R, within global SARS-CoV-2 variants sampled from January 2020 to February 2021. However, we observed a rapid increase followed by a fast decline in the number of Nucleocapsid sequences containing R203K/G204R mutation from May 2020 to September 2020. This fluctuation in the number of viral variants is possibly due to: 1) immunological control; and/or 2) cross-immunity from previous exposure to other seasonal human coronaviruses that contribute to the persistence of viral variants that contain R203K/G204R mutation.
- None of the highly networked T-cell epitope derived peptides identified from Nucleocapsid contained P67S/P199L and/or R203K/G204R mutations.

6 T-cell epitope derived peptides identified from Nucleocapsid elicit CD8+ T-cell polyfunctional/effector response in SARS-CoV-2 convalescent participants

- When peripheral blood mononuclear cells obtained from participants 1-2 months postrecovery were stimulated by using two Nucleocapsid-derived peptides, we observed robust production of interleukin-2 (IL-2), interferon gamma (IFN-g), tumour necrosis factor alpha (TNF-alpha) and a marker for degranulation of CD8+ T-cells (CD107a/b).
- Importantly, 28-62% of the responding CD8+ T-cells were polyfunctional exhibiting four effector functions simultaneously.



7 Conclusions

- Our immunoinformatics analysis pipeline defined 57 SARS-CoV-2 immunogenic peptides within highly networked (i.e. topologically important regions of Nucleocapsid and Spike proteins that avoid genetic mutations that enhance viral fitness or infectivity.
- Of these, 18 had limited homology to seasonal human coronaviruses and therefore are promising candidates for distinguishing SARS-CoV-2-specific immune response from pre-existing coronavirus immunity.
- Importantly, CD8+ T-cells obtained from COVID-19 survivors exhibited polyfunctional/effector responses to highly networked T-cell epitope derived peptides identified from Nucleocapsid protein, providing a proof of concept that our immunoinformatics analysis pipeline selects novel immunogens which can elicit polyfunctional SARS-CoV-2-specific T-cell response.

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