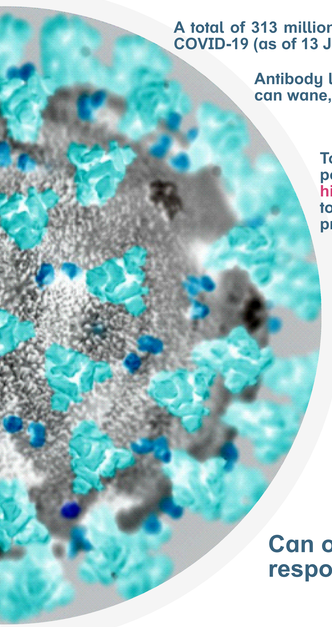
00319

Defining Immunogenic Peptides Targeting SARS-CoV-2 Variants of Concern

Eunok Lee[1,2], Gabriel Duette[1,2], Gabriela Martins Costa Gomes[1], Katie Tungatt[1], Chloe Doyle[1,2], Ashley Lee[1], Vicki V. Stylianou[1,2], Rajiv Khanna[3,4], Kerrie Sandgren[1,2], Anthony L. Cunningham[1,2] and Sarah Palmer[1,2]

[1]Centre for Virus Research, The Westmead Institute for Medical Research, Westmead, New South Wales, Australia; [2]Sydney Medical School, Faculty of Medicine and Health, The University of Sydney, Camperdown, New South Wales, Australia; [3]Faculty of Medicine, The University of Queensland, Brisbane, Queensland, Australia; [4]QIMR Berghofer Centre for Immunotherapy and Vaccine Development, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia

BACKGROUND



A total of 313 million cases for SARS-CoV-2 infection and 5.5 million deaths due to COVID-19 (as of 13 Jan 2022)

Antibody levels within the individuals who have recovered from COVID-19 can wane, however, cellular immunity against SARS-CoV-2 can persist.

To test the longevity of the T-cell immunity against SARS-CoV-2, post COVID-19 recovery and post vaccination, out group selected highly networked T-cell epitope derived peptides within topologically important regions of Spike and Nucleocapsid proteins.

SARS-CoV-2 has been evolving during the COVID-19 pandemic, which contributed to the establishment of Variants of Concern (VOCs):

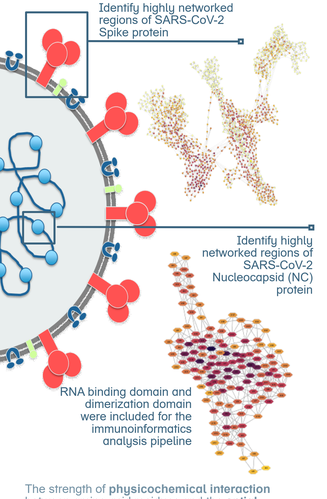
alpha, beta, delta, and omicron

Did our immunoinformatics analysis pipeline correctly identified highly networked T-cell epitope derived peptides that avoid mutations associated with the VOCs?

Can our selected peptides induce T-cell immune responses?

METHODS

PROTEIN NETWORK ANALYSIS → PEPTIDE SELECTION → MUTATIONAL ANALYSIS/ EX VIVO ASSESSEMENT



The strength of **physicochemical interaction** between amino acid residues and the **sptial location** of each residue within a viral protein were quantified as "**network score**".

This **network score** represents **a degree of topological importance** for **each residue** within a protein.

There are **protein regions** that contain many residues with high network scores. The peptides from these protein regions are termed **highly networked peptides**.

DTU netMHCpan and netMHCIIpan to select HLA binders from the highly networked regions

Post analysis for the selected highly networked peptides:
- Population coverage
- HLA-I antigen processing
- HLA-I Immunogenicity

List of peptides that are predicted to be immunogenic

For ≥12-mers:
Determine the regions within the Spike and NCproteins that contain clusters of highly networked T-cell epitope derived peptides

For 9-mers:
Select highly networked T-cell epitope derived peptides that have a broad range of predicted time required for the peptide to dissociate from HLA-I molecules

Select other peptides that were previously characterized for T-cell immune response

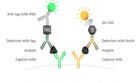
Total of 32 peptides for *ex vivo* assessment

Total of 32 peptides combined for *ex vivo* assessment

GISAID Compare mutations associated with SARS-CoV-2 (VOCs) to the 32 peptides

PBMCs from COVID-19 post-recovery donors (n=23)

Incubate PBMCs and the customized peptide pool



Fluorospot for IFNγ and IL-2 response to the peptide pool

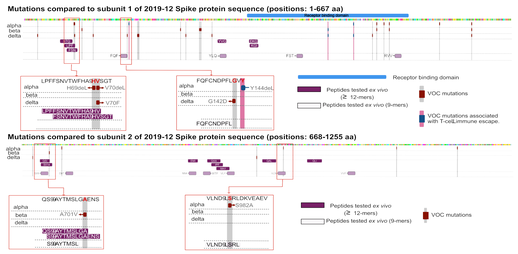
ICS to assess IFNγ, IL-2, TNFα and degranulation marker (CD107a/b) response from CD4 and CD8 T-cells to the peptide pool

RESULT FROM PROTEIN SEQUENCE ANALYSIS

SPIKE

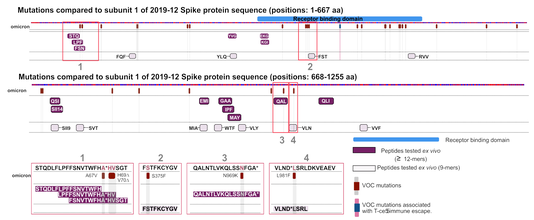
ALPHA, BETA, AND DELTA VOCs

80% OF OUR SELECTED PEPTIDES **avoided** the **mutations** associated with alpha, beta, and delta VOCs.



OMICRON

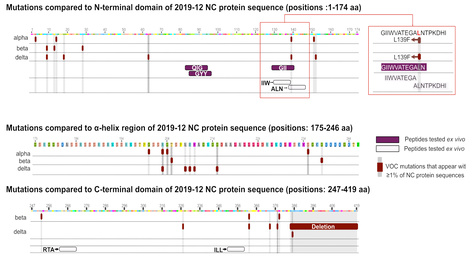
80% OF OUR SELECTED PEPTIDES **avoided** the **mutations** associated with a new VOC, omicron.



NC

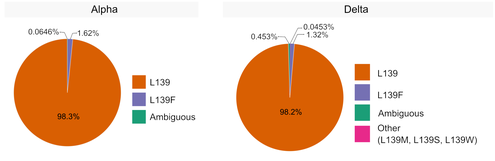
ALPHA, BETA, AND DELTA VOCs

2/7 OF NC-DERIVED PEPTIDES SELECTED FOR *EX VIVO* ASSESSMENT contained the mutation associated with alpha and delta VOCs.



HOWEVER...

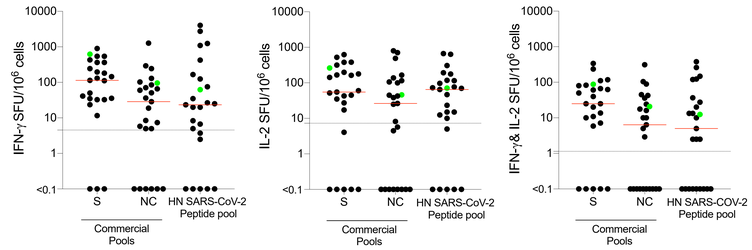
<2% of alpha and delta VOCs contained the mutation, L139F, which was found within the NC-derived peptides.



Omicron VOC contained **mutations at 203 and 204 aa** within the α-helix region of the NC protein.
The α-helix was excluded during an initial stage of the immunoinformatics analysis pipeline.

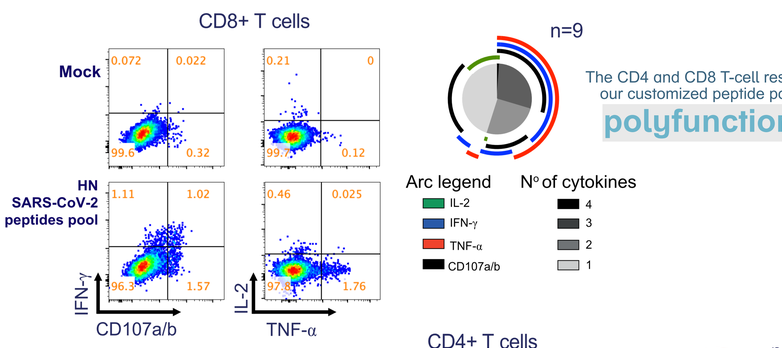
RESULT FROM *EX VIVO* ASSESSMENT

CUSTOMIZED PEPTIDE POOL vs COMMERCIAL

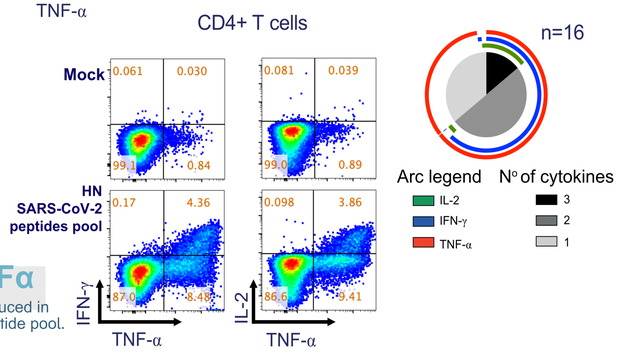


17 out of 23 COVID-19 post recovery donors responded to our customized peptide pool at a **similar degree** to the commercial Spike or NC peptide pool.

CD4 & CD8 T-CELL RESPONSE



The CD4 and CD8 T-cell response to our customized peptide pool was **polyfunctional**.



IFNγ and TNFα were the main cytokines produced in response to the customized peptide pool.

CONCLUSION

Our immunoinformatics analysis pipeline allowed us to select peptides from the Spike and NC proteins which avoid a majority of mutations associated with alpha, beta, delta and omicron SARS-CoV-2 VOCs.

Our customized pepitde pool elicted a polyfunctional T-cell response, making an ideal candidate for assessing the duration of cellular immunity induced by SARS-CoV-2 variants and vaccines.

ACKNOWLEDGEMENTS

We acknowledge with gratitutde the participants of this study.