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Epitope Identification for Vaccine Development SARS-CoV-2 Case Study

The current COVID-19 pandemic, caused by the SARS-CoV-2 virus, has placed an urgent demand on vaccine development, and a number of public and private initiatives are focused on this task. Immunitrack is committed to playing its role in the fight against COVID-19 by applying its technology to support vaccine development and immune monitoring in infected and recovered COVID-19 patients.

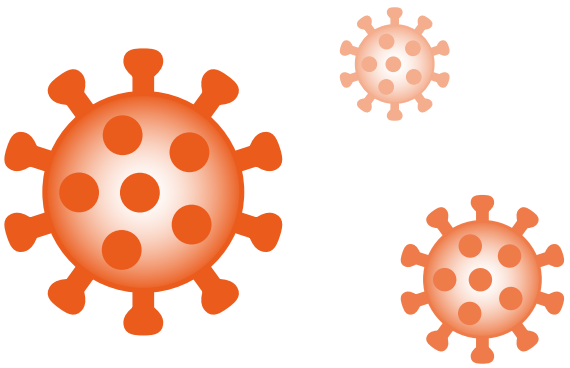
SARS-CoV-2

Most SARS-CoV-2 vaccine development efforts focus on targeting the Spike protein, which is present on the viral surface. Several studies on influenza viruses as well as hepatitis C virus (HCV) and cytomegalovirus (CMV) have demonstrated that effective viral clearance during viral infection and reduction in symptom severity are dependent on proper CD4 and CD8 T cell activation (1-3).

While the CD8 T cell response is important for clearing virally-infected human cells, the presence of CD4 T cells is critical for immune system memory of infection. Thus, we believe that a successful vaccine development strategy against SARS-CoV-2 depends on a strong and lasting activation of both the CD4 and CD8 T cell populations.

Immunitrack is involved a number of projects related to SARS-CoV-2 vaccine development, for example:

- **Novel SARS-CoV-2 Epitope Study:** In March 2020, we performed an initial study to identify novel candidate CD4 and CD8-stimulating epitopes from SARS-CoV-2. The report summarised on Page 3 outlines the rational for our efforts and includes a link to the final dataset as a free download.
- **Spike Epitope Study:** We are currently finalising screening efforts including 1,200 overlapping epitopes from the SARS-CoV-2 Spike protein to identify candidate CD4 and CD8 T cell epitopes against a panel of human and murine MHC alleles (See Table 1 for details).



Human MHC I	Human MHC II	Murine MHC I	Murine MHC II
A*01:01	DRB1*01:01	Kb	IA-b
A*02:01	DRB1*03:01	Db	IA-d
A*03:01	DRB1*04:01	Kd	IE-d
A*11:01	DRB1*11:01	Ld	
A*24:02	DRB1*13:01	Dd	
B*07:02	DRB1*15:01		
B*08:01	DRB3*01:01		
C*04:01	DRB3*02:02		
C*07:01	DRB4*01:01		
C*07:02	DRB5*01:01		
	DPA1*0103/DPB1*0401		

Table 1. 1,200 peptides derived from Spike are currently being analysed for stably binding epitopes to the MHC alleles class I and class II for human and mouse models listed here.

Identify CD4 and CD8 T Cell Epitopes With NeoScreen®

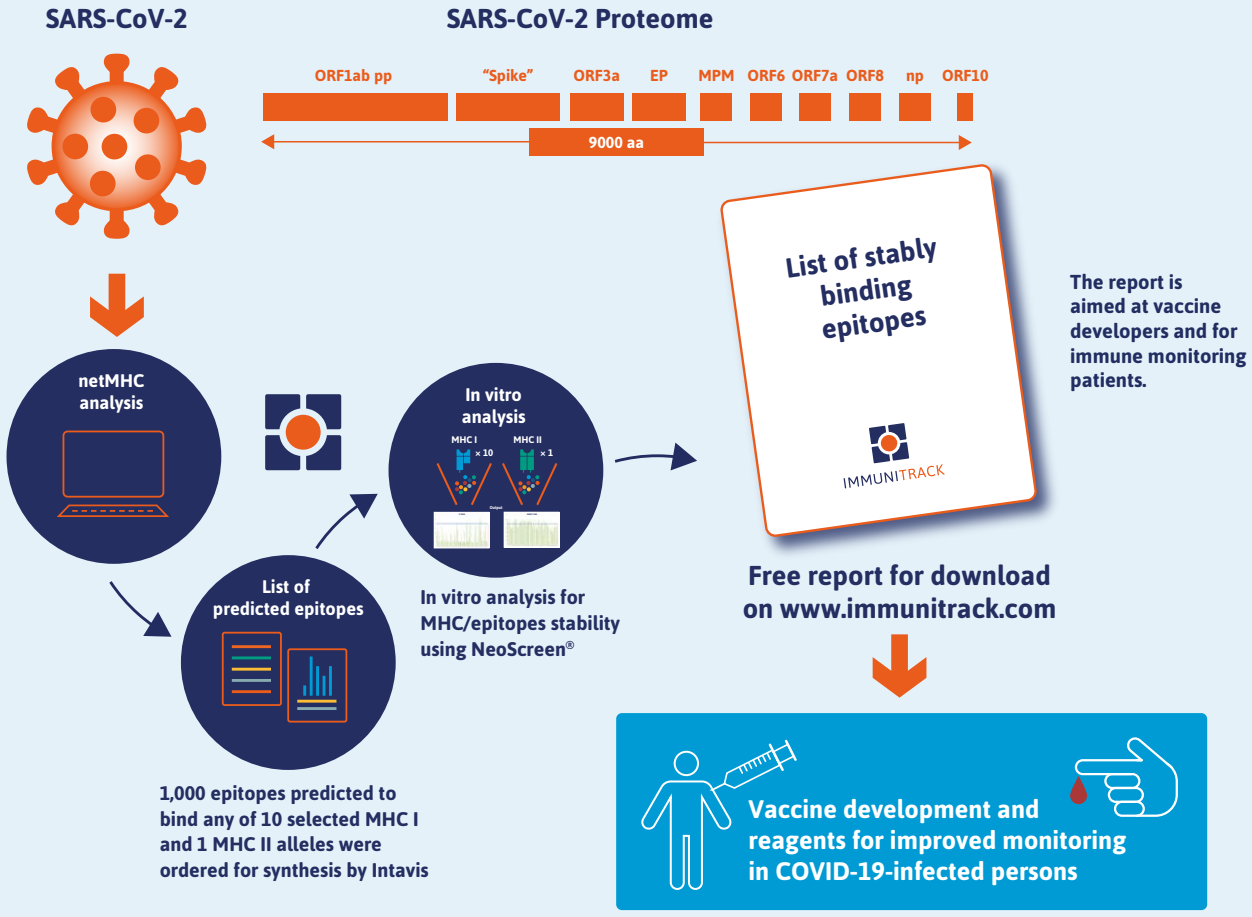
At Immunitrack, we believe that the key to developing powerful vaccines is to combine epitopes that stimulate an antibody response with epitopes that stimulate a cellular response. However, finding out which epitopes lead to highly effective immune responses and are thus worth pursuing for vaccine development is a challenge.

There are a number of epitope prediction algorithms available but these generally only perform well for a subset of Caucasian alleles i.e. these tools are not always reliable for, e.g., MHC-C subtype (HLA-C) and most MHC class II alleles.

The majority of T cell epitopes used in vaccine development are identified through *in silico* prediction platforms that typically incorporate multiple aspects of MHC class-specific affinity. Although widely used, affinity measurements alone may yield false positive predictions, because for any epitope to be truly immunogenic, it must be able to bind a compatible MHC molecule and remain bound for long enough to be presented to and recognised by T cells to elicit an immune response (4-8).

Our NeoScreen® immunogenicity prediction platform **combines affinity and stability assays to eliminate the false positives** that may arise when conducting affinity assays alone. Identifying such false positives early during vaccine development enables optimisation to include immunogenic epitopes, ultimately leading to vaccines with greater efficacy.

Fig. 2 **Report listing epitopes from Coronavirus SARS-CoV-2 likely to stimulate an immune response**



Screening SARS-CoV-2 Epitopes Against Asian MHC Alleles

In collaboration with researchers at the University of Copenhagen, we initially used netMHC to make *in silico* predictions of epitopes presented by 10 MHC I and 1 MHC II alleles covering approx. 90 % of the Asian population. Using this approach, we compiled a list of 100 candidate epitopes for the following MHC alleles: A*0101, A*0201, A*0301, A*11:01 A*2402, B*40:01, C*01:02, C*04:01, C*0701, C*0702, and DRB1*0401, resulting in 1,100 MHC/peptide binding studies.

For this project we partnered with Intavis, who synthesised the SARS-CoV-2 epitopes assessed in this study. Using our unique NeoScreen® technology we conducted in vitro binding studies of the epitopes. Our study identifies 159 epitopes that stably bind MHC I alleles, and 22 that bind the tested MHC II allele (see report and Table 2).

Allele	Number of predicted epitopes with min. 60 % stability
A*0101	14
A*0201	15
A*0301	41
A*1101	49
A*2402	30
B*4001	30
C*0102	3
C*0401	1
C*0701	3
C*0702	3
DRB1*0401	22

Table 2. SARS-CoV-2 Epitopes

Download Study Data

We anticipate that this new data will aid stakeholders in identifying which T cell-stimulating epitopes are worth pursuing in a COVID-19 vaccine. We narrowed down the number of epitopes of interest per allele as listed in Table 1.

Download the report containing all stability data obtained for these epitopes.

The stability cutoff was 60 % of a reference peptide. Please consult the report for further explanation.

The report can be downloaded here:
https://bit.ly/30PzMa5

References

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NeoScreen®

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www.immunitrack.com/vaccines/covid19-vaccine-efforts

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About Immunitrack
Immunitrack is founded upon world-leading research on MHC-epitope binding. Our proprietary epitope screening platform NeoScreen® measures the affinity and stability of MHC/epitope interactions, with capacity to rapidly screen libraries with thousands of (neo-)epitopes for applications within immuno-oncology, vaccine production, T cell therapies and immune monitoring.

Immunitrack's mission is to provide the pharmaceutical industry and research community with technology and reagents to select or redesign drug candidates during early R&D and to monitor the effects of lead drug candidates on patient immune responses.