A multi target, T-cell epitope peptide vaccine candidate against SARS-CoV-2 and related mutant variants

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Introduction

The ongoing pandemic caused by the SARS-CoV-2 coronavirus has triggered the development of a vast number of vaccine candidates, based on different technology platforms, to combat this unprecedented disease. While neutralizing antibodies are able to block infectious virus entry and lead to protection, the emergence of mutant variants reduces the antibody-mediated immunity elicited by vaccination or infection. The role of cell-mediated immunity is instrumental in addressing this limitation, as T-cells have the capacity to eliminate infected cells and help viral clearance.

We set out to develop a multi-epitope, T-cell peptide-based vaccine with the goal to address all circulating mutants and potentially new emerging variants. Applying immunogenicity prediction tools, we selected a set of epitopes from structural and non-structural viral proteins that are well-conserved and synthesized them using solid-phase peptide synthesis. Using peripheral blood mononuclear cells from infected individuals, we tested the immunogenic potential of said peptides by measuring the IFN-γ response following peptide restimulation. We chose eight of these peptides to design "branched" versions and we conjugated them to a lipopeptidic backbone carrying elements to spontaneously drive the formation of Synthetic Virus-Like Particles (SVLPs), namely a lipid tail and a coiled-coil domain. We characterized the particles using dynamic light scattering and mass spectrometry. Next, we immunized two different mouse strains to determine the particles' efficacy in triggering robust immune responses. Cytokine levels in splenocytes were determined with a cytokine multiplex assay and with ELISPOT, 24h and 48h after peptide restimulation.

These ongoing analyses and further *in vivo* studies will allow us to proceed with the development of a novel T-cell based vaccine candidate. Such a multi epitope-based vaccination approach that relies on a safe, validated in man delivery platform, could be used either to initiate immune responses or boost an existing immune response.

Results

Synthetic Virus-Like Particles for T-cell vaccine delivery

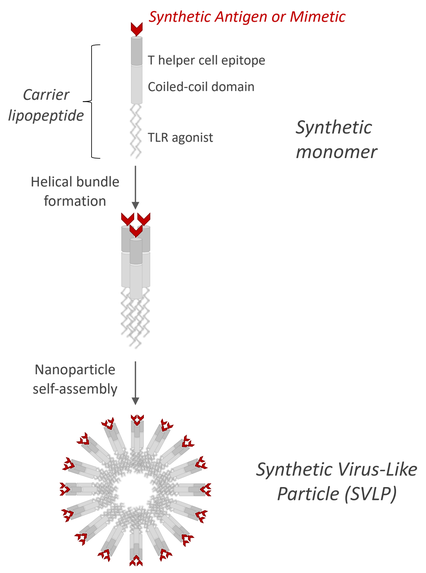


Figure 1. The Virometix SVLP technology platform and its use for the delivery of a SARS-CoV-2 T-cell vaccine

A set of well-conserved T-cell epitopes from structural and non-structural viral proteins were rationally chosen and synthesized using SPPS. Subsequently they were conjugated to a lipopeptidic backbone carrying elements to spontaneously drive the formation of SVLPs (WO 2020/127728 A1).

Incorporation of eight CD8⁺/CD4⁺ epitopes in two different SVLP formulations for animal immunization

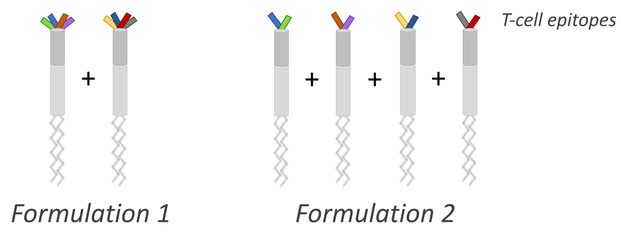


Figure 3. Multi-target T-cell epitope presentation on SVLPs

Two different formulations were tested for the delivery of 8 CD8⁺/CD4⁺ T-cell epitopes. The scheme shows the monomeric units which drive the formation of 2 or 4 different particles. The vaccine candidates were used to immunize BALB/c and C57BI/6 mice with a prime-boost regimen.

Stimulation of PBMC from SARS-CoV-2-seropositive individuals with the universal T-cell epitopes leads to IFN-γ production revealing antigen-specific T-cell responses

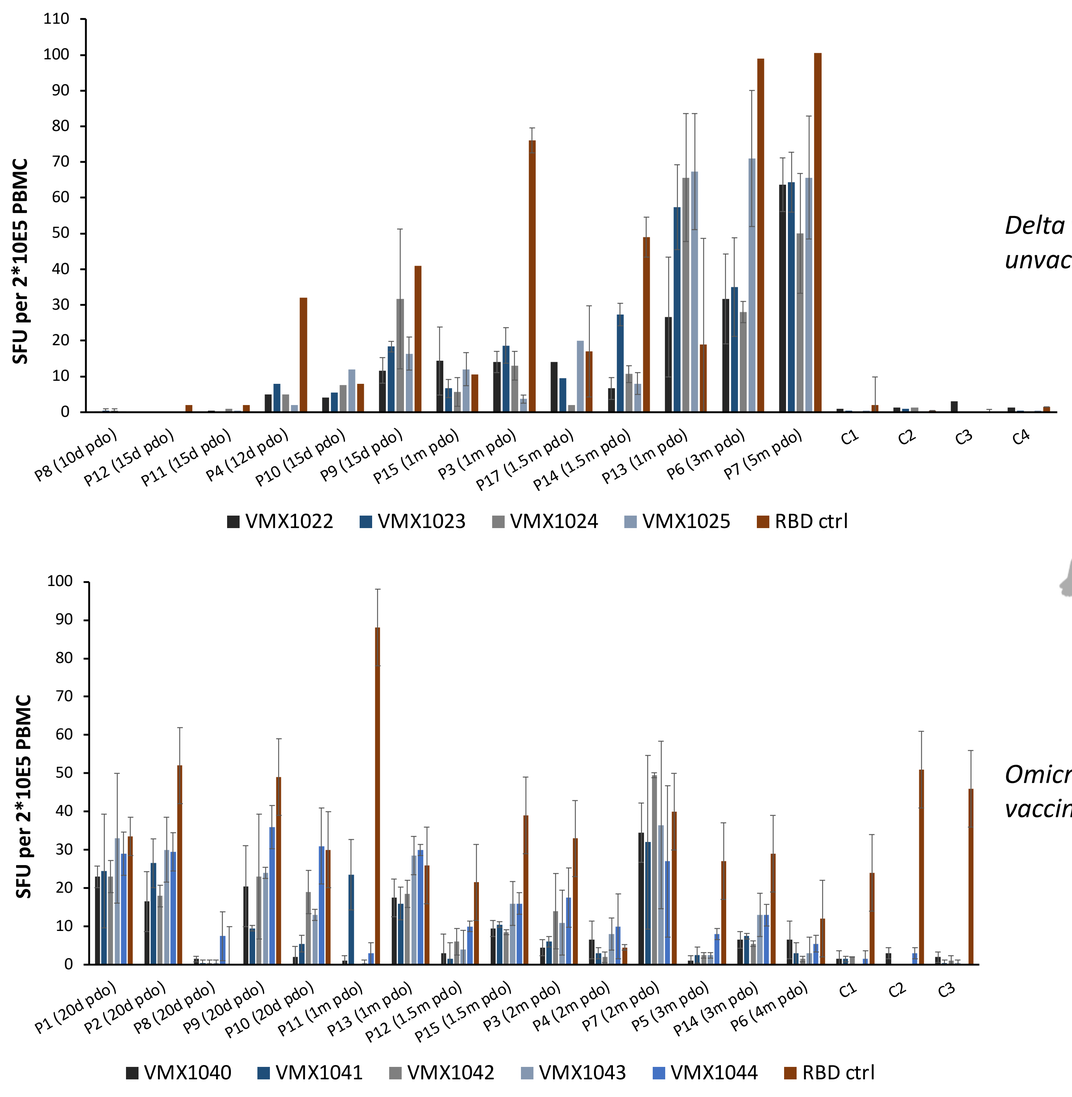
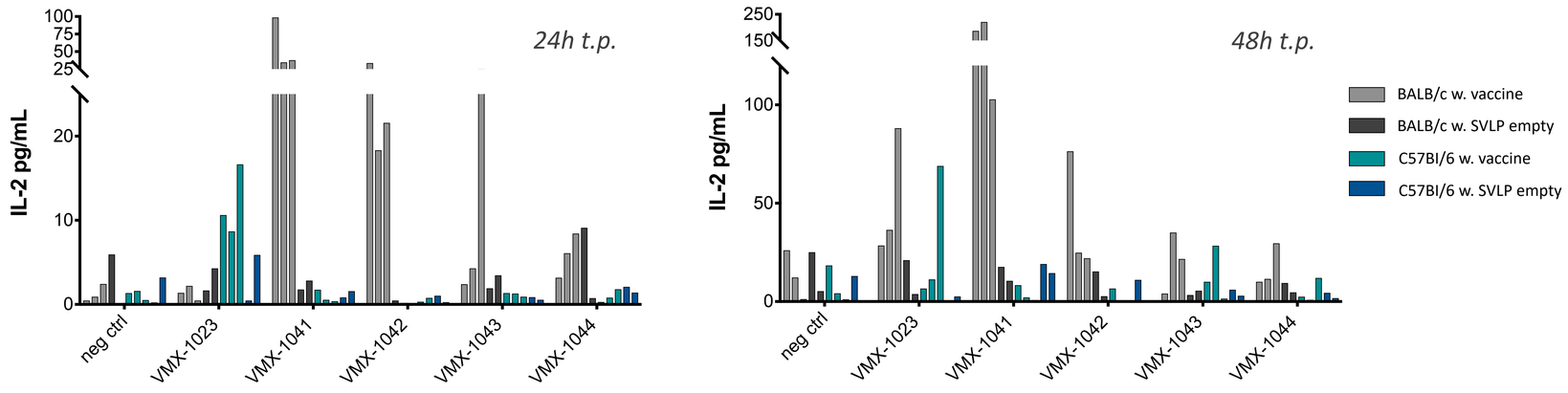


Figure 2. Immunogenic potential of the universal T-cell peptide epitopes

PBMC from infected individuals were restimulated with nine individual peptides and the IFN-γ response was measured with ELISPOT. The RBD domain from Spike was used as positive control. C1-C3 designate non-infected PBMC donors.

Mice immunization with SVLPs presenting the T-cell epitopes drives measurable cellular responses



	IFN-γ, 48h		IFN-γ, 24h		IL-2, 48h		IL-2, 24h		TNF-α, 48h		IL-6, 48h		IL-12p70, 48h		KC/GRO, 48h	
	vaccine	empty	vaccine	empty	vaccine	empty	vaccine	empty	vaccine	empty	vaccine	empty	vaccine	empty	vaccine	empty
VMX-1023					51.1	12.4	1.4	3.0								
VMX-1025													940.04	0.00		
VMX-1040													296.35	0.00		
VMX-1041	0.40	0.17	0.44	0.04	170.1	14.1	57.2	2.3	9.60	3.99	32.80	1.66			1.72	0.44
VMX-1042					41.1	9.1	24.6	0.3								
VMX-1043					20.3	4.5	10.6	2.7							1.86	1.08
VMX-1044					17.1	7.1	5.9	4.9								
negative ctrl	0.07	0.01	0.02	0.07	13.2	15.2	1.3	3.0	4.84	6.84	0.82	2.93	8.01		0.75	0.95

Figure 4. Cytokine responses in mice immunized with the T-cell vaccine

Antigen-specific T-cell responses were assessed by MSD multiplex assay in restimulated mice splenocytes. IL-2 responses from individual animals are shown with bar graphs, whereas average values from various cytokines are presented in the table.

Conclusions

We designed and produced a multi-epitope, T-cell based synthetic vaccine against SARS-CoV-2 using our proprietary SVLP nanoparticle technology and demonstrated the following:

- The single CD8⁺/CD4⁺ peptide epitopes elicit robust IFN-γ responses in infected with more than one viral variant individuals
- The entire T-cell vaccine formulation triggers polyfunctional cytokine responses in naïve immunized mice

Completion of the ongoing immunological readouts and further *in vivo* analyses will allow the non-clinical development of a novel T-cell based vaccine candidate against all the circulating SARS-CoV-2 variants.