V-212, a novel peptide-based, serotype-independent vaccine candidate against *Streptococcus pneumoniae*

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Introduction

Despite the introduction of large-scale vaccination programs and the clinical success of PCVs, *S. pneumoniae* continues to be a significant threat with global burden. In response to serotype replacement and emergence of resistance, efforts to improve vaccines' breadth of coverage and efficacy are underway. We are developing V-212, a peptide-based vaccine candidate consisting of a small number of well-conserved antigenic epitopes, presented on a synthetic virus-like particle (SVLP), previously proven safe and immunogenic in man.

V-212 is immunogenic in mice and rabbits with durable epitope-specific serum antibody responses. Said antibodies bind several *Spn* serotypes, supporting native epitope recognition on bacterial surfaces and the V-212 serotype-independent potential. In a serotype 3 lethal sepsis mouse model, V-212 prevents pneumococcal disease by reducing bacterial load in lungs and blood, and extends animal survival. Moreover, it protects against bacteremia-induced death in a serotype 8 sepsis model. Following peripheral immunization, V-212 antibodies are also found in the lung and nasal tissues of animals, suggesting the triggering of respiratory mucosal responses. In an *in vitro* phagocytic uptake model, V-212-induced antibodies mediate the uptake of *Spn* by human granulocytes and monocytes, a prerequisite for *in vivo* bacterial clearance. Moreover, V-212 antibodies reduce adherence of *Spn* on epithelial cell lines, suggesting the vaccine's effect on pneumococcal colonization.

V-212 is a novel, peptide-based candidate for a broad-spectrum *Spn* vaccine. Having completed a GLP toxicity study and GMP manufacturing, V-212 is advancing toward clinical development with a first-in-human clinical trial.

Results

1. The peptide epitopes of the V-212 vaccine candidate are delivered using Synthetic Virus-Like Particles (SVLP)

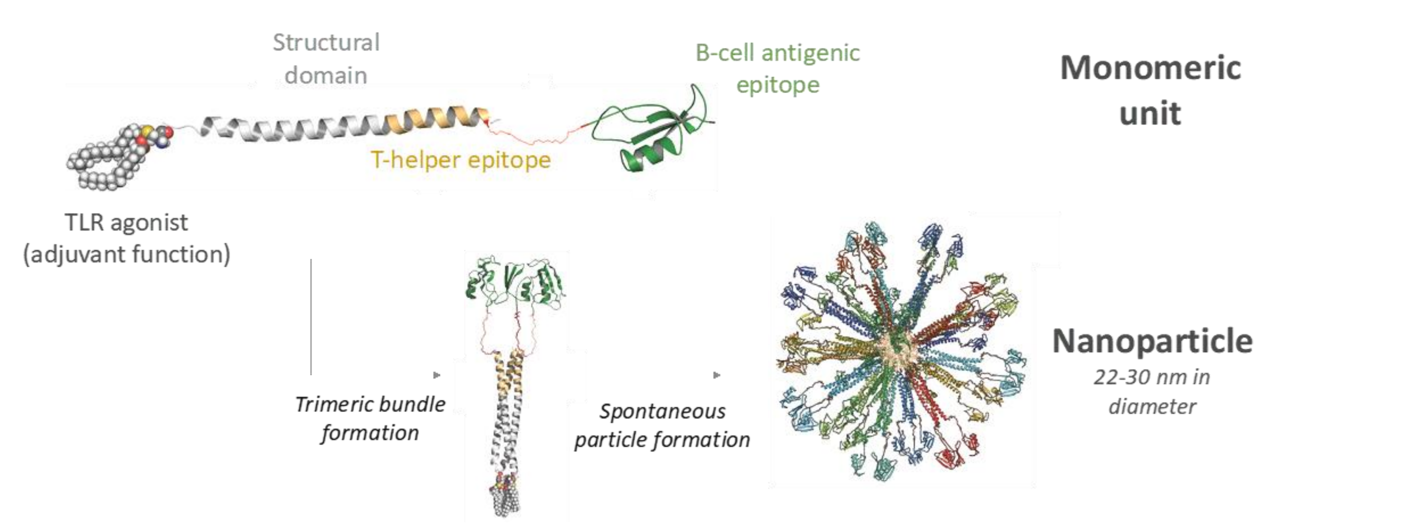


Figure 1. The Virometix SVLP technology platform and its use for the V-212 delivery

Well-conserved B-cell epitopes from *S. pneumoniae* surface 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 (PCT/EP2024/069620).

2. V-212 confers protection against serotype 3 and 8 bacterial lethal challenge in mice

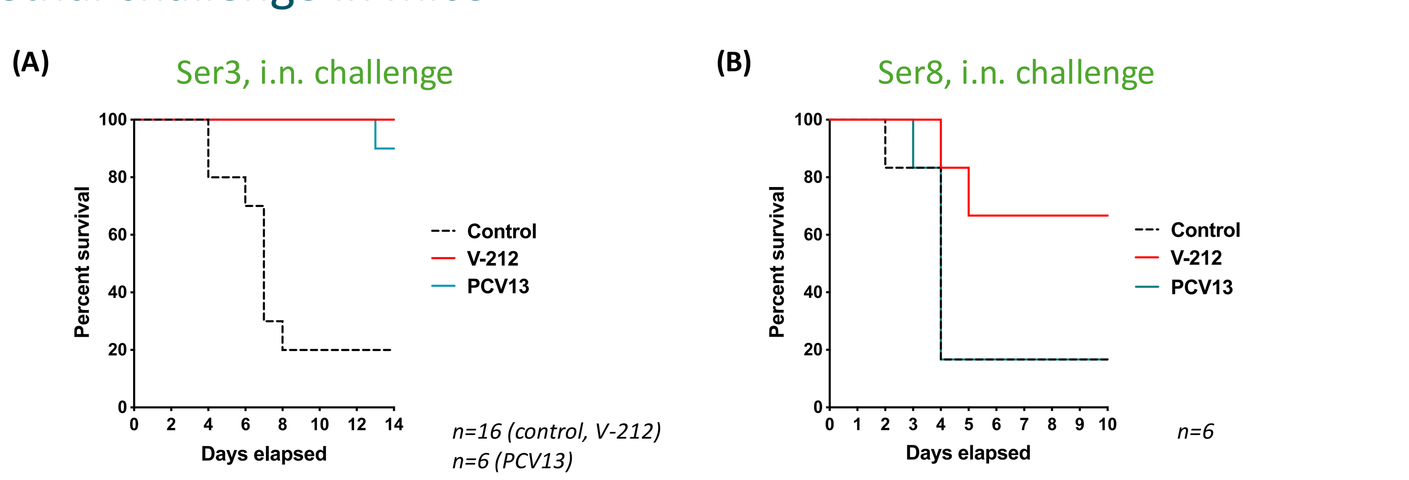


Figure 2. Vaccine efficacy against lethal pneumococcal challenge

BALB/c mice were immunized three times s.c. with V-212, PCV13 (Prevnar 13 vaccine) or a control SVLP particle. Two weeks after the last immunization mice were intranasally challenged with a lethal dose of two *S. pneumoniae* serotypes. Kaplan-Meier survival analysis is depicted for serotype 3 (A) and serotype 8 (B).

3. V-212 blocks bacterial lung colonization and invasive disease from serotype 3 in mice

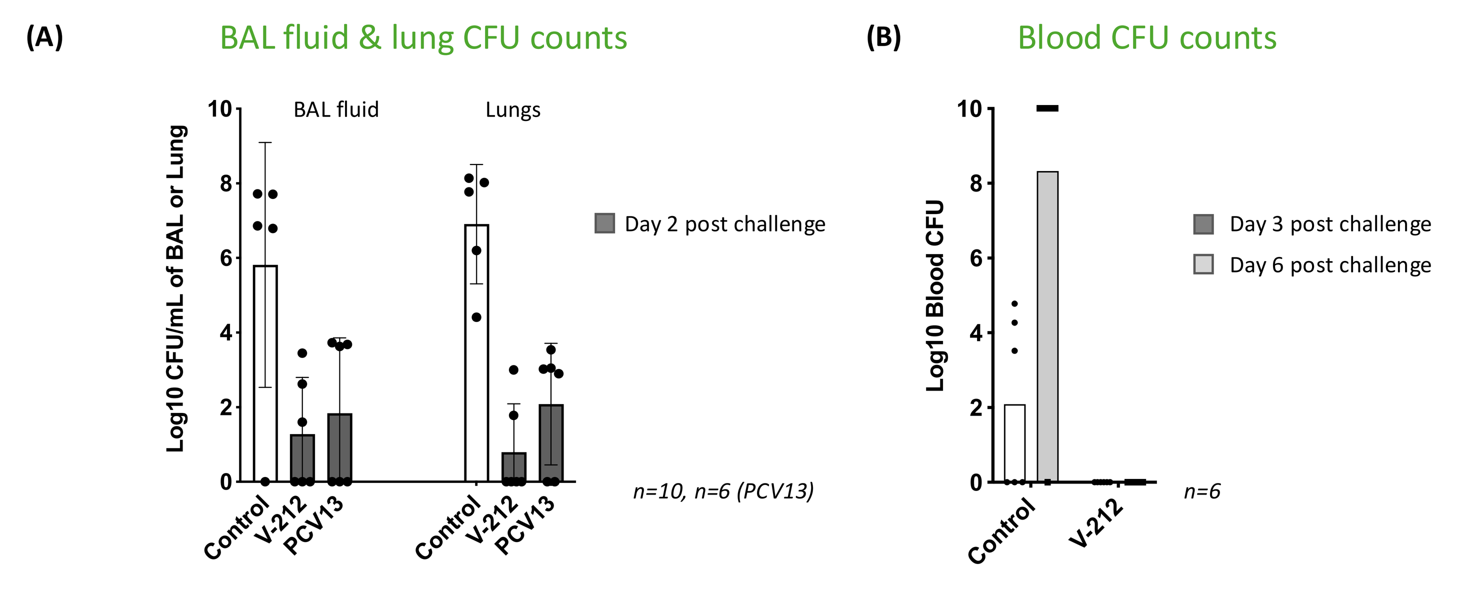


Figure 3. Vaccine efficacy against organs colonization and bacteremia

V-212 significantly reduced *S. pneumoniae* colonization of the lungs. Three times V-212- or control-immunized BALB/c mice were intranasally infected with serotype 3. CFU values were obtained (A) from lung tissue and BAL (bronchoalveolar lavage) fluid 2 days post challenge (two-way ANOVA; p<0,001), and (B) from the animals' blood 3 and 6 days post challenge (two-way ANOVA; p<0,001).

4. V-212 is immunogenic in mice with durable antibody responses and IgG present in the lung tissue

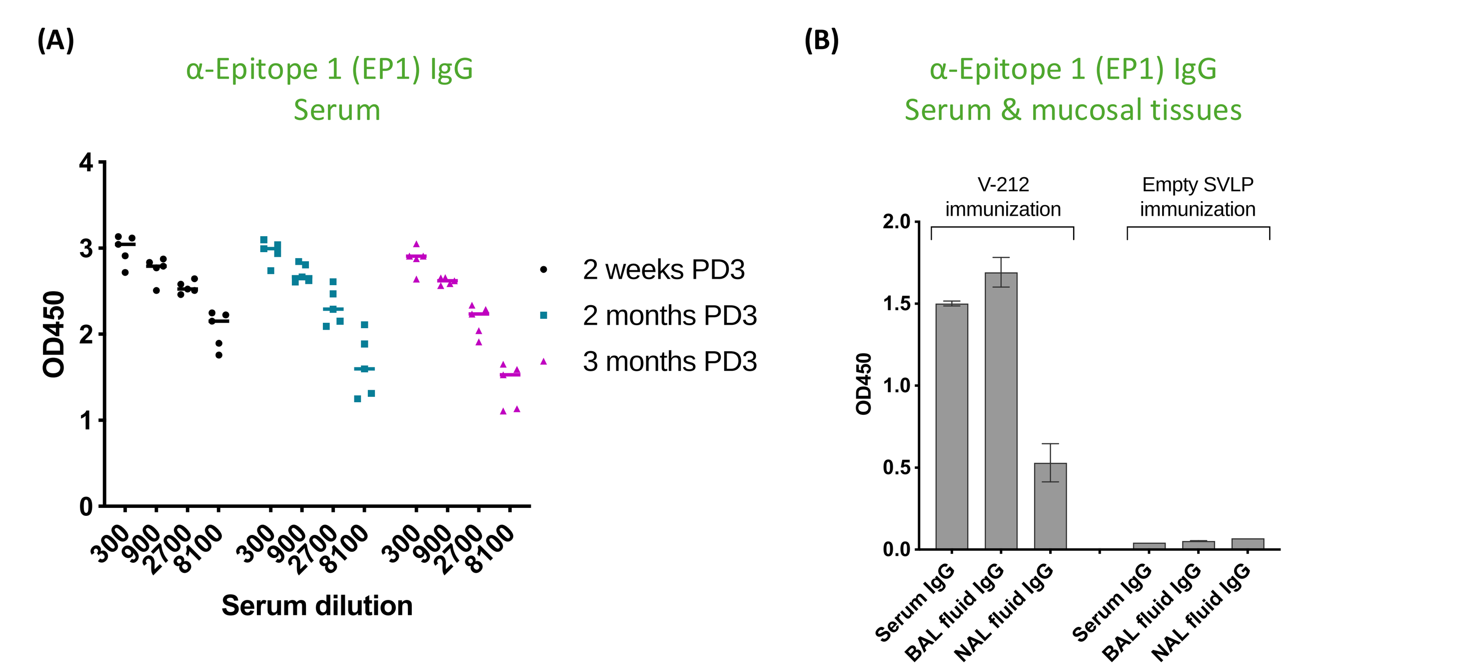


Figure 4. V-212 antibody levels in mouse serum, BAL and NAL (nasal wash) fluids

IgG produced after a 3x i.m. immunization of BALB/c mice with V-212 were measured with ELISA. Example shows serum IgG levels against EP1 at different time-points post last immunization (A) or levels in serum, BAL and NAL fluids one week post last immunization (B).

5. V-212-induced antibodies recognize PCV13 serotypes and serotypes not included in the vaccine

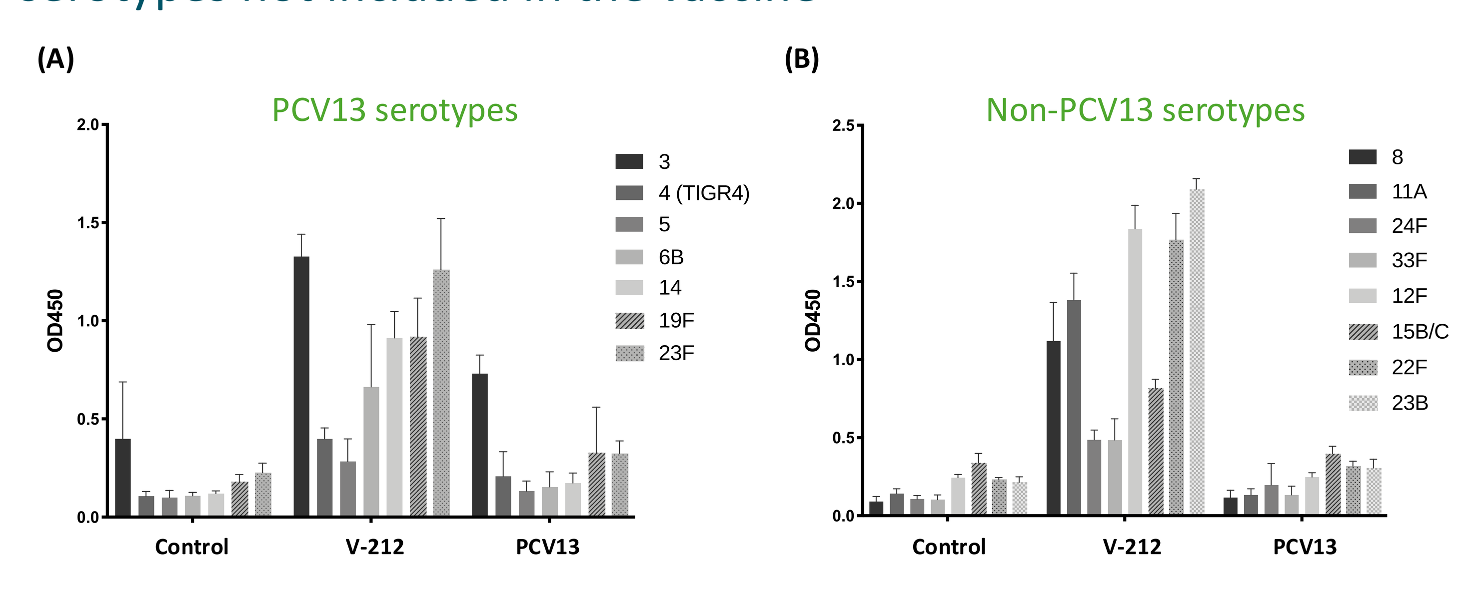


Figure 5. IgG binding to different *S. pneumoniae* serotypes

Antibodies produced on day 42 effectively bound several *S. pneumoniae* serotypes included (A) and not included (B) in the PCV13 vaccine, in an *in vitro* whole bacterial cell ELISA assay.

6. V-212 antibodies mediate phagocytic uptake of *S. pneumoniae* by human PBMC

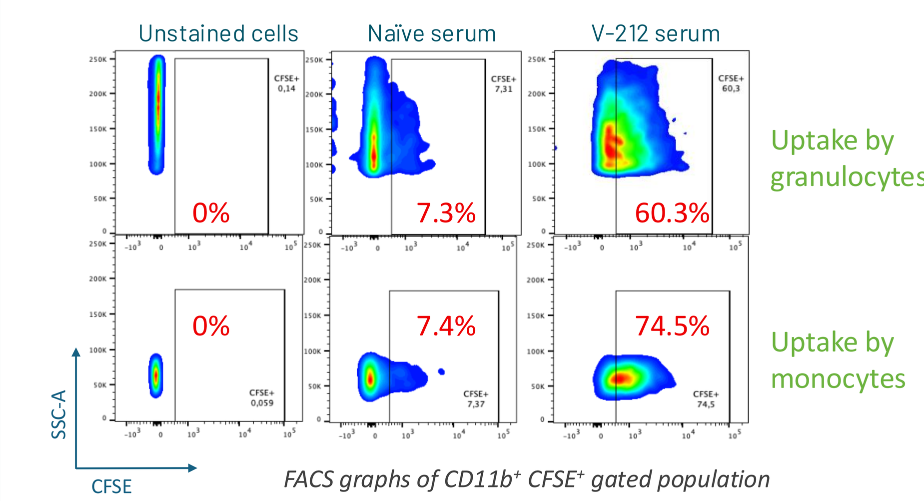
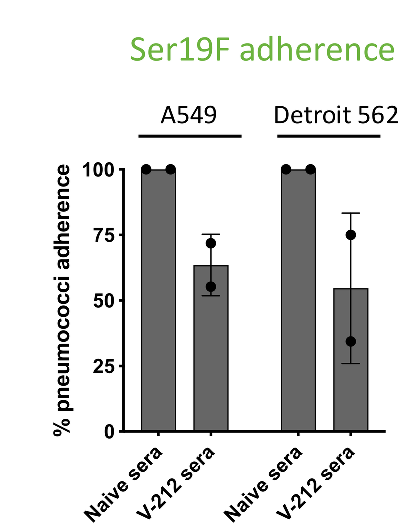


Figure 6. V-212 antibody-mediated phagocytic uptake of serotype 19F

Ser19F bacterial uptake was performed using serum from 3x i.m. V-212-immunized mice, or from naïve mice. Following CFSE-stained bacteria and sera co-incubation with freshly isolated human donor PBMC cells (both the granulocyte and the monocyte fraction), uptake was monitored using flow cytometry.

7. V-212 antibodies reduce adherence of *S. pneumoniae* onto lung and nasopharyngeal epithelial cells



Serotype	A549	D562
Ser3	26%	58%
Ser6B	62%	46%
Ser19F	36%	45%

Figure 7. V-212 antibody-mediated inhibition of adherence

Serum from 3x i.m. V-212-immunized mice, or from naïve mic e was used to assess adherence of serotypes 3, 6B and 19F onto lung A549 and nasopharyngeal Detroit 562 epithelial cells. Bar graph on the left shows V-212 effect on Ser19F adherence (dots designate biological replicates). Table on the right depicts vaccine performance on two more serotypes.

8. V-212 triggers cellular responses in the lung, in form of IFN-γ

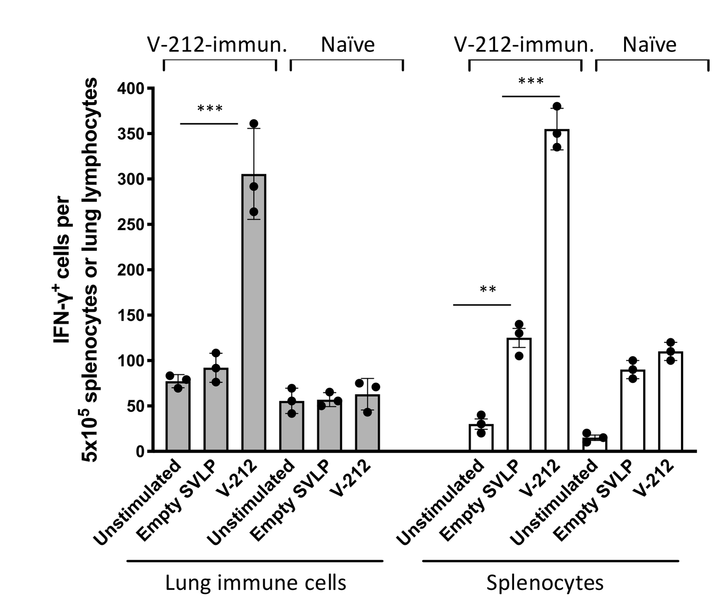


Figure 8. IFN-γ responses in immunized mice

Immune cells isolated from lung tissues or splenocytes, both originating from 1x V-212-immunized C57BL/6 or naïve mice, were restimulated with the agents shown on the x-axis. IFN-γ ELISPOT readout was performed in on day 13. Results correspond to pooled samples from 4 animals per group in triplicates. Statistics: unpaired t-test; ** p≤ 0.01, *** p≤ 0.001

Conclusions – Next steps

V-212 is a novel, peptide-based candidate for a broad-spectrum *S. pneumoniae* vaccine, developed with the Virometix' proprietary SVLP nanoparticle technology. V-212:

- Protects against lethal infection of mice with serotypes 3 and 8, preventing organ colonization and bacteremia caused by serotype 3
- Generates durable serum IgG responses in mice, and elicits IgG in the lung tissue after peripheral (i.m.) immunization which likely contribute to the protection seen
- Generates IgG that recognize multiple *Spn* serotypes *in vitro*, including non-PCV13 serotypes
- Mediates phagocytic uptake of *Spn* by human immune cells, indicative of the mode of bacterial clearance
- Reduces pneumococcal adherence on epithelial cells, suggestive of the mechanism of protection
- Triggers IFN-γ responses, supportive of humoral responses and likely the mechanism of bacterial clearance

V-212 is advancing toward clinical development with a randomized, placebo-controlled, double-blind first-in-human clinical trial.