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

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Introduction

Streptococcus pneumoniae (*Spn*) is a leading cause of a wide range of bacterial infections including otitis media, community-acquired pneumonia, meningitis and bacteremia, with considerable morbidity and mortality worldwide. Current prophylaxis is based on capsular polysaccharide vaccines, plain or conjugated to protein carriers, that are periodically updated to cover for the circulating serotypes. Young children under 2 years of age, the elderly and individuals with weakened immune systems are still vulnerable to infection due to either emerging serotypes not covered by current vaccines, or to poor coverage of certain vaccine serotypes. To circumvent the need for new vaccines composition and to provide broad serotype coverage, we are developing V-212, a peptide-based vaccine candidate based on a small number of well-conserved antigenic epitopes presented on a synthetic virus-like particle (SVLP). The antigenic epitopes, parts of Spn virulent surface protein domains shared among serotypes, are synthesized using solid-phase peptide synthesis and subsequently conjugated to a lipopeptidic backbone carrying elements to activate innate and adaptive immune responses. This SVLP vaccine delivery platform has previously been proven to be safe and immunogenic in man.
V-212 is immunogenic in mice and rabbits with durable epitope-specific antibody responses. The vaccine is also able to induce IFN-γ, indicative of a cellular immune response which supports the strong humoral responses and might contribute to efficacy. In a lethal sepsis mouse model, administration of V-212 prevents pneumococcal disease by reducing bacterial presence in lungs, and extends animal survival by blocking bacterial dissemination in blood, after intranasal challenge with serotype 3. Moreover, V-212 protects against bacteremia-induced death in a serotype 8 sepsis model. Antibodies triggered upon V-212 immunization recognize and bind several different *S. pneumoniae* serotypes – including non-PCV13 vaccine serotypes – tested in a whole cell ELISA assay, supporting native epitope recognition on bacterial surfaces and the pan-serotype potential of V-212. Additional studies to elucidate the mechanism of protection conferred by V-212, as well as studies to evaluate its potential to be combined with approved polysaccharide-conjugate vaccines are ongoing. Furthermore, the V-212 vaccine is currently being tested in a GLP safety toxicology study in rabbits in preparation for a clinical trial application.

Results

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

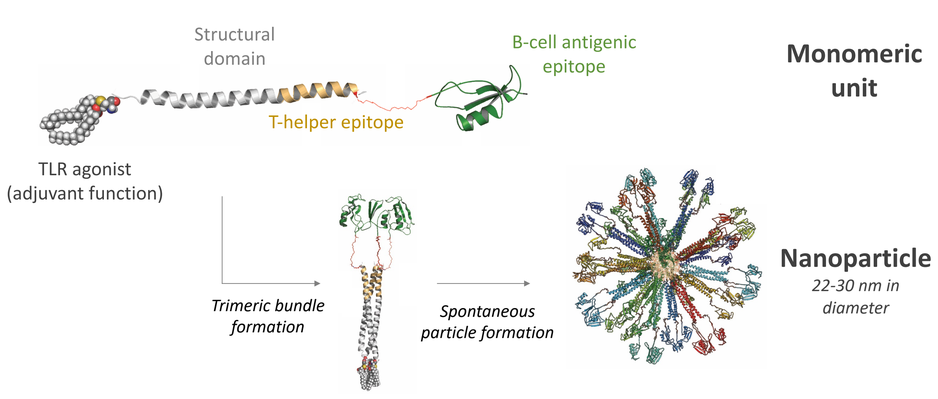

Figure 1. The Virometix SVLP technology platform and its use for the V-212 delivery

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 (WO 2020/127728 A1).

V-212 is immunogenic in rabbits with durable antibody responses

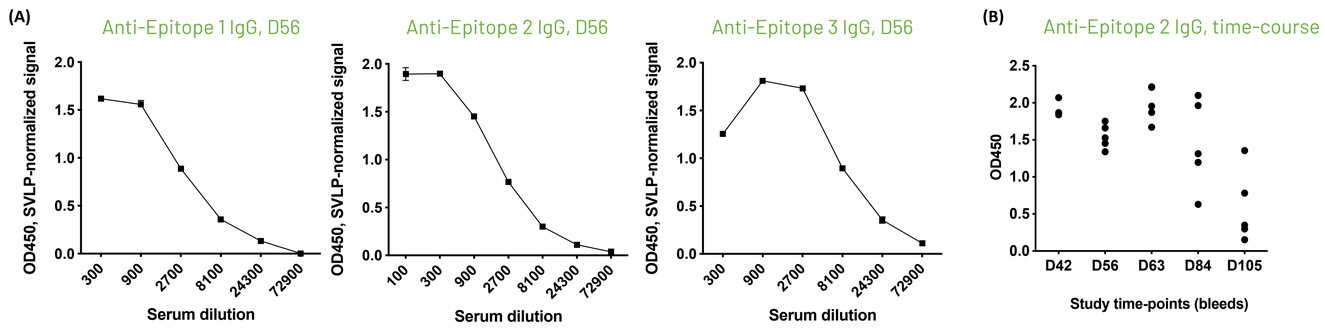

Figure 4. V-212 antibody levels in rabbits

Antibodies produced on days 42, 56, 63, 84 and 105 after a triple immunization of rabbits with V-212 were measured with ELISA. Graphs show IgG levels on D56 against epitopes 1, 2 and 3 within V-212 (A), and IgG levels against epitope 2 in a time-course manner

V-212 triggers IFN-γ release, indicative of a T-cell-dependent immune response

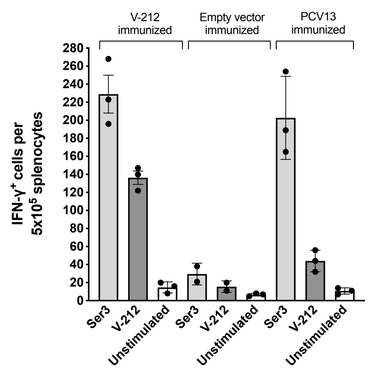

Figure 5. IFN-g responses in immunized mice

Splenocytes were collected from mice 12 days after they were intramuscularly immunized 1x with V-212, the empty vector or PCV13, and were restimulated with serotype 3, the V-212 vaccine or were left unstimulated. The amount of IFN-γ release was measured in terms of positive cells per $5x10^5$ splenocytes in an ELISPOT assay. The immunized animal groups are indicated on top of the graph, whereas the restimulation factor is shown on the x-axis.

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

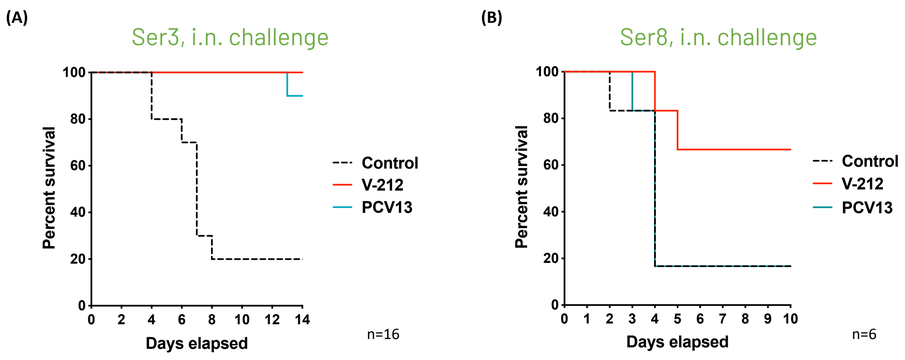

Figure 2. Vaccine efficacy against lethal pneumococcal challenge

BALB/c mice were immunized three times s.c. with V-212, PCV13 (the polysaccharide-based 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).

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

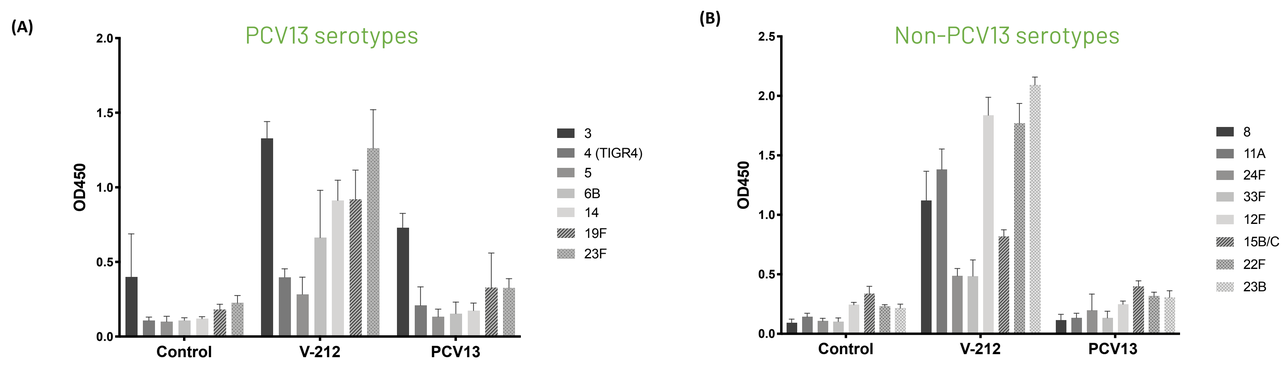

Figure 6. IgG binding to different *S. pneumoniae* serotypes

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.

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

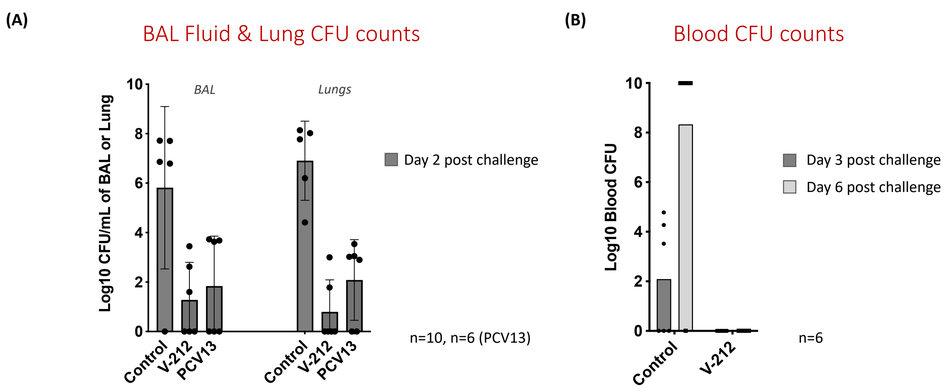

Figure 3. Vaccine efficacy against organs colonization and bacteremia

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; $p<0,001$).

Conclusions – Next steps

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

- Is immunogenic in mice and rabbits with durable antibody responses
- Protects against lethal infection of mice with serotypes 3 and 8, preventing organ colonization and bacteremia caused by serotype 3
- Triggers IFN-γ responses after *in vitro* restimulation of splenocytes, supportive of strong humoral responses
- Elicits antibodies that recognize multiple *Spn* serotypes *in vitro*, including non-PCV13 serotypes

V-212 is a novel candidate for a broad-spectrum *S. pneumoniae* vaccine. Additional mechanistic and PCV13 combination studies are ongoing to conclude the candidate's non-clinical development.