A peptide-based, serotype-independent vaccine against *Streptococcus pneumoniae*

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Introduction

Streptococcus pneumoniae 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. The current prophylaxis is based on capsular polysaccharide vaccines, plain or conjugated to protein carriers, that are periodically updated to cover for the circulating serotypes. However, young children under 2 years of age, the elderly and individuals with weakened immune systems are still vulnerable to infection, due to emerging serotypes not covered by current vaccines. To circumvent the need for new vaccines composition and to provide broad serotype coverage, we set out to investigate peptide-based vaccine candidates based on a small number of well-conserved antigenic epitopes.

To this end, we designed single and "branched" peptides from bacterial virulent surface protein domains shared among serotypes, and synthesized them using solid-phase peptide synthesis. We evaluated the epitopes' functionality in binding assays using convalescent sera from patients with invasive pneumococcal disease. Subsequently we conjugated them to a lipopeptidic backbone carrying elements to spontaneously drive the formation of Synthetic Virus-Like Particles (SVLPs), and characterized the particles with dynamic light scattering and mass spectrometry.

We used the lethal mouse pneumonia model to assess prevention of pneumococcal disease upon vaccination with various SVLP combinations and after intranasal challenge with serotype 3. Mice were monitored for post-challenge survival, bacterial burdens in the blood and organs, IgG production against the specific SVLP-conjugated epitopes, as well as for safety (bodyweight and clinical signs) during the immunization rounds. Antibodies produced upon immunization were functionally assessed for binding onto whole bacterial cells of serotype 3 and of seven vaccine and non-vaccine serotypes.

Results

Synthetic Virus-Like Particles for vaccine delivery

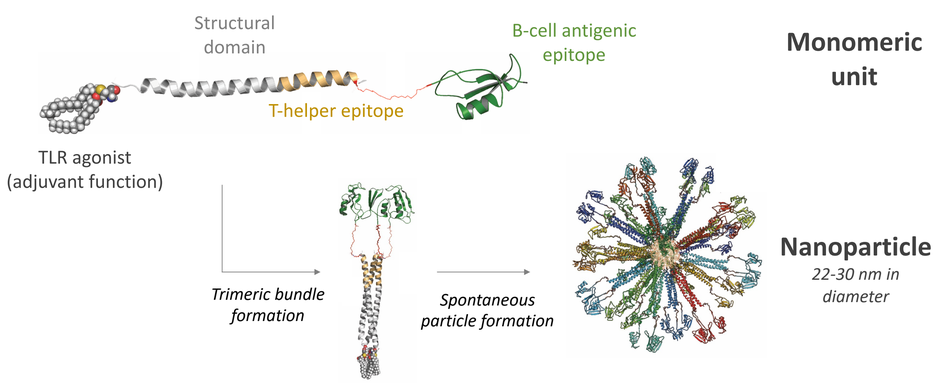


Figure 1. The Virometix SVLP technology platform and its use for the delivery of a serotype-independent peptide-based vaccine

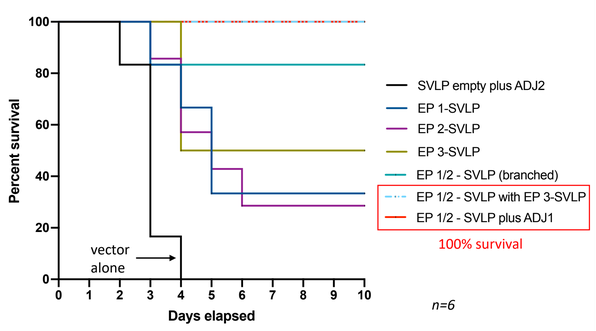
A set of well-conserved B-cell epitopes from *S. pneumoniae* surface proteins were rationally chosen and synthesized using SPPS. Subsequently they were conjugated to a lipopeptidic backbone carrying elements to spontaneously drive the formation of SVLPs (WO 2020/127728 A1).

Three epitopes on SVLPs confer 100% survival upon serotype 3 bacterial lethal challenge

(A) *All animal groups*

Epitopes in SVLPs	10-day survival
SVLP	0%
SVLP+ADJ2	0%
EP 1	33%
EP 2	17%
EP 3	50%
EP 4	17%
EP 1/2 (branched)	83%
EP 1/2 + EP 4	67%
EP 1/2 + EP 3 + EP 4	83%
EP 1/2 + EP 3 plus ADJ1	83%
EP 1/2 + EP 4 plus ADJ1	83%
EP 1/2 plus ADJ2	83%
EP 1/2 + EP 4 plus ADJ2	83%
EP 1/2 + EP 3	100%
EP 1/2 plus ADJ1	100%
EP 1/2 + EP 3 + EP 4 plus ADJ1	100%
EP 1/2 + EP 3 plus ADJ2	100%
EP 1/2 + EP 3 + EP 4 plus ADJ2	100%

(B) *Example with selected groups*



(C)

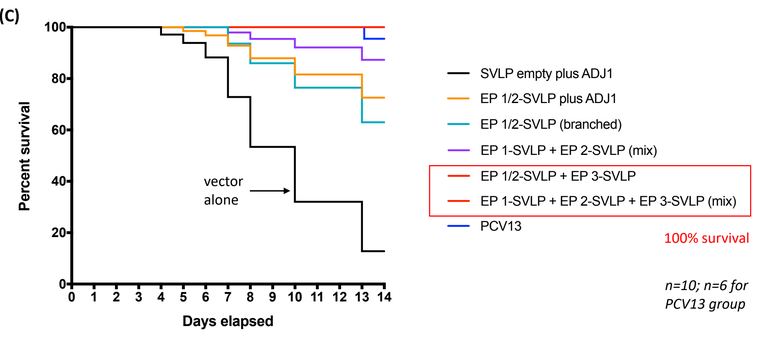


Figure 2. Vaccine efficacy against lethal pneumococcal challenge

BALB/c mice were immunized three times s.c. and challenged with *S. pneumoniae* serotype 3 two weeks after the last immunization. (A) Survival rates after challenge of the various epitope combinations on SVLPs. EP 1 refers to epitope 1 displayed on a single SVLP particle and EP 1/2 refers to epitopes 1 and 2 displayed as a branched epitope on the particle. (B-C) Kaplan-Meier survival analysis of selected groups. A control mice group received the polysaccharide-based PCV13 vaccine (Prevnar 13).

Immunization leads to increasing IgG titers over time

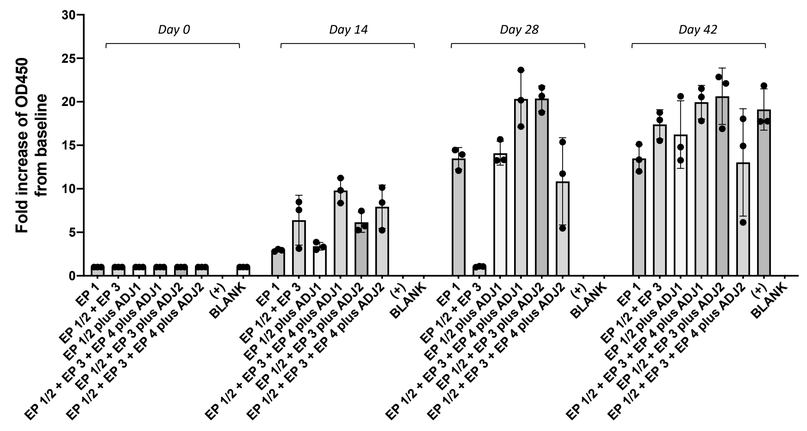


Figure 4. Antibody titers against different epitopes

Antibodies produced on days 0, 14, 28 and 42 were measured with ELISA. Example shown here refers to antibodies against epitope 1 (EP 1), in groups where 100% survival was observed.

Immunization-induced IgG antibodies recognize PCV13 serotypes and serotypes not included in the vaccine

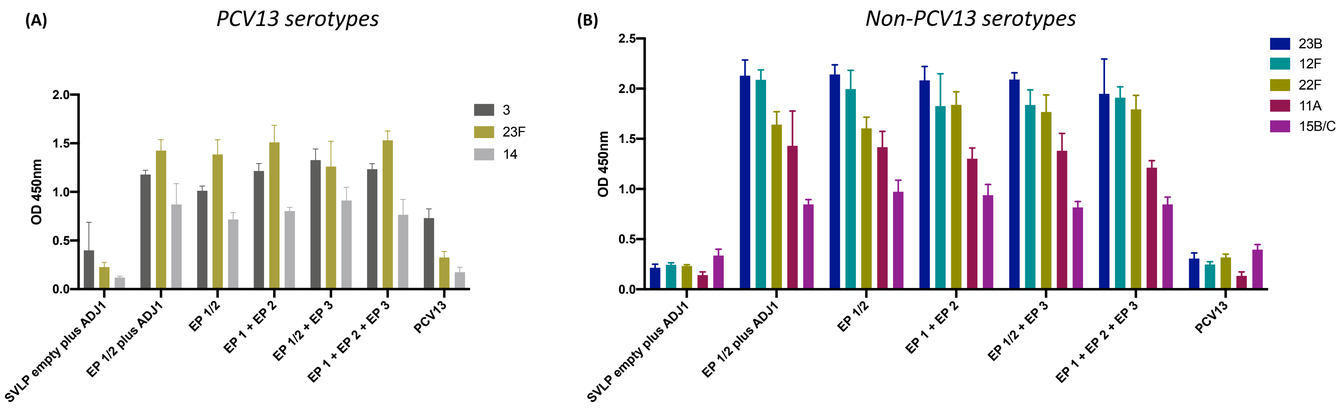


Figure 5. IgG binding to different *S. pneumoniae* serotypes

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

Immunization does not result in any abnormal clinical signs – vaccine is safe in animals

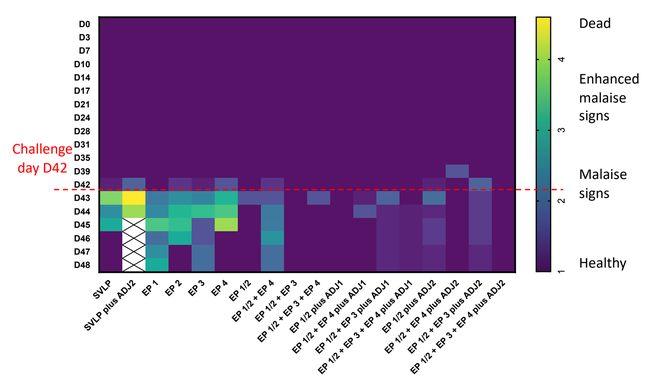


Figure 6. Safety aspects in immunized groups

Safety was monitored during the immunization rounds in all animal groups using a pathoscore 1-5 scale.

Increased animal survival is accompanied by low or cleared bacterial counts in blood and organs

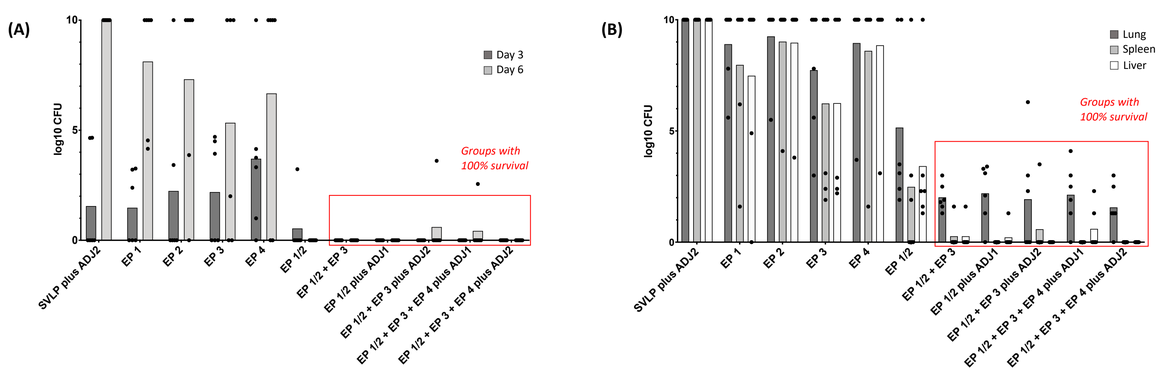


Figure 3. Vaccine efficacy against bacteremia and organs colonization

Five epitope combinations significantly reduced *S. pneumoniae* (A) blood CFUs (two-way ANOVA; p<0,001; p≤0,0042) and (B) lung (one-way ANOVA; p≤0,0024; p≤0,0322), liver (p<0,0001; p≤0,0023) and spleen (p<0,0001) CFUs at 3- and 6-days post-infection.

Conclusions

We generated novel, peptide-based prophylactic vaccine candidates against *S. pneumoniae* using our proprietary SVLP nanoparticle technology. The combination of three such universal epitopes is sufficient to:

- Elicit antibodies that recognize multiple serotypes, even ones that are not included in current polysaccharidic vaccines
- Prevent organ colonization and bacteremia
- Confer complete protection against lethal infection, at least equivalent to PCV13

Completion of the ongoing studies in higher animals will allow the entry to the clinical development of a broad-spectrum *S. pneumoniae* vaccine.