

Safety and immunogenicity of a novel multiple antigen pneumococcal vaccine in adults: A Phase 1 randomised clinical trial

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Abstract

Background

Pneumococcal vaccines, combining multiple protein antigens, provide an alternative approach to currently marketed vaccines and may provide broader protection against pneumococcal disease. This trial evaluated the safety and immunogenicity of a novel vaccine candidate PnuBioVax in healthy young adults.

Methods

In a Phase 1 double-blind study, 36 subjects (18–40 years) were randomised to receive 3 doses of PnuBioVax, 28 days apart, at one of three dose levels (50, 200, 500 µg) or placebo. Safety assessments included rates of emergent adverse events (AEs), injection site and systemic reactions. Immunogenicity endpoints included antibody titre against PnuBioVax and selected pneumococcal antigens.

Results

In the placebo (n = 9) and PnuBioVax (n = 27) vaccinated subjects, there were 15 and 72, reported TEAEs, respectively. The majority of TEAEs were classified as common vaccine related AEs. There were no serious AEs. Common vaccine-related AEs occurred in 13 PnuBioVax (48%) and 2 placebo (22%) subjects and were all headaches (mild and moderate). Injection site reactions, mostly pain and tenderness (graded mild or moderate) were reported, in particular in the 200 µg and 500 µg PnuBioVax groups. There were no clinically significant changes in vital signs, ECG or blood chemistries. Subjects receiving the higher dose (200 and 500 µg) demonstrated a greater fold increase in IgG titre compared with the starting dose (50 µg) or the placebo group. The fold-increase was statistically significantly higher for 200 and 500 µg PnuBioVax vs 50 µg PnuBioVax and placebo at each timepoint post-immunisation. Most subjects receiving 200 and 500 µg PnuBioVax demonstrated a ≥2-fold increase in antibody against pneumolysin (Ply), Pneumococcal surface antigen (PsaA), PiaA (Pneumococcal iron acquisition), PspA (Pneumococcal surface protein A) and pilus proteins (RrgB and RrgA).

Conclusions

All dose levels were considered safe and well tolerated. There was a statistically significant increase in anti-PnuBioVax IgG titres at the 200 and 500 µg dose levels compared to 50 µg and placebo. Trial registration number: [NCT02572635](https://www.clinicaltrials.gov)
<https://www.clinicaltrials.gov>.

Abbreviations: AEs, adverse events; ELISA, enzyme-linked immunosorbent assay; SD, standard deviation; TEAEs, treatment emergent adverse events; TMB, 3,3',5,5'-tetramethylbenzidine; EPT, end-point titre; MSD, mesoscale discovery; ECL, electrochemiluminescence; Ply, pneumolysin; PspA, pneumococcal surface protein A; PCV, polysaccharide conjugate vaccines; MedDRA, medical dictionary for regulatory activities; PsaA, pneumococcal surface antigen; PiuA, pneumococcal iron uptake; PiaA, pneumococcal iron acquisition; IPD, invasive pneumococcal disease; OM, otitis media; WT, wild type

Keywords: Pneumococcal infection; Phase I; PnuBioVax; Immunogenicity; Safety; Multiple antigen vaccine

1 Introduction

Pneumonia remains the leading global cause of death among children under age five, killing more than 900,000 children in 2013 and accounting for 15% of all child deaths [1]. *Streptococcus pneumoniae* (pneumococcus) is the most common cause of severe pneumonia in infants and a leading cause of morbidity and mortality in the elderly. Pneumococcus also causes sepsis and meningitis and is one of the most common causes of bacterial otitis media (OM). Pneumococci are transmitted by direct contact from infected patients or healthy asymptomatic carriers, most commonly by oral or nasal mucosal secretion. In the general population, pneumococci are commonly restricted to the nasopharyngeal mucosa, and can persist in the host for several days or weeks. Individual hosts may carry more than one pneumococci serotype at any one time and colonisation precedes but rarely leads to disease. The introduction of polysaccharide conjugate vaccines (PCVs) in the paediatric age group has had a dramatic effect on rates of carriage and invasive pneumococcal disease (IPD) caused by vaccine serotypes (VT). The reduction is not only seen in vaccine targeted age groups but also in the non-vaccinated population. This indirect effect is mediated through a reduction in nasopharyngeal carriage and transmission [2].

There are two main limitations of the currently available vaccines. Firstly, despite a clear overall benefit following introduction of PCVs, increasing pneumococcal disease caused by non-vaccine serotypes (NVT), through serotype emergence or replacement, has been well documented in many countries where PCVs have been introduced [3]. Replacement of first generation PCVs with vaccines covering additional serotypes (PCV-10 and PCV-13) has helped contain serotype replacement in the short term [4], but capsule switching and the emergence of new variants through recombination remains a concern particularly in environments associated with high carriage rates and a broad spectrum of serotypes causing disease [5,6]. The second limitation is the complexity and cost of PCVs, which are difficult to manufacture and without considerable financial assistance, their affordability and accessibility is restricted for low and middle-income countries [7].

Pneumococcal vaccines that are more affordable and provide either protection against serotypes prevalent in the developing world or, ideally, broad protection across all pneumococcal serotypes would be highly desirable. A relatively new approach is to move away from using polysaccharide antigens to target conserved surface proteins, common to most or all pneumococcal strains. One conserved protein, the cholesterol-binding haemolytic cytotoxin pneumolysin (Ply), is closely associated with the development of invasive disease and inflammation [8]. Non-toxic Ply mutants have been shown to give protection in animal models [9]. The most advanced protein vaccine candidates, including combinations of recombinant antigens and killed whole cell preparations, have been tested clinically, including Phase 2 trials in infants and elderly adults [10,11]. By their nature these vaccines have multiple mechanisms of action potentially moderating IPD and preventing carriage and transmission. The impact of protein vaccines on nasopharyngeal colonisation is under investigation in regions with high carriage rates in Africa [12] and Asia but it remains unclear when these vaccines will progress into larger trials to assess the impact on IPD.

The objective of this Phase 1 trial was to investigate in adults the safety and immunogenicity of a new, novel pneumococcal vaccine at 3 dose levels. PnuBioVax, described in this manuscript, is a vaccine produced from genetically-modified *S. pneumoniae* TIGR4 in whom the Ply gene has been mutated to remove toxicity whilst retaining immunogenicity of the Ply protein [13]. The bacteria are subjected to stress during fermentation, induced by a temperature shift from 30 °C to 37°. This temperature shift is designed to mimic the translocation of *S. pneumoniae* from the nasopharynx to the circulatory system and from a commensal to an invasive phenotype thereby upregulating proteins that may be relevant for provoking protective immune responses as a consequence of infection. Following detergent extraction, the filtrate is processed by anion exchange chromatography to enrich for surface exposed antigens, including, but not limited to, PspA, pilus proteins RrgB and RrgA, PsaA and non-toxic Ply [14]. In a pre-clinical GLP toxicity study in rabbits, PnuBioVax was safe and immunogenic. PnuBioVax is under development as a vaccine against *S. pneumoniae* infection and offers the potential for broad-based protection against a wide range of pneumococcal strains and lower production costs compared to PCVs.

2 Methodology

2.1 Study vaccine

PnuBioVax is a sterile liquid formulated in 40 mM Tris, 150 mM NaCl, pH 8.0 at a protein concentration of 1 mg/mL. For 50 and 200 µg dose levels, the vaccine (PnuBioVax: Batch 15100) was diluted with 40 mM Tris, 150 mM NaCl, pH 8.0 prior to administration. This diluent also served as the placebo. PnuBioVax is non-adjuvanted. Vaccine and placebo were administered at a 0.5 mL volume by intramuscular injection, and all subsequent doses were confirmed following interim review of the safety data. The trial was performed at a single site, Simbec Research Limited.

2.2 Study participants & study design

The trial (SPV-001) was a first-in-human randomised, placebo-controlled, parallel group, double-blind, dose escalation trial to evaluate the safety and immunogenicity of PnuBioVax administered on three occasions 28 days apart, at 50, 200 and 500 µg in adult subjects. Thirty-six healthy males and females, aged 18 to 40 years, were recruited and divided into three cohorts of 12 subjects (9 receiving PnuBioVax and 3 placebo). Subjects were allocated to treatment groups according to a randomisation code using the PROC PLAN procedure of SAS® version 9.1.3.

After the first dose and at pre-determined times on each dosing day and follow-up visit, a safety review was conducted for dose escalation purposes. Dose escalation only proceeded following satisfactory review of the blinded day 8 safety data that included adverse events (AEs), routine laboratory assessments, vital signs, lymph node assessment, injection site reactions and concomitant medication from at least 9 evaluable subjects in the preceding cohort.

All 36 subjects received at least one PnuBioVax or placebo dose and these constitute the safety population. Prior to unblinding, protocol deviations were identified in 7 subjects that were considered likely to affect the scientific interpretation of immunogenicity data. Therefore the immunogenicity analysis is based on the per protocol population (29 subjects).

The trial protocol and all relevant amendments, together with subject information and consent documents were reviewed and approved by Wales Research Ethics Committee 2. Clinical Trials Authorisation was obtained from the Medicines and Healthcare Regulatory Agency (MHRA). The clinical trial (NCT02572635) was performed in accordance with the Declaration of Helsinki (Brazil, 2013) and the principles of Good Clinical Practice.

2.3 Safety and reactogenicity assessment

The primary assessment was based on the incidence and severity of all treatment emergent adverse events (TEAEs). Secondary assessments were (i) The incidence and severity of common systemic vaccine related AEs (anorexia, nausea/vomiting, diarrhoea, headache, fatigue, myalgia, fever); (ii) The incidence and severity of injection site reactions (pain, tenderness, erythema, induration, pruritus); and (iii) Changes in laboratory parameters (biochemistry, haematology and urinalysis) or physical examination from day 1 (baseline) to day 64 that were considered to be clinically significant.

2.4 Immunogenicity assessment

2.4.1 Anti-PnuBioVax IgG

Sera were prepared from blood samples taken on days 1 (pre-dosing), 29 (post dose 1), 57 (post dose 2) and 85 (post dose 3) to assess IgG antibody responses by ELISA. The assay was qualified and conducted by Simbec Research. Briefly, 96-well plates were coated with PnuBioVax, washed, and incubated with serially diluted duplicate samples of test serum. Bound antibody was detected with peroxidase-conjugated donkey anti-human IgG and developed with TMB. Absorbance values were plotted against the reciprocal value of the serum dilution, using a 4 parametric logistic regression with a semi-logarithmic scale to obtain titration curves for each sample. The end-point titre was determined as the titre where the absorbance was equal to a pre-determined cut-point of 0.5.

The proportion of subjects who developed an immune response to PnuBioVax were determined. An immune response was defined in two ways. Firstly, as at least a 2-fold increase in antibody titre from the baseline. Secondly, to compare the change in immune responses in PnuBioVax immunised subjects with any change observed in placebo subjects at the same time point. A response was defined as a change in antibody titre that was ≥ 2 x standard deviation (SD) of the placebo change at the same time point.

2.4.2 Multiplexed electrochemiluminescence (ECL) assay using MSD technology

This exploratory investigation measured antibody production against specific pneumococcal proteins at days 1 and 85. This procedure was conducted at UCL (University College London) GOS Institute of Child Health, London and utilises MesoScale Discovery (MSD, Rockville, MD, USA). The serum IgG antibodies to pneumococcal protein antigens were measured using a direct binding electrochemiluminescence- based multiplex assay [15] on customised 8 antigen plates. Antibody interaction with immobilised recombinant antigen was converted into ECL signal and read using an ECL detector. ECL signal is relative to antibody concentration.

2.4.3 Ply neutralisation assay

The ability of anti-Ply antibodies, raised against the non-toxic Ply, to inhibit haemolysis was also investigated. Samples containing antibody were serially diluted and added to a lysate prepared from whole *S. pneumoniae* (WT) TIGR4 which has been subjected to multiple rounds of freeze/thawing. Lysates were also prepared from *S. pneumoniae* 6B and 11A. Lysates, which contain native Ply, were first titrated to obtain a dilution at which maximum lysis of a fixed number of red blood cells (RBC) was achieved. IgG prepared from human sera samples was incubated in the presence of the lysate (at the selected concentration) and then added to RBC and incubated. Plates were centrifuged to pellet the remaining RBC and supernatant transferred to a 96 well plate and absorbance (OD_{450}) measured. IgG dilution was plotted against absorbance and an end-point titre, defined as 50% inhibition of the maximum lysis, obtained.

2.5 Statistical methods

AEs were coded using Medical Dictionary for Regulatory Activities (MedDRA) version 18.1. The incidence of TEAE and vaccine-related TEAEs was summarised by organ system, preferred term, severity and relationship to study drug. Abnormal laboratory safety, vital signs and 12-lead ECG data were tabulated. Absolute and change from baseline haematology, biochemistry and vital signs parameters were summarised descriptively. Lymph node and injection site reaction assessments were also summarised by frequency for each category.

Anti-PnuBioVax IgG titre responses were summarised descriptively and a comparison between treatments carried out using an analysis of covariance (ANCOVA) model with fixed effect for treatment and the log transformed baseline as a covariate. Fold increase in antibody titres were summarised and a ratio of the geometric means between the baseline and post-baseline visit calculated. A comparison of the mean fold increase was carried out between treatments using an analysis of variance (ANOVA) model with fixed effect for

TEAE Leading to withdrawal	0 (0.0)	0 (0.0)	0 (0.0)	1 (11.1)	1 (3.7)	1 (2.8)
Number (%) of subjects with TEAE by severity						
Mild	2 (22.2)	1 (11.1)	4 (44.4)	2 (22.2)	7 (25.9)	9 (25.0)
Moderate	3 (33.3)	3 (33.3)	3 (33.3)	5 (55.6)	11 (40.7)	14 (38.9)
Severe	1 (11.1)	1 (11.1)	1 (11.1)	1 (11.1)	3 (11.1)	4 (11.1)

The most commonly occurring TEAE was headache. This was the only TEAE classified as a common systemic vaccine related TEAE that was reported (Table 3). When the number of subjects reporting any TEAE was compared, a higher% of subjects reported TEAEs following PnuBioVax when compared to placebo, 21 (77.8%) vs 6 (66.7%), with a tendency towards an increase in the incidence of moderate headache with increasing dose of PnuBioVax (0 (0.0%), 2 (22.0%), 3 (33.3%) and 5 (55.6%) for placebo, 50, 200 and 500 µg PnuBioVax, respectively), although the majority were considered unlikely to be or unrelated to the vaccine. Table 4

Table 3 Common vaccine related AEs.

Preferred term		Number of subjects (%) reporting TEAEs					
		Placebo (n = 9)	50 µg PnuBioVax (n = 9)	200 µg PnuBioVax (n = 9)	500 µg PnuBioVax (n = 9)	All PnuBioVax (n = 27)	Overall (n = 36)
Headache	Number of TEAEs	2	3	11	15	29	31
	Number (%) of subjects reporting ≥1 headache	2 (22.2)	2 (22.2)	5 (55.6)	6 (66.7)	13 (48.1)	15 (41.7)
	Number (%) subjects with headache by severity						
	Mild	2 (22.2)	0 (0.0)	2 (22.2)	1 (11.1)	3 (11.1)	5 (13.9)
	Moderate	0 (0.0)	2 (22.2)	3 (33.3)	5 (55.6)	10 (37.0)	10 (27.8)
	Severe	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 4 Injection site reactions.

Parameter	Time	Number of subjects (%) reporting any grade of injection site reaction				
		Placebo (n = 9)	50 µg PnuBioVax (n = 9)	200 µg PnuBioVax (n = 9)	500 µg PnuBioVax (n = 9)	All PnuBioVax (n = 27)
Pain	Days 1–64	4 (44.4)	7 (77.8)	9 (100.0)	9 (100.0)	25 (92.6)
Tenderness	Days 1–64	1 (11.1)	4 (44.4)	9 (100.0)	9 (100.0)	22 (81.5)
Erythema	Days 1–64	0 (0.0)	0 (0.0)	0 (0.0)	2 (22.2)	2 (7.4)
Induration/Swelling	Days 1–64	1 (11.1)	0 (0.0)	0 (0.0)	3 (33.3)	3 (11.1)
Pruritus	Days 1–64	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Injection site reactions (mostly pain and tenderness graded mild or moderate) were commonly reported, in particular in the 200 µg and 500 µg PnuBioVax groups. 2 subjects withdrew on the basis of moderate or severe reactions.

Across the entire assessment period (days 1–64), a higher number of subjects who received PnuBioVax reported pain (25 (92.6%) vs 4 (44.4%)) and tenderness (22 (81.5%) vs 1 (11.1%)) when compared to placebo. The pain and tenderness was mostly mild in intensity (grade 1) in that it did not interfere with activity and caused mild discomfort to touch.

On each dosing day, there appeared to a delayed onset in pain and tenderness, with higher (change to ' with a higher % of subjects) % subjects reporting pain and tenderness at 4, 8 and 20 hours post-dose when compared to 1 and 2 hours post-dose. Moderate (grade 2) pain and tenderness was reported by 4 (14.8%) subjects who received PnuBioVax, 2 (22.2%) following 200 µg and 2 (22.2%) following 500 µg. By day 7 post each dose, all reactions reported as moderate had subsided with the exception of one subject who recorded moderate pain and tenderness on day 29, which progressed to severe on day 31 resulting in them being withdrawn from the trial.

No subject reported any severe (grade 3) or potentially life threatening (grade 4) injection site reactions at their scheduled visits.

Across the entire assessment period (days 1–64), the incidence of erythema, induration and swelling was low, with 2 (7.4%) subjects who received PnuBioVax reporting mild transient erythema, both after 500 µg and 4 (11.1%) subjects reporting mild induration and swelling, of which 1 (11.1%) received placebo and 3 (11.1%) received 500 µg PnuBioVax. No subject reported any moderate (grade 2), severe (grade 3) or potentially life threatening (grade 4) erythema or induration and swelling following PnuBioVax or placebo. No subject reported pruritus at the injection site following PnuBioVax or placebo.

There were no vaccine related clinically significant changes in biochemistry, physical examination, lymph node assessment, vital signs or 12 lead ECG parameters during the trial.

3.3 Immunogenicity

3.3.1 Anti-PnuBioVax IgG titres

32 subjects received 3 immunisations of which 29 were considered 'per-protocol' prior to unblinding. Total IgG response to PnuBioVax was measured by ELISA. For the per protocol population, IgG endpoint titres are presented as geometric mean titre (with 95% confidence intervals) for groups at days 1 (baseline), 29, 57 and 85 (Table 5). As expected, significant baseline titres were found in the adult subjects and therefore data were analysed by comparing titres post- and pre-immunisation as a ratio (fold increases). In addition, the number of subjects in each group achieving an immune response, as defined in Methodology, is reported (Table 5).

Table 5 IgG response to PnuBioVax in per-protocol population.

	Placebo (n = 9)	50 µg PnuBioVax (n = 8)	200 µg PnuBioVax (n = 7)	500 µg PnuBioVax (n = 5)
Pre-vac GMT (95% CI) Day 1	2686.9 (1891.2, 3817.3)	2343.7 (1725.5, 3183.2)	2592.4 (1597.9, 4206.1)	2833.1 (1704.8, 4707.9)
Post dose 1 GMT (95% CI) Day 29	2584.7 (1868.8, 3575.0)	2524.2 (1905.0, 3344.7)	3222.2 (1956.8, 5306.1)	3888.9 (2676.2, 5651.1)
GMT ratio	0.96	1.08	1.24	1.37
Number (%) subjects ≥2-fold increase	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Number (%) subjects ≥2 SD of placebo fold increase	0 (0.0)	2 (25.0)	5 (71.4)	4 (80.0)
Post dose 2 GMT (95% CI) Day 57	2474.5 (1778.8, 3442.3)	2839.2 (2135.1, 3775.5)	4359.6 (2287.1, 8310.2)	5448.3 (4199.7, 7068.3)
GMC ratio	0.92	1.21	1.68	1.92
Number (%) subjects ≥2-fold increase	0 (0.0)	0 (0.0)	1 (14.3)	2 (40.0)
Number (%) subjects ≥2 SD of placebo fold increase	0 (0.0)	7 (87.5)	7 (100.0)	5 (100.0)
Post dose 3 GMT (95%CI)Day 85	2595.4 (1889.4, 3565.3)	2932.7 (2187.7, 3931.3)	4742.0 (2668.0, 8428.4)	6379.9 (4886.9, 8329.0)
GMT ratio	0.97	1.25	1.83	2.25
Number (%) subjects ≥2-fold increase	0 (0.0)	0 (0.0)	4 (57.1)	3 (60.0)
Number (%) subjects ≥2 SD of placebo fold increase	1 (11.1)	4 (50.0)	6 (85.7)	5 (100.0)

Subjects receiving the higher dose (200 and 500 µg) demonstrated a greater fold increase in IgG titre compared with the starting dose (50 µg) or the placebo group. The fold-increase was statistically significantly higher for 200 and 500 µg PnuBioVax vs 50 µg PnuBioVax and placebo at each timepoint post-immunisation, Comparing with placebo at day 85, p values for the 200 and 500 µg groups were 0.0004 and <0.0001, respectively. There was no evidence of a difference in fold-increase between 200 and 500 µg PnuBioVax or 50 µg PnuBioVax and placebo.

Combining the 200 and 500 µg groups, 12 (100%) and 11 (91.7%) subjects who received PnuBioVax exhibited a fold increase in IgG titre of ≥2 SD of placebo on days 57 (after 2 doses) and 85 (after 3 doses). Similarly in these groups, 3 (25%) and 7 (58.3%) subjects exhibited a ≥2-fold increase in IgG titre on days 57 and 85.

3.3.2 Antibody responses against pneumococcal proteins

29 subjects were evaluated in the per protocol population. Antibody responses against pneumococcal antigens PspA, Ply, PsaA, PiaA, PiuA the pilus proteins RrgB and RrgA were measured by MSD assays (Table 6).

Table 6 Antibody responses against pneumococcal proteins. The number of subjects achieving a ≥ 2 -fold increase from day 1 (baseline) to day 85 in antibody titres against individual pneumococcal proteins.

Antibody against	Number of subjects with ≥ 2 -fold increase (%)			
	Placebo (n = 9)	50 μg dose (n = 8)	200 μg dose (n = 7)	500 μg dose (n = 5)
PspA2	0	1 (12.5)	4 (57.1)	5 (100.0)
Ply	0	1 (12.5)	5 (71.4)	3 (60.0)
PsaA	0	0 (0.0)	4 (57.1)	1 (20.0)
PiaA	1 (11.1)	0 (0.0)	5 (71.4)	5 (100.0)
PiuA	0	0 (0.0)	1 (12.5)	0 (0.0)
RrgBT4	0	8 (100.0)	7 (100.0)	5 (100.0)
RrgB6B	0	8 (100.0)	7 (100.0)	5 (100.0)
RrgAT4	0	8 (100.0)	7 (100.0)	5 (100.0)

All subjects in PnuBioVax 50, 200 and 500 μg dose groups had increases of ≥ 2 -fold against the vaccine type pilus proteins RrgBT4 and RrgAT4 (TIGR4 protein sequences). Strong responses were also seen against the heterologous non-vaccine type pilus protein RrgB6B (serotype 6B protein sequence). Greater than 50% of subjects in the 200 and 500 μg dose groups (n = 12) achieved a ≥ 2 -fold increase in ECL signal against the antigens PspA2 (family 2 protein sequence), Ply and PiaA, and 5 out of 12 had responses against the PsaA protein. One subject in the PnuBioVax vaccinated groups recorded a > 2 -fold increase in ECL signal against PiuA.

The number of subjects achieving a ≥ 2 -fold increase from day 1 (baseline) to day 85 in antibody titres against individual pneumococcal proteins by ECL assay.

3.3.3 Ply neutralisation

Anti-Ply antibodies were purified and analysed to confirm functional activity in the haemolysis inhibition assay. Results using the TIGR4 lysate are shown in Table 7. In the 200 and 500 μg groups (n = 12), 50% of subjects achieved a ≥ 2 -fold increase in endpoint titre (defined as 50% inhibition of haemolysis); in comparison, in the placebo group (n = 9), no subjects recorded an increase of ≥ 2 -fold from baseline levels. Using antibodies from subjects in the 200 μg group, the same levels of inhibition of haemolysis by lysates prepared from *S. pneumoniae* 6B and 11A were obtained.

Table 7 Number of subjects achieving a ≥ 2 -fold increase from day 1 (baseline) to day 85 in Ply neutralisation titres.

Anti-Ply response	Number of subjects (%)			
	Placebo (n = 9)	50 μg dose (n = 8)	200 μg dose (n = 7)	500 μg dose (n = 5)
Inhibition of haemolytic activity	0 (0.0)	2 (25.0)	4 (57.1)	2 (40.0)

4 Discussion

In this first investigation of PnuBioVax in humans, the vaccine was considered safe and well tolerated following intramuscular injection of 50, 200 or 500 μg on 3 occasions to healthy adult subjects. There was a tendency towards more frequent and more severe injection site reactions with higher doses and an increased incidence of headache as the dose increased. Of particular note was the absence of other typical vaccine-related adverse events such as anorexia, nausea/vomiting, diarrhoea, fatigue, myalgia (non-injection site) or fever. It can be concluded that vaccine safety would not preclude clinical investigation of PnuBioVax in target populations.

The antibody titre data demonstrated measurable IgG (specific for PnuBioVax) pre-dose at baseline. In adults this was predicted and is the result of previous exposure to *S. pneumoniae* both as infants during the acquisition of natural immunity and continued exposure into adulthood. Titres at 28 days following the final PnuBioVax immunisation were used to calculate the fold increase to account for the varying baseline titres. In the small subject numbers in this trial, results indicated that significant increases in IgG titres were achieved at the 200 and 500 μg dose levels. In contrast to polysaccharide-based pneumococcal vaccines, there is no definition of a protective immune response for novel protein-based vaccines, such as PnuBioVax.

Post-immunisation results demonstrate increases in antibody levels against several pneumococcal antigens. Importantly, antibodies against the non-toxic Ply were capable of neutralising the haemolytic activity of native Ply derived from several pneumococcal strains. There is evidence that neutralisation of circulating Ply in mice can significantly attenuate cardiac injury during IPD [16].

A multi-antigen vaccine such as PnuBioVax may be effective in several modes of action. Antibodies directed against ABC transporter pneumococcal proteins, PiaA, PiuA and PsaA, have the potential to interfere with nutrient uptake [17,18], whereas antibodies against the pilus proteins, RrgB and RrgA, may block adhesion [19,20]. The role of PspA is to inhibit complement deposition on the pneumococcal surface and therefore antibodies to PspA can promote the opsonisation and killing of pneumococci [21].

In infants, colonisation and the emergence of immunity results in a reduction in the incidence of IPD. The kinetics of acquired immunity would suggest that anti-protein responses play a significant role, however the relative importance of anti-capsular, anti-protein or cellular mechanisms remain elusive [15]. Healthy adults are not generally susceptible to IPD and there are several lines of evidence that this protection is provided by naturally acquired IgG against protein antigens [22]; for example, an anti-protein response to PspA and not a homotypic anti-capsular response was shown to correlate with a reduction in experimental carriage [23].

In the first-in-human trial of PnuBioVax, there were significant baseline IgG against protein antigens present in the vaccine and this response was boosted by PnuBioVax immunisation. As the main target group for a broadly protection pneumococcal vaccine is the paediatric population with low natural immunity, the immunogenicity provided by PnuBioVax in this group requires investigation in Phase 2 studies.

Conflict of interest

CE, SH, YP, MJ, AM, CC, CB are employees of ImmunoBiology Ltd. DG has received consultancy fees from ImmunoBiology Ltd and is an NIHR Senior Investigator. DG and PDe G's laboratory have been in receipt of research grants from ImmunoBiology Ltd.

Author's contributions

CE, SH, MJ and CB participated in study design, execution of the study, review of data and editing of the manuscript.

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Highlights

- Diseases caused by *Streptococcus pneumoniae* (pneumococcus) constitute a major global public health problem.
- Pneumococcal vaccines, combining multiple protein antigens, provide an alternative approach to currently marketed vaccines and may provide broader protection against pneumococcal disease.
- In a Phase I clinical trial in healthy adults, PhnBioVax, a novel multiple antigen vaccine was shown to be safe and immunogenic.

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