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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.

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

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, pneumococcalironacquisition; IPD, invasive pneumococcal disease; OM, otitis media; WT, wild type.

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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 18–40 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

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