Vaccine

journal homepage: www.elsevier.com/locate/vaccine

A randomized, double-blind trial to evaluate immunogenicity and safety of 13-valent pneumococcal conjugate vaccine given concomitantly with trivalent influenza vaccine in adults aged ≥65 years

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ARTICLE INFO

ABSTRACT

This randomized, double-blind study evaluated concomitant administration of 13-valent pneumococcal conjugate vaccine (PCV13) and trivalent inactivated influenza vaccine (TIV) in adults aged ≥65 years who were naïve to 23-valent pneumococcal polysaccharide vaccine. Patients (N = 1160) were randomized 1:1 to receive PCV13 + TIV followed by placebo, or Placebo + TIV followed by PCV13 at 0 and 1 months, with blood draws at 0, 1, and 2 months. Slightly lower pneumococcal serotype-specific anticapsular polysaccharide immunoglobulin G geometric mean concentrations were observed with PCV13 + TIV relative to PCV13. Concomitant PCV13 + TIV demonstrates acceptable immunogenicity and safety compared with either agent given alone.

1. Introduction

Diseases caused by *Streptococcus pneumoniae* are a major health problem. The World Health Organization has estimated that 1.6 million people die annually from pneumococcal disease. For individuals aged ≥65 years, the reported worldwide incidence of invasive pneumococcal disease (IPD) ranges from 24 to 85 per 100,000 persons [1]. As the treatment of pneumococcal disease is limited by the continuous increase in antimicrobial resistance of *S. pneumoniae*, vaccination is considered an important preventive strategy [1,2].

Currently, a 23-valent pneumococcal polysaccharide vaccine (PPV) is available for the protection of older persons against pneumococcal disease. PPV has the drawbacks of poorly defined vaccine efficacy, lack of priming, risk of hyporesponsiveness, and a decline of antibodies over 5 years at different rates for the

23 serotypes [1–4]. A 7-valent pneumococcal conjugate vaccine (PCV7; Prevnar®/Prevenar®; Pfizer Inc) is available for infants and children. Since PCV7's licensure in 2000 in the USA, the incidence of IPD caused by vaccine serotypes has decreased not only in those aged <2 years, but also among adults because of the indirect effects of herd immunity [5]. Nevertheless, IPD death rates in adults aged >50 years still remain 11- to 28-fold higher than in children aged 1 year [6]. Additionally, adults with certain comorbid conditions may benefit less than healthier adults from the indirect effects of the pneumococcal conjugate vaccine [7].

Pfizer is developing a 13-valent pneumococcal conjugate vaccine (PCV13; serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) for adults and children to prevent pneumococcal disease caused by the vaccine serotypes. PCV13 has been approved for use in infants and young children in the United States, Europe, and other countries. Like PCV7, PCV13 is manufactured using glycoconjugate technology. By conjugating the purified capsular saccharides of *S. pneumoniae* to an immunogenic protein carrier, the normally T-cell-independent response elicited by free polysaccharides is converted to a T-cell-dependent immune response. In children, PCV7 induces immunologic memory and boosts antibody responses upon repeated vaccination, overcoming the limitations of the

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nonconjugated PPV. Pneumococcal conjugate vaccines, including PCV13, have demonstrated immunogenicity and safety in older adults [4,8,9].

PPV and the trivalent inactivated influenza vaccine are commonly recommended for older adults [10]. The ability to administer both vaccines concomitantly, when appropriate, is an important way to facilitate immunization. Compatibility of the nonconjugated PPV coadministered with the influenza vaccine has been demonstrated previously [10,11].

The current study evaluates the safety and immunogenicity of PCV13 when administered concomitantly with the trivalent inactivated influenza vaccine (TIV) in adults aged ≥65 years who are naïve to PPVs. This study was performed as part of an ongoing program to develop PCV13 for use in adults. It was carried out before the start of a large scale efficacy study to establish the efficacy of PCV13 to prevent a first episode of vaccine serotype-specific pneumococcal community-acquired pneumonia, and to establish a protective antibody level in adults aged ≥65 years in The Netherlands [12]. In the efficacy study, some participants received PCV13 and TIV concomitantly.

2. Patients and methods

This was a parallel-group, randomized, double-blind, multicenter trial conducted at 39 sites (3 hospital clinics and 36 general practices) in Germany, The Netherlands, Belgium, and Hungary. The trial was registered at Clinicaltrials.gov as number NCT00492557.

2.1. Participants

Participants were recruited by infectious disease specialists and general practitioners. Some sites used flyers and office advertisements to draw attention to the study. Potential participants received written information about the study (informed consent document) to review. Before signing the informed consent for study participation, the study physician answered all study-related questions. Participants were not paid for their participation, but were reimbursed for expenses for their travel, parking, and meals. The study vaccines (PCV13 and TIV) were supplied free of charge. All recruitment documents and anticipated costs for reimbursement payments were reviewed and approved by the ethics committees concerned. Participants were enrolled from October 2007 to February 2008. The trial was conducted in accordance with the ethical principles of the Declaration of Helsinki and all participants provided written informed consent before enrollment.

Healthy men and women aged ≥65 years were eligible for enrollment. Participants were ineligible if they had: a history of *S. pneumoniae* infection within the previous 5 years; were previously vaccinated with any pneumococcal vaccine, or vaccinated with influenza- or diphtheria-containing vaccine within 6 months of study vaccine; had received blood products or immunoglobulins within the previous 6 months; had known or suspected immunodeficiency or suppression; had serious chronic illness with pulmonary, renal, or cardiac failure; had evidence of severe cognitive impairment; or were residents in a nursing home or other long-term care facility.

2.2. Interventions

Eligible participants received either PCV13 given concomitantly with TIV (PCV13 + TIV) followed 1 month (day 29–43) later by placebo (PCV13 + TIV/Placebo) or placebo given concomitantly with TIV (Placebo + TIV) followed 1-month (day 29–43) later by PCV13 (Placebo + TIV/PCV13). Vaccinations (0.5-mL dose) were given intramuscularly into the left (PCV13 or Placebo) and right

Table 1
Study schedule.

Group	Month 0 Dose 1	Month 1 Dose 2	Month 2
PCV13 + TIV/Placebo	Blood draw PCV13 + TIV	Blood draw Placebo	Blood draw
Placebo + TIV/PCV13	Blood draw Placebo + TIV	Blood draw PCV13	Blood draw

PCV13, 13-valent pneumococcal conjugate vaccine; TIV, trivalent inactivated influenza vaccine.

(TIV) deltoid muscle. Three blood samples were taken; at baseline and 1 month after each vaccination (Table 1).

2.3. Vaccines administered

PCV13 contains saccharides from pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9 V, 14, 18C, 19A, 19F, and 23F individually conjugated to nontoxic diphtheria toxin cross-reactive material 197 (CRM_{197}). The vaccine is formulated at pH 5.8 with 5 mM succinate buffer, 0.85% sodium chloride and 0.02% polysorbate 80, and is formulated to contain 2.2 μg of each saccharide, except for 4.4 μg of 6B per 0.5-mL dose. The vaccine also contains 0.125 mg aluminum as aluminum phosphate per 0.5-mL dose.

The placebo was formulated similarly, but without the CRM_{197} conjugated pneumococcal saccharides. PCV13 and placebo were filled in identical containers, so that their appearances matched.

The split virion, inactivated TIV (Fluarix® 2007/2008, GlaxoSmithKline Biological SA), contains strains of influenza viruses that are antigenically equivalent to the annually recommended strains of one influenza A/H1N1 virus (15 μg), one A/H3N2 virus (15 μg), and one B virus (15 μg) per 0.5-mL dose of vaccine.

2.4. Study objectives

This study was designed to test whether the immune responses induced by the concomitant administration of PCV13 + TIV to antigens A/H1N1, A/H3N2 and B are noninferior to those induced by TIV alone (TIV + Placebo), and that the immune responses to the PCV13 serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) induced by PCV13 + TIV are noninferior to those induced by PCV13 administered 1 month after TIV.

The safety profile of PCV13 + TIV compared with that of each agent alone was also assessed.

2.5. Immunogenicity assessments

The immune responses induced by PCV13 + TIV were compared with those of TIV alone (Placebo + TIV), as measured by the standard hemagglutination inhibition (HAIs) assays for the TIV strains (A/H1N1, A/H3N2, and B) 1 month after TIV vaccination, and with PCV13 alone in a subset of 605 participants, as measured by a standardized enzyme-linked immunosorbent assay for serotype-specific immunoglobulin G (IgG) 1 month after PCV13 vaccination [13].

For TIV antigens (A/H1N1, A/H3N2, and B), a responder was defined as a participant achieving a ≥4-fold increase in HAI titres from prevaccination to 1 month postvaccination. A comparison between the two treatment groups (PCV13 + TIV relative to Placebo + TIV) was based on the difference in proportions of responders. Noninferiority was declared if the lower limit of the 2-sided 95% confidence interval (CI) for the difference in the proportion of responders between groups ([PCV13 + TIV] – [Placebo + TIV]) was greater than −0.10 consistent with existing literature [14].

Serotype-specific anticapsular polysaccharide IgG geometric mean concentrations (GMCs) were calculated for each of the 13

pneumococcal serotypes. A comparison between the two treatment groups (PCV13 + TIV relative to PCV13) was based on the ratio of GMCs for each of the pneumococcal serotypes. Noninferiority was declared if the lower limit of the 2-sided 95% CI for the GMC ratio ([PCV13 + TIV]:PCV13) was >0.5 (2-fold criterion) calculated 1-month after PCV13 vaccination. PCV13 efficacy data in the adult populations are not yet available. For the purpose of comparing groups administered PCV13 with and without TIV, a 0.5 margin was applied. This definition was considered to be reasonable on the basis of GMC ratios of 2- to 3-fold seen among serotypes, and across several of the infant PCV7 or PCV9 efficacy trials [15]. These differences are not manifested as differences in efficacy among the serotypes. Therefore, geometric mean immune response values that are within a 2–3-fold range are unlikely to manifest as a clinically significant change in the effectiveness of the vaccine. This noninferiority margin was consistent with relevant publications at the time of study design [14].

Additionally, the immune response of PCV13 + TIV was assessed based on the European Medicines Agency (EMA) "Note for Guidance on Harmonisation of Requirements for Influenza Vaccines" [16]. This includes the proportion of responders to A/H1N1, A/H3N2, and B based on a significant increase in antihemagglutinin antibody titre >30%; geometric mean fold rise (GMFR) >2.0; and the proportion of participants achieving an HAI titre ≥40 is >60%.

2.6. Safety assessments

Local reactions (redness, swelling, pain, and limitation of arm movement) at the PCV13 injection site and systemic events, including fever (oral temperature ≥38 °C), chills, fatigue, headache, vomiting, decreased appetite, rash, and new and aggravated generalized muscle or joint pain, and the use of antipyretic and pain medications to treat symptoms, was recorded for 14 days in an electronic diary by the participants. Other adverse events, which were collected by the investigator in response to direct questioning of the subject on his/her health since the last visit, were documented on the case report form at each visit throughout the study; the investigator assessed each adverse event for severity, for serious criteria, and causality.

2.7. Sample size

Sample size estimation was based on the proportion of responders (achieving at least a 4-fold increase in HAI titre) in each group for TIV comparisons, and the GMCs in each group for PCV13 comparisons. Sample sizes were calculated using nQuery Advisor® 6.0 (Statistical Solutions, Ltd., Cork, Ireland). This study was powered to show noninferiority of PCV13 + TIV relative to Placebo + TIV and PCV13 alone.

For TIV comparisons, sample size calculations assumed power of at least 80%; a noninferiority criterion of −0.10 for the difference in proportions of responders; no difference in true responses between the groups ([PCV13 + TIV]–[Placebo + TIV alone]); a 2-sided, type-I error rate of 0.05; and a dropout rate of ≤7%. With these assumptions, 511 evaluable participants per group were needed for TIV comparisons. A total of 1160 participants were randomly assigned to ensure 1022 evaluable participants for TIV comparisons.

For IgG comparisons, sample size calculations assumed power of approximately 90%; 2-fold noninferiority criterion for GMCs; no difference in true responses between the groups ([PCV13 + TIV] – [PCV13 alone]); a 2-sided, type-I error of 0.05; and a dropout rate of ≤7%. With these assumptions, 281 evaluable participants per group were needed for pneumococcal comparisons.

2.8. Randomization

Eligible participants were randomly assigned in a 1:1 ratio to receive PCV13 + TIV/Placebo or Placebo + TIV/PCV13 through the sponsor's internet-based enrollment system. This system was accessed through the internet or an interactive voice-response system by authorized site staff. The randomization schedule used a randomized block design in which treatment sequences were randomly ordered within each block. All participants, study staff, and those assessing outcomes were blinded to the group assignment.

The selection for inclusion in the IgG subset analysis occurred after all participants were enrolled. Participants were randomly ordered within treatment groups and assigned a rank (1, 2, 3, etc.), then chosen in rank order, which facilitated replacement when no sera were available; the next participant in the sequence from the same treatment group was used as a replacement.

2.9. Statistical analysis

The statistical analyses were performed by the sponsor. For the 3 influenza virus subtypes contained in TIV, exact, 2-sided 95% CIs based on the procedure of Chan and Zhang [17] were computed on the difference in proportions of responders ([PCV13 + TIV] − [Placebo + TIV]).

For the comparison of PCV13 + TIV to PCV13, IgG concentrations for each vaccine group and serotype were logarithmically transformed for analysis, and GMC was computed. Corresponding 2-sided 95% CIs for the GMCs were constructed by back transformation of the CI for the mean of logarithmically transformed assay results, which were computed using the Student's t distribution. Noninferiority was evaluated using the ratio of postvaccination GMCs (PCV13 + TIV:PCV13) and corresponding 2-sided 95% CIs, and was declared if the lower limit of the 2-sided 95% CI for the GMC ratio was >0.5. For the GMC ratio, the CI was computed by back transforming the CI for the mean difference of the measures on the natural log scale which used the Student's t distribution.

The fold rises in antibody concentrations from before vaccination to 1 month after vaccination were summarized by geometric means and CIs, and were computed using the logarithmically transformed assay results.

Safety comparisons between groups were based on the 95% CI using Chan and Zhang [17] methodology, with a difference noted between the 2 groups if the 95% CI for the difference excluded zero.

3. Results

3.1. Baseline characteristics and participant disposition

A total of 1190 participants were enrolled. There were 29 screen failures and 1 participant with no signed informed consent. A total of 1160 participants were randomly assigned in a 1:1 ratio to the PCV13 + TIV/Placebo group (n = 580) or Placebo + TIV/PCV13 group (n = 580) (Fig. 1). The evaluable immunogenicity population included 1096 participants (PCV13 + TIV/Placebo group n = 549 and Placebo + TIV/PCV13 group n = 547), each of whom adhered to the protocol requirements, had valid and determinate assay results, and had no other major protocol violations. The all-available immunogenicity population included all participants who had ≥1 valid and determinate assay result. Demographics for the evaluable immunogenicity population are presented in Table 2. IgG analysis was performed in a subset of 605 participants.

The safety population (n = 1151) included any participant who received at least 1 dose of the study vaccine (PCV13 + TIV/Placebo group n = 576 and Placebo + TIV/PCV13 group n = 575).
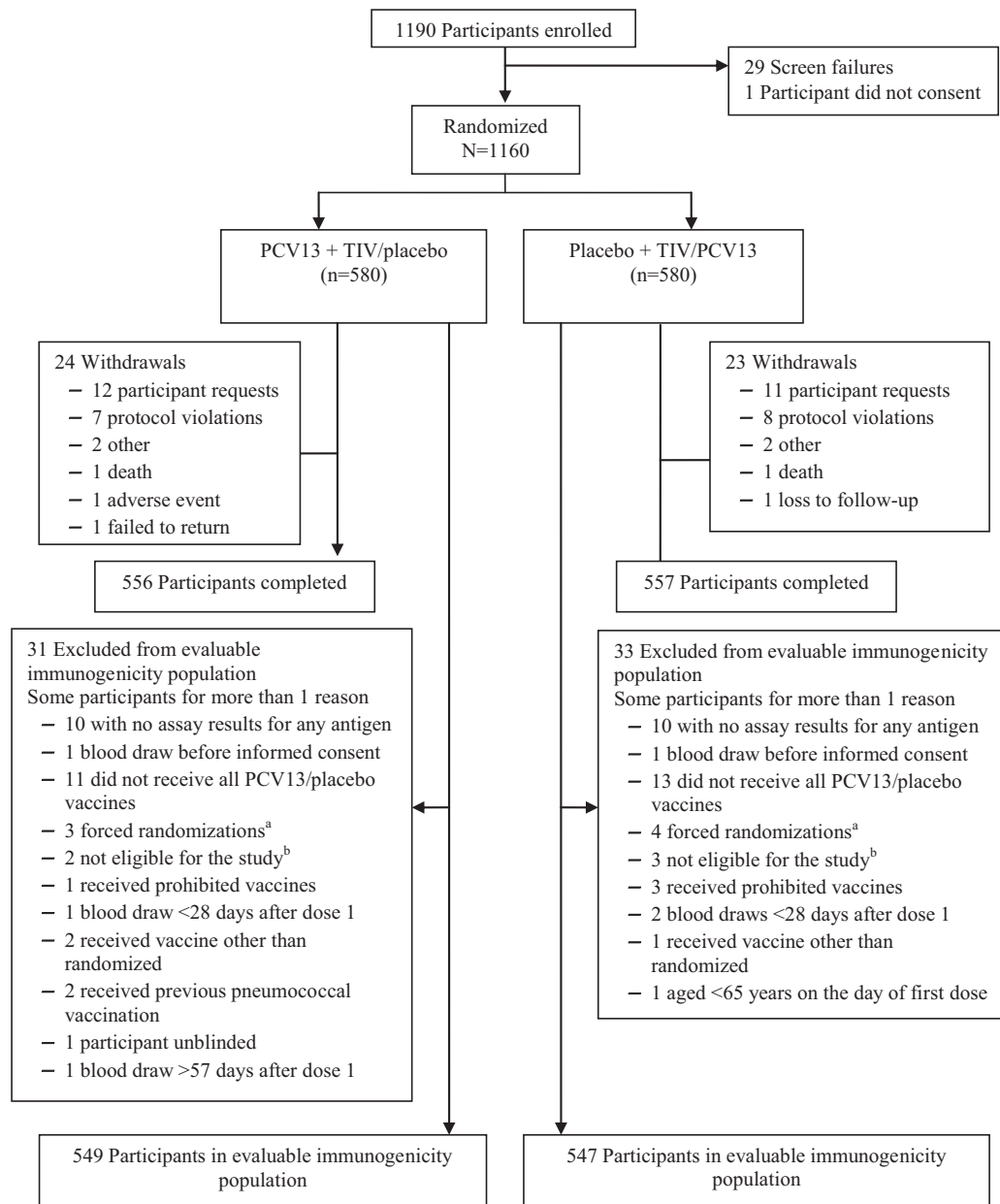
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┌──────────────────────────────┐
│  1190 Participants enrolled  │
└──────────────────────────────┘
                │              ┌──────────────────────────────────┐
                │─────────────▶│ 29 Screen failures               │
                │              │ 1 Participant did not consent    │
                ▼              └──────────────────────────────────┘
       ┌──────────────────┐
       │   Randomized     │
       │    N=1160        │
       └──────────────────┘
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┌─────────────────────────┐              ┌─────────────────────────┐
│  PCV13 + TIV/placebo    │              │  Placebo + TIV/PCV13    │
│      (n=580)            │              │      (n=580)            │
└─────────────────────────┘              └─────────────────────────┘
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┌──────────────────────────────┐        ┌──────────────────────────────┐
│ 24 Withdrawals               │        │ 23 Withdrawals               │
│  − 12 participant requests   │        │  − 11 participant requests   │
│  − 7 protocol violations     │        │  − 8 protocol violations     │
│  − 2 other                   │        │  − 2 other                   │
│  − 1 death                   │        │  − 1 death                   │
│  − 1 adverse event           │        │  − 1 loss to follow-up       │
│  − 1 failed to return        │        │                              │
└──────────────────────────────┘        └──────────────────────────────┘
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┌──────────────────────────────┐        ┌──────────────────────────────┐
│  556 Participants completed  │        │  557 Participants completed  │
└──────────────────────────────┘        └──────────────────────────────┘
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┌──────────────────────────────────────┐  ┌──────────────────────────────────────┐
│ 31 Excluded from evaluable           │  │ 33 Excluded from evaluable immunogenicity│
│ immunogenicity population            │  │ population                           │
│ Some participants for more than 1    │  │ Some participants for more than 1    │
│ reason                               │  │ reason                               │
│  − 10 with no assay results for any  │  │  − 10 with no assay results for any  │
│    antigen                           │  │    antigen                           │
│  − 1 blood draw before informed      │  │  − 1 blood draw before informed      │
│    consent                           │  │    consent                           │
│  − 11 did not receive all PCV13/     │  │  − 13 did not receive all PCV13/     │
│    placebo vaccines                  │  │    placebo vaccines                  │
│  − 3 forced randomizations[a]        │  │  − 4 forced randomizations[a]        │
│  − 2 not eligible for the study[b]   │  │  − 3 not eligible for the study[b]   │
│  − 1 received prohibited vaccines    │  │  − 3 received prohibited vaccines    │
│  − 1 blood draw <28 days after dose 1│  │  − 2 blood draws <28 days after      │
│  − 2 received vaccine other than     │  │    dose 1                            │
│    randomized                        │  │  − 1 received vaccine other than     │
│  − 2 received previous pneumococcal  │  │    randomized                        │
│    vaccination                       │  │  − 1 aged <65 years on the day of    │
│  − 1 participant unblinded           │  │    first dose                        │
│  − 1 blood draw >57 days after dose 1│  │                                      │
└──────────────────────────────────────┘  └──────────────────────────────────────┘
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┌──────────────────────────────────────┐  ┌──────────────────────────────────────┐
│ 549 Participants in evaluable         │  │ 547 Participants in evaluable         │
│ immunogenicity population             │  │ immunogenicity population             │
└──────────────────────────────────────┘  └──────────────────────────────────────┘
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Fig. 1. Participant disposition.
[a]Forced randomization occurred when a participant received study vaccine from the available study vaccine supply without being randomized. The vaccine recipient is then assigned a randomization number for this treatment group. [b]Participants had known or suspected immunodeficiency or suppression. PCV13, 13-valent pneumococcal conjugate vaccine; TIV, trivalent inactivated influenza vaccine.

Table 2
Demographic characteristics for the evaluable immunogenicity population.

	PCV13 + TIV/Placebo (n = 549)	Placebo + TIV/PCV13 (n = 547)	Total (N = 1096)
Sex, n (%)			
Female	276 (50.3)	274 (50.1)	550 (50.2)
Male	273 (49.7)	273 (49.9)	546 (49.8)
Race, n (%)			
White	543 (98.9)	543 (99.3)	1086 (99.1)
Asian	3 (0.5)	2 (0.4)	5 (0.5)
Other	2 (0.4)	1 (0.2)	3 (0.3)
Black or African-American	1 (0.2)	1 (0.2)	2 (0.2)
Mean age at vaccination, years ± SD	72.0 ± 5.5	72.0 ± 5.4	72.0 ± 5.4

PCV13, 13-valent pneumococcal conjugate vaccine; SD, standard deviation; TIV, trivalent inactivated influenza vaccine.

Demographic characteristics in the safety population were similar to those in the evaluable immunogenicity population.

Participants were followed up for approximately 1 month (29–43 days) after each vaccination.

3.2. Immunogenicity

3.2.1. Response to TIV

The proportions of responders (participants achieving a ≥4-fold increase in HAI titre for each TIV subtype) were similar after PCV13 + TIV compared with Placebo + TIV for A/H1N1 (80.3% and 78.6%, respectively), A/H3N2 (58.0% and 62.6%, respectively), and B (52.2% and 54.0%, respectively). Noninferiority (lower limit of the 95% CI greater than −10%) was met for A/H1N1 and B. For A/H3N2, the difference in the proportions of responders was −4.6%, with a lower limit of the 95% CI of −10.4% (Table 3). The proportion of responders in the PCV13 + TIV group for A/H1N1 (80.3%), A/H3N2 (58.0%), and B (52.2%) exceeded the EMA guidance value of >30% (Table 3) [16].

The HAI geometric mean titres (GMTs) were similar in the 2 groups (PCV13 + TIV compared with Placebo + TIV) at baseline and rose substantially after vaccination. Of note, baseline HAI GMTs for A/H3N2 in both groups was higher than those for A/H1N1 and B in both groups (Table 4). The GMFR in the PCV13 + TIV group was ≥4.1 and exceeded the EMA guidance value of >2.0 [16].

The proportion of participants achieving HAI titres ≥40 for A/H1N1, A/H3N2, and B in the PCV13 + TIV group exceeded the EMA guidance value of >60% (Table 5) [16].

3.2.2. Response to PCV13

Baseline antibody GMC for pneumococcal serotypes ranged from 0.21 µg/mL (serotype 4) to 2.67 µg/mL (serotype 19A) in the PCV13 + TIV group, and 0.19 µg/mL (serotype 4) to 2.77 µg/mL (serotype 19A) in the Placebo + TIV group (data not shown). One month after administration of PCV13 in each group, the overall IgG GMCs were lower in the PCV13 + TIV group relative to the PCV13 group (administered 1 month after Placebo + TIV). The noninferiority criterion for IgG GMC ratios was met for all serotypes except 19F, for which the lower limit of the 95% CI was 0.49, just below the predetermined lower limit of >0.5 (2-fold criterion) (Table 6).

3.3. Safety

Local reactions at the pneumococcal injection site were similar after PCV13 + TIV relative to after PCV13 (administered 1 month after Placebo + TIV) and were 46.9% and 46.6%, respectively; the majority was mild (Table 7).

Systemic events were reported more frequently after PCV13 + TIV relative to Placebo + TIV (60.1% vs. 50.5%), with statistical evidence of a percentage difference between the two groups for any systemic event (95% CI 3.4; 15.7), chills (95% CI 0.5; 8.9), rash (95% CI 0.4; 6.6), and new muscle pain (95% CI 4.9; 15.6) (Table 8).

Systemic events were reported more frequently after PCV13 + TIV relative to PCV13 alone (60.1% vs. 48.5%), with statistical evidence of a percentage difference for any systemic event (95% CI 5.4; 17.8), fatigue (95% CI 2.8; 14.9), headache (95% CI 2.1; 13.8), chills (95% CI 0.4; 9.0), decreased appetite (95% CI 1.0; 10.2), new joint pain (95% CI 0.1; 9.2), and any aggravated joint pain (95% CI 2.7; 11.4) (Table 8).

Overall, fever rates were low and fever was mild or moderate in severity. There were no vaccine-related serious adverse events (SAEs) during the study. One SAE (angina pectoris with ST-segment elevation on day 10) after placebo in the PCV13 + TIV/Placebo group caused withdrawal of a participant. Two SAEs resulted in death; 1 case of cardiac failure on day 3 after placebo

(PCV13 + TIV/Placebo) and 1 case of gastrointestinal hemorrhage with perforated duodenal ulcer and peritonitis on day 29 after PCV13 (Placebo + TIV/PCV13).

4. Discussion

In this study, which included predominantly white adults aged ≥65 years who were naïve to PPV, the immunogenicity and safety responses to the three viral subtypes in TIV (A/H1N1, A/H3N2, and B) and each of the 13 serotypes (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) in PCV13 after concomitant administration of PCV13 and TIV were directly compared with TIV (and placebo) or PCV13 administered after TIV.

A clinically meaningful, empirically determined level of antibodies against pneumococcal or influenza antigens that is protective against disease in adults is lacking. A correlation between antibody levels and protection against invasive pneumococcal disease was demonstrated previously in children [18]. Therefore, as in most vaccine trials, the endpoints of the present trial were based on a comparison of the relative changes in immune response between administration of the vaccines separately or together [19–21]. For TIV antigens, the immune response correlates of protection are considered to be acceptable levels of serum antibody to the individual vaccine hemagglutinins as measured by HAI and described in "Note for Guidance on Harmonisation of Requirements for Influenza Vaccines" [16].

The analysis of TIV (A/H1N1, A/H3N2, and B) immune responses, based on the proportion of responders achieving at least a 4-fold rise in HAI titre, showed that noninferiority of PCV13 + TIV relative to TIV was met for A/H1N1 and B; for A/H3N2, the difference in proportions of responders was −4.6%, with a lower limit of the 95% CI of −10.4%, which was slightly lower than the more than −10.0% predefined margin of noninferiority. However, it was noted that in contrast with the other two virus subtypes, the mean predose-1 titres for A/H3N2 were quite high, perhaps reflecting pressure from A/H3N2 epidemics that occurred in the years prior to the study. In the regions where the study was conducted, H3N2 predominated over H1N1 and B in the 2006–2007 season [22]. Higher pre-immunization titres may limit the likelihood of demonstrating 4-fold responses, and the lower frequency of response would be expected to impact the ability to demonstrate noninferiority. Notably, H3N2 responder rates at an HAI titre ≥40 were comparable in the PCV13 + TIV and Placebo + TIV groups, indicating a high likelihood of protection against H3N2. In fact, all criteria proposed in the EMA "Note for Guidance on Harmonisation of Requirements for Influenza Vaccines" [16] were exceeded for all three TIV antigens (H1N1, H3N2, and B) when TIV was administered with PCV13. The data support the conclusion that TIV is sufficiently immunogenic when given concomitantly with PCV13, and that protection against influenza is likely to be clinically indistinguishable from that provided by TIV alone.

The IgG GMCs of the 13 serotypes after PCV13 + TIV were uniformly lower compared with the responses after PCV13 alone. However, the lower responses were still within the 2-fold GMC criterion for noninferiority for all pneumococcal serotypes, with the exception of 19F, which was just below the noninferiority margin. The lower immune response observed by concomitant administration of these vaccine antigens is not easily understood. Such interactions are thought to be caused by complex, multi-factorial interactions, including antigen competition, and the effects of other vaccine components on the immune response [23]. A possible mechanism could be that vaccine antigens interfere with the MHC class I and II antigen processing and presentation pathways, leading to a uniformly reduced response to PCV13 serotypes [24]. Further research is required to better understand this phenomenon.

Table 3
Proportion of participants with a ≥4-fold increase in titer after dose 1 for TIV antigens—evaluable immunogenicity population.

	PCV13 + TIV/Placebo		Placebo + TIV/PCV13		Difference
	n/N	% (95% CI)	n/N	% (95% CI)	% (95% CI)
A/H1N1	440/548	80.3 (76.7; 83.5)	429/546	78.6 (74.9; 81.9)	1.7 (−3.1; 6.5)
A/H3N2	316/545	58.0 (53.7; 62.2)	341/545	62.6 (58.4; 66.6)	−4.6 (−10.4; 1.3)
B	286/548	52.2 (47.9; 56.4)	295/546	54.0 (49.7; 58.3)	−1.8 (−7.8; 4.1)

CI, confidence interval; n, number of participants with an antibody titer that met the prespecified criteria; N, number of participants with a determinate antibody titer for the given concomitant vaccine antigen; PCV13, 13-valent pneumococcal conjugate vaccine; TIV, trivalent inactivated influenza vaccine.

Table 4
Standard hemagglutination inhibition assay GMTs before and after dose 1 and GMFR after dose 1—evaluable immunogenicity population.

TIV antigen vaccine received	Predose 1		Postdose 1		Postdose 1/predose 1	
	n	GMT (95% CI)	n	GMT (95% CI)	n	GMFR (95% CI)
A/H1N1						
PCV13 + TIV	548	21.6 (19.9; 23.6)	548	195.5 (176.6; 216.4)	548	9.0 (8.1; 10.1)
Placebo + TIV	547	22.5 (20.5; 24.6)	546	191.9 (173.4; 212.5)	546	8.5 (7.7; 9.5)
A/H3N2						
PCV13 + TIV	545	62.9 (55.8; 70.9)	545	327.4 (293.5; 365.2)	545	5.2 (4.6; 5.9)
Placebo + TIV	546	65.3 (57.5; 74.1)	545	413.2 (370.5; 460.8)	545	6.3 (5.5; 7.2)
B						
PCV13 + TIV	548	22.3 (20.4; 24.3)	548	90.4 (81.6; 100.2)	548	4.1 (3.7; 4.5)
Placebo + TIV	547	22.5 (20.6; 24.6)	546	88.5 (79.8; 98.1)	546	3.9 (3.6; 4.3)

GMFR, geometric mean fold rise; GMT, geometric mean titer; n, number of participants with valid and determinate assay results at the given visit; PCV13, 13-valent pneumococcal conjugate vaccine; TIV, trivalent inactivated influenza vaccine.

Table 5
Proportion of participants achieving hemagglutination inhibition assay titer ≥40 after dose 1—evaluable immunogenicity population.

TIV antigen	PCV13 + TIV/Placebo		Placebo + TIV/PCV13	
	n/N	% (95% CI)	n/N	% (95% CI)
A/H1N1	515/548	94.0 (91.6; 95.8)	514/546	94.1 (91.8; 96.0)
A/H3N2	526/545	96.5 (94.6; 97.9)	531/545	97.4 (95.7; 98.6)
B	449/548	81.9 (78.5; 85.1)	444/546	81.3 (77.8; 84.5)

N, number of participants with valid and determinate antibody titer for the concomitant vaccine antigen; n, number of participants with an antibody titer that met the prespecified level; PCV13, 13-valent pneumococcal conjugate vaccine; TIV, trivalent inactivated influenza vaccine.

Local reactions at the PCV13 injection site were comparable. Although systemic events were more common after PCV13 + TIV relative to TIV or PCV13 alone, this is probably because of the additive effects of both TIV and PCV13 systemic events. Overall, fever rates were low, and there were no vaccine-related SAEs during the study.

Although immune responses to vaccine antigens were observed after receipt of both vaccines, the lack of knowledge about the threshold level of antibodies needed to protect against pneumococcal disease in adults is a limitation of the study. The results from the efficacy study of PCV13 being conducted in adults aged ≥65 years in The Netherlands are awaited to help establish an

Table 6
Comparison of pneumococcal IgG GMC 1 month after dose 1 for PCV13 + TIV/Placebo and 1 month after dose 2 for Placebo + TIV/PCV13—evaluable immunogenicity population.

Serotype	Postdose 1[a] PCV13 + TIV/Placebo		Postdose 2[a] Placebo + TIV/PCV13		Vaccine comparison
	n[b]	GMC, μg/mL	n[b]	GMC, μg/mL	Ratio (95% CI)
1	276	2.52	270	3.20	0.79 (0.60; 1.04)
3	272	1.08	273	1.15	0.94 (0.78; 1.13)
4	279	2.15	278	3.24	0.66 (0.51; 0.87)
5	253	4.74	255	6.90	0.69 (0.55; 0.86)
6A	272	4.61	264	6.10	0.76 (0.61; 0.94)
6B	278	6.24	264	6.43	0.97 (0.75; 1.25)
7F	273	7.63	253	9.04	0.84 (0.67; 1.07)
9V	250	4.97	247	6.21	0.80 (0.63; 1.02)
14	272	8.95	277	12.44	0.72 (0.53; 0.97)
18C	247	8.88	261	11.07	0.80 (0.64; 1.01)
19A	266	11.93	255	17.10	0.70 (0.56; 0.87)
19F	277	4.78	276	7.39	0.65 (0.49; 0.85)
23F	277	5.82	265	6.11	0.95 (0.71; 1.27)

[a] Comparisons were made 1 month (i.e. 29–43 days) after participants received PCV13; this occurred 1 month (29–43 days) after dose 1 in the PCV13 + TIV/Placebo group and 1 month (29–43 days) after dose 2 in the Placebo + TIV/PCV13 group.
[b] Number of participants with valid and determinate antibody concentrations for each serotype.
GMC, geometric mean concentration; IgG, immunoglobulin G; n, number of participants with valid and determinate assay results at the given visit; PCV13, 13-valent pneumococcal conjugate vaccine; TIV, trivalent inactivated influenza vaccine.

Table 7
Percentage of participants with local reactions at the PCV13 injection site within 14 days after vaccination—safety population.

Reaction	PCV13 + TIV % (n/N)	PCV13 alone % (n/N)	Difference (%) (95% CI[a])
Any	46.9 (229/488)	46.6 (219/470)	0.3 (−6.0; 6.7)
Redness	16.6 (73/440)	12.3 (53/432)	4.3 (−0.4; 9.0)
Swelling	13.8 (61/441)	10.2 (44/431)	3.6 (−0.7; 8.0)
Pain	40.0 (192/480)	43.4 (204/470)	−3.4 (−9.7; 3.0)
Limitation of arm movement	13.9 (62/445)	14.8 (64/432)	−0.9 (−5.6; 3.8)

[a] Exact 2-sided CI for the difference in proportions, (PCV13 + TIV) − (Placebo + TIV/PCV13), expressed as a percentage.
CI, confidence interval; PCV13, 13-valent pneumococcal conjugate vaccine; TIV, trivalent inactivated influenza vaccine.

Table 8
Percentage of participants with systemic events within 14 days of vaccination—Safety population.

Systemic events	PCV13 + TIV % (n/N)	Placebo + TIV % (n/N)	Difference % (95 CI) PCV13 + TIV vs. Placebo + TIV	PCV13 % (n/N)	Difference % (95 CI) PCV13 + TIV vs. PCV13
Any fever ≥38 °C	4.2 (18/431)	3.2 (14/433)	0.9 (−1.7; 3.6)	3.6 (15/422)	0.6 (−2.1; 3.3)
Fever ≥38 °C but <38.5 °C	3.0 (13/430)	1.9 (8/431)	1.2 (−1.0; 3.4)	3.1 (13/422)	−0.1 (−2.5; 2.4)
Fever ≥38.5 °C but <39 °C	1.4 (6/425)	1.2 (5/430)	0.2 (−1.5; 2.0)	1.0 (4/420)	0.5 (−1.2; 2.2)
Fever ≥39 °C but ≤40 °C	0 (0/423)	0.2 (1/428)	−0.2 (−1.3; 0.6)	0 (0/419)	0.0 (−0.9; 0.9)
Fever >40 °C	0 (0/423)	0 (0/428)	0 (−0.9; 0.9)	0 (0/419)	0 (−0.9; 0.9)
Fatigue	37.4 (178/476)	31.9 (154/483)	5.5 (−0.5; 11.5)	28.5 (130/456)	8.9 (2.8; 14.9)
Headache	32.6 (154/472)	29.7 (139/468)	2.9 (−3.0; 8.9)	24.7 (111/449)	7.9 (2.1; 13.8)
Chills	13.8 (61/443)	9.1 (40/440)	4.7 (0.5; 8.9)	9.1 (39/429)	4.7 (0.4; 9.0)
Rash	6.9 (30/433)	3.4 (15/436)	3.5 (0.4; 6.6)	6.8 (29/427)	0.1 (−3.3; 3.6)
Vomiting	3.0 (13/432)	3.4 (15/437)	−0.4 (−2.9; 2.0)	1.7 (7/424)	1.4 (−0.7; 3.6)
Decreased appetite	16.9 (76/450)	14.6 (66/452)	2.3 (−2.5; 7.1)	11.3 (49/434)	5.6 (1.0; 10.2)
New muscle pain	26.9 (126/468)	16.7 (76/456)	10.3 (4.9; 15.6)	23.4 (105/448)	3.5 (−2.2; 9.1)
Any aggravated muscle pain	18.7 (85/454)	14.0 (63/449)	4.7 (−0.2; 9.6)	15.0 (66/439)	3.7 (−1.2; 8.7)
New joint pain	16.2 (73/452)	13.1 (59/451)	3.1 (−1.6; 7.7)	11.5 (50/435)	4.7 (0.1; 9.2)
Any aggravated joint pain	15.7 (71/452)	13.0 (58/447)	2.7 (−1.9; 7.4)	8.6 (37/428)	7.1 (2.7; 11.4)
Any systemic event	60.1 (306/509)	50.5 (254/503)	9.6 (3.4; 15.7)	48.5 (236/487)	11.7 (5.4; 17.8)

CI, confidence interval; PCV13, 13-valent pneumococcal conjugate vaccine; TIV, trivalent inactivated influenza vaccine.

effective antibody level against pneumococcal disease in adults [12].

Overall, the concomitant administration of PCV13 and TIV was demonstrated to be immunogenic and safe. If PCV13 is determined to add value in a comprehensive immunization strategy against pneumococcal disease, the ability to coadminister PCV13 and TIV would facilitate the immunization of older adults.

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