CLINICAL STUDY REPORT

A follow-on, multi-centre, open-label, clinical, phase 4 trial to investigate the persistence of serotype-specific antibodies at 40 months of age in children who have received either' the 7-valent or the 13-valent pneumococcal conjugate vaccine at 2,4 and 12 months of age and assessing the immunogenicity of a 13-valent pneumococcal conjugate vaccine booster dose given at 40 months of age.

Protocol Number:	OVG 2009/04
Ethics Approval:	Oxfordshire Research Ethics Committee C OxREC Ref: 10/H0606/9
Eudract Number	2009-017498-39
Investigational products:	Prevenar 13 – *Streptococcus pneumoniae* serotypes 1,3,4,5,6A,6B,7F,9V,14,18C,19A, 19F,23F individually conjugated to CRM_{197} Manufactured by: Pfizer, EU/1/09/590/0016, First authorisation 09/12/2009
Indication:	Active immunisation for the prevention of invasive disease, pneumonia and acute otitis media caused by *S. pneumoniae* in infants and children from 6 weeks to 5 years of age.
Sponsor	University of Oxford
Developmental Phase:	Phase IV
Study Initiation:	May 2010
Study Completion:	December 2010
Investigator:	Professor Andrew Pollard
Date of the report:	29/05/2012
Author of the report:	Dr Johannes Truck

Contents

1. List of Abbreviations and Definitions of Terms

AE	Adverse Event
CI	Chief Investigator
GCP	Good Clinical Practice
GMC	Geometric Mean Concentration
GMT	Geometric Mean Titres
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
IgG	Immunoglobulin G
OPA	Opsonophagocytic Assay
PCV-7	7-valent Pneumococcal Vaccine
PCV-13	13-valent Pneumococcal Vaccine
PI	Principal Investigator
REC	Research Ethics Committee
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions

2. Study administrative structure

2.1. Principal investigator

Andrew J Pollard FRCPCH PhD,

Professor of Paediatric Infection & Immunity

University of Oxford, Oxford, United Kingdom

2.2. Study Sites

1) Oxford Vaccine Group, Department of Paediatrics, University of Oxford, UK
2) Bristol Children's Vaccine Centre, Department of Paediatrics, University of Bristol, UK
3) St. George's Vaccine Institute, University of London, UK
4) Wellcome Trust Clinical Research Facility, University of Southampton, UK

2.3. Staff involved in the conduct of the study

The trial was administered by employees of The Oxford Vaccine Group, Department of Paediatrics, University of Oxford. Study monitoring was administered by employees of the Oxford Vaccine Group, Department of Paediatrics, University of Oxford.

Monitors examined the study files on a periodic basis and performed verification of source documentation for each participant.

Table 1 lists persons whose participation materially affected the conduct of the study.

Table 1 Staff and Responsibilities

Activity	**Name**
Chief Investigator	Prof Andrew Pollard
Investigators	Dr Matthew Snape (Oxford) Prof Adam Finn (Bristol) Dr Saul Faust (Southampton) Dr Paul Heath (London)
Study Monitors	Simon Kerridge, Lucy Nicklin
Laboratory Assays	Dr Johannes Truck, Dr Elizabeth Clutterbuck, Jaclyn Barel, Amber Thompson

Lead Research Doctors	Dr Florencia Tatangeli Dr Clarissa Oeser (London) Dr Jennifer Oliver (Bristol) Dr Woolfe Walker (Southampton)
Lead Research Nurses	Lily Norman (Oxford) Sandra Dymond (Bristol) Emma Macleod (Southampton) Nigel Butter (London)
Clinical Trials Assistants	Emma Godfrey, Rebecca Beckley
Administration	Shirley Ashmore, Saima Khalid

Table 2 lists the laboratories which performed immunological assays for the study.

Table 2 Central Laboratories for Immunological Assays

Immunological Assay	**Central Laboratory**
ELISA and OPA	Pfizer, Inc. 401 N. Middletown Rd., Pearl River NY 10965 USA
Serum separation, ELISpot	Paediatric Infection and Immunity Laboratory Centre for Clinical Vaccinology and Tropical Medicine Churchill Hospital Oxford, UK

3. Protocol synopsis

The encapsulated bacterium Streptococcus pneumoniae (pneumococcus) is responsible for a considerable burden of disease in young children causing invasive disease including meningitis as well as localised infections such as otitis media, pneumonia and others. More than 14 million episodes of serious pneumococcal disease and about 800 000 deaths in children under the age of five occur annually. In the elderly, pneumonia is the most common manifestation affecting 10 per 1000 individuals over 65, and having a significant morbidity with community-acquired pneumonia ending up in log-term institutionalised care. Furthermore, the mortality of IPD is much higher than in other age groups and can be as high as 37% in those over 80 years of age. The health, social and economic burden of pneumococcal infections increase year on year as the population continues to age at a rate that shows no signs of slowing.

Healthy preschool children frequently carry pneumococci in the nasopharynx. The vast majority of these children remain asymptomatic and resulting pneumococcal disease is very rare. However, these children are thought to be the main transmitters of pneumococci to unvaccinated and susceptible individuals including neonates and the elderly, which may result in disease in these populations. Maintaining adequate antibody levels in preschool children is the key of sustained direct protection of vaccinated individuals as well as achieving high levels of herd immunity as both are presumed to be mediated by antibody. Pneumococcal conjugate vaccines (PCV) are highly effective in preventing disease and the carriage of the serotypes contained in the vaccine. So far, research has focussed on direct protection of the vaccinated individual although using a schedule intended to optimise population immunity might further reduce morbidity and mortality in the (unvaccinated) population, especially the elderly. Previous immunogenicity studies showed that with the currently used immunisation schedules immunity wanes rapidly during the 2nd year of life and will presumably fall thereafter. However, none of the published studies assessed the persistence of antibody beyond the second year of life in children vaccinated with any PCV immunisation schedule. Data from conjugate vaccines against other polysaccharide-encapsulated organisms suggest that even after several doses of vaccine effectiveness declines rapidly along with waning antibody levels.

Several UK sites have previously taken part in a parallel-group, randomised, active-controlled, double-blind, multicentre trial to evaluate the safety, tolerability and immunogencity of a 13-

valent pneumococcal conjugate vaccine (PCV-13) in healthy infants given with routine paediatric vaccinations, which lead together with results from other studies to the licensure of this vaccine followed by its implementation into the routine immunisation schedules of many countries.

We evaluated in a follow-on of this trial the persistence of serotype-specific antibody up to around 3,5 years of age in individuals having received either the 7-valent pneumococcal conjugate vaccine (PCV-7) or PCV-13 in a 2+1 schedule at 2, 4 and 12 months of age. In addition, we investigated the immunogenicity of a PCV-13 vaccine booster at this age comparing individuals having been primed with either vaccine.

4. Study Design

Eligible study participants were recruited at the age of approximately 3.5 years having previously participated in a randomised controlled trial (RCT) comparing the immunogenicity of either a 13-valent pneumococcal conjugate vaccine (Prevenar 13®, Pfizer) or a 7-valent pneumococcal conjugate vaccine (Prevenar®, Pfizer) given at 2, 4 and 12 months of age. In the current study a booster dose of PCV-13 was given to all children and serum antibodies for PCV-13 serotypes were measured before and 1 month after the booster. All laboratory personnel were blinded to the type of vaccine these children have received in the initial study.

5. Ethical conduct of the study

5.1. Ethics Committee

Prior to study commencement, the Investigator provided the Oxfordshire Research Ethics Committee C and the Sponsor with all appropriate material, including the protocol, informed consent form, other written participant information and advertising material. The trial was only initiated once written OxREC C approval had been received by the Investigator and Sponsor. The Investigator submitted all subsequent protocol amendments to the OxREC C and the Sponsor.

5.2. GCP compliance

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki (last amended October 2000, with additional footnotes added 2002 and 2004), in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical

Practice (CPMP/ICH/135/95) July 1996 and regulatory requirements in accordance with the United Kingdom's MHRA Medicines for Human Use (Clinical Trials) Regulations, 2004.

The study was conducted in accordance with procedures identified in the protocol which was reviewed and approved by the OxREC. The study was conducted by scientifically and medically qualified persons. Standard Operating Procedure (SOPs) were used at all clinical and laboratory sites. Regular monitoring was performed according to ICH GCP. Following written SOPs, the monitors verified that the clinical trial was conducted and data generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The Investigator site provided direct access to all trial related source data/documents and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

5.3. Subject Information and Consent

Written informed consent, in accordance with the principles of the Declaration of Helsinki, ICH-GCP, and applicable UK regulations, was obtained from the parent/guardian of each participant prior to entering the participant into the trial. A copy of the signed informed consent form was provided to the participant and a copy was kept by the Investigator in the participant's clinical trial record.

The Investigator ensured that the participant's anonymity was maintained. The participant was identified only by initials and a participant's ID (enrolment) number on the CRF. All documents are being stored securely and kept in strict confidence in compliance with the Data Protection Act.

6. Regulatory approval

Initial approval was obtained from OXREC C on 18th March 2010. An overview of subsequent substantial amendments is listed in Table 3 below.

Table 3 Overview of substantial amendments to the study protocol

No.	Date of submission	Summary of amendment	Date of approval
1	6th April 2010	Study timelines amended to study. Background to the study amended to reflect recent incorporation of the 13-valent pneumococcal conjugate vaccine into the routine infant immunisation schedule in the UK. Incorporation of the possibility that sites other than Oxford will be able to provide samples for B cell analysis, by the use of frozen blood samples Changes to the methods of informing parents of their child's results from the previous study and inviting them to participate in this new study; this has now been changed to contacting all families by mail with a follow-up telephone call shortly after. The refinement of temporary exclusion criteria relating to receipt of recent immunisations, to distinguish the exclusion period for live vaccines (remains at 28 days) and non-live vaccines (reduced to 7 days). Change to the needle to be used for vaccine administration to 0.6 x 25 mm 23 gauge needle; the previous needle size of 21 gauge was written in error. Change to the handling of blood samples from participants whose B cell response to immunisation is to be assessed, such that for these participants any blood sample up to 5ml will be collected into 'red top' serum separator tubes and any sample beyond 5ml will be stored in a heparinised tube for B cell analysis. The definition of a lack of response to the PCV13 vaccine received in this study has been formalised to state that this	15th April 2010

		refers to a PCV13 serotype specific IgG < 0.35 mcg/ml.	
2	27th April 2010	As PCV13 is now a licensed vaccine in the UK, it is felt that a safety review by a study specific Data Monitoring Committee was no longer required for this study. Instead review of any SAEs will be performed by the Oxford Radcliffe Hospitals Trust / University of Oxford Trial Safety Committee (TSG). The B cell section of laboratory methods has been altered to allow the analysis of B cell responses to vaccines received in the study to be performed at local study sites as well as at Oxford.	21st June 2010

7. Study participants

7.1. Recruitment

Out of 191 potential participants 108 children were enrolled into the study and received the booster vaccine. 1 child withdrew consent before visit 2 and 1 child was lost to follow-up leaving 106 participants completing visit 2 (Figure 1). At visit 1, age and time since last pneumococcal conjugate vaccination at 12 months of age did not differ significantly between the two study groups. Similarly, the proportion of study participants being female or White Caucasian (the dominant ethnicity) and the time interval between Visit 1 and Visit 2 were similar in both groups (Table).

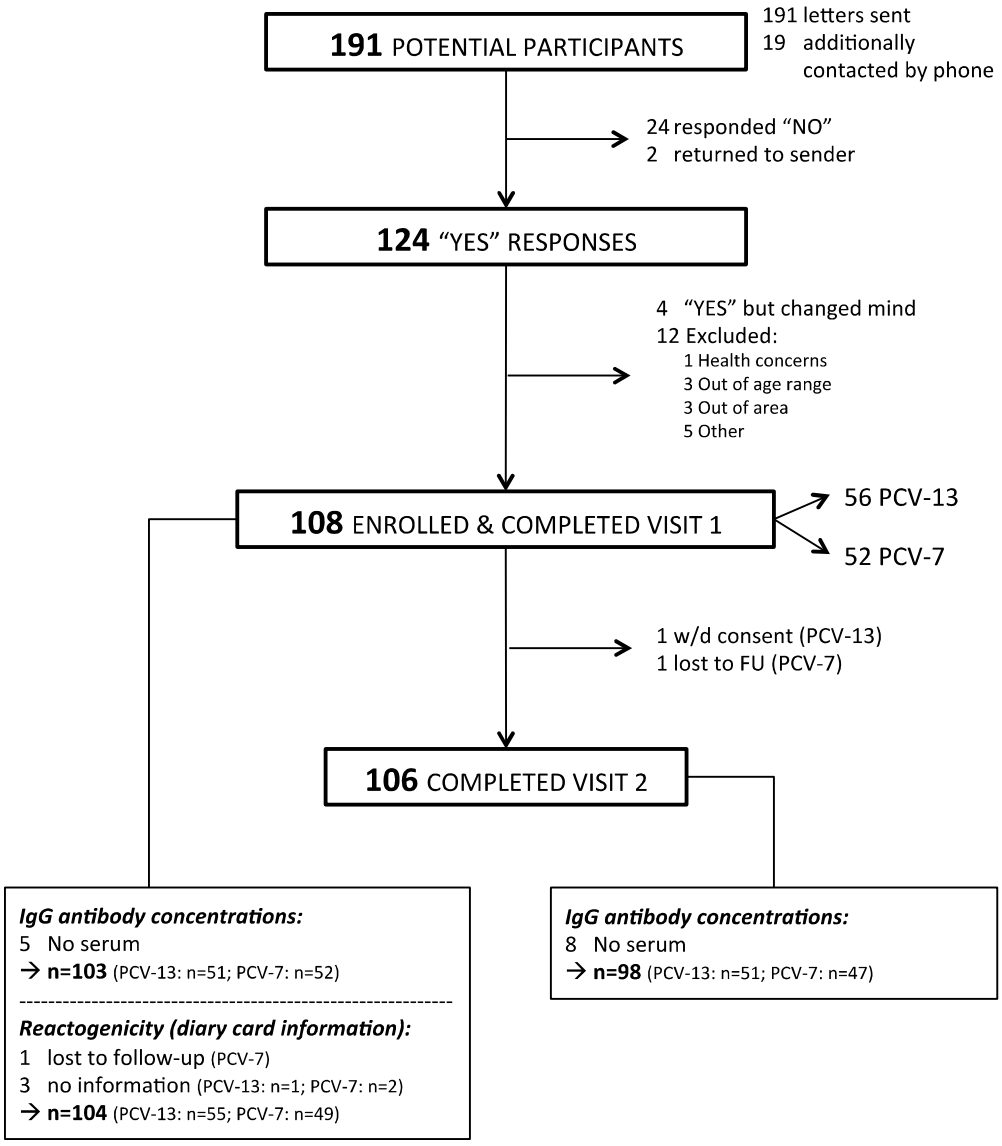

Figure 1 Flow chart of participants through the study

	PCV-13 (N=56)	PCV-7 (N=52)
Proportion of female study participants	28 (50%)	28 (54%)
Proportion of White Caucasian study participants	54 (96%)	48 (92%)
Mean age at Visit 1 in years (range)	3.50 (3.16 - 4.09)	3.51 (3.15 - 4.04)
Mean time since 12-month booster to Visit 1 in years (range)	2.49 (2.13 - 3.07)	2.51 (2.15 - 2.97)
Mean time interval between Visit 1 - Visit 2 in days (range)	34.9 (26.5 - 56.5)	35.0 (27.6 - 42.6)

Table 4 Summary of study participants' characteristics

8. Eligibility criteria

8.1. Inclusion Criteria:

Participants must meet the following conditions in order to be enrolled:

- Participant completed the Wyeth-sponsored PCV13 infant trial study (6096A1-007) at one of the study sites participating in this follow-on study.
- Aged 39-46 months (inclusive) at time of enrolment.
- Available for entire study period and whose parent/legal guardian can be reached by telephone.
- Healthy children as determined by medical history, physical examination, done by a study nurse (and/or study doctor if required, depending on the medical history of the participant and physical assessment), and judgment of the investigator.
- Parent/legal guardian must be able to complete all relevant study procedures during study participation.

9. Exclusion Criteria:

Participants with any of the following conditions or characteristics will be excluded from study enrolment:

- Has received further doses of pneumococcal vaccination with licensed or investigational pneumococcal vaccine other than those given as part of the Wyeth-sponsored PCV13 infant trial study (6096A1-007).
- A previous anaphylactic reaction to any vaccine or vaccine-related component.
- Contraindication to vaccination with pneumococcal conjugate vaccine.
- Bleeding diathesis or condition associated with prolonged bleeding time that would contraindicate intramuscular injection.
- Known or suspected immune deficiency or suppression.
- History of culture-proven invasive disease caused by *S. pneumoniae*.
- Major known congenital malformation or serious chronic disorder.

- Significant neurologic disorder or history of seizures including febrile seizure, or significant stable or evolving disorders such as cerebral palsy, encephalopathy, hydrocephalus, or other significant disorder.
- Receipt of blood products or gamma-globulin (including hepatitis B immunoglobulin and monoclonal antibodies; eg, synagisB).
- Participation in another investigational study other than the Wyeth-sponsored PCV13 infant trial study (6096A1-007). Participation in purely observational studies is acceptable.
- Child who is a direct descendant (child, grandchild) of the study site personnel.

10. Study vaccines

10.1. Vaccines and interventions

PCV-13 is formulated in a similar manner to the PCV-7. It contains saccharides from pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F with each of the polysaccharides being covalently conjugated to a carrier protein, a nontoxic mutant of the diphtheria toxin called CRM197. The concentration of each saccharide is 2.2µg, except for polysaccharide 6B (4.4 µg); all in a 5.0 mM succinate with 0.125 mg of aluminium as aluminium phosphate per 0.5-mL dose. All vaccines were presented in prefilled syringes by Pfizer. PCV-13 was administered to all participants intramuscularly into the deltoid muscle during visit 1. Routine preschool booster immunisations were also administered to those who required it following the UK immunization programme at V2. The vaccines used were Infanrix-IPV (GlaxoSmithKline Biologicals) containing diphtheria and tetanus toxoid, pertussis toxoid, pertactin, filamentous haemagglutinin and inactivated poliovirus types 1, 2 and 3; and MMR Vaxpro (Sanofi Pasteur-MSD) containing live, attenuated measles, mumps and rubella viruses. These vaccines were administered intramuscularly in opposite deltoid muscles at 0.5 mL per dose.

10.2. Vaccine Accountability

The study vaccines Prevenar 13 were supplied by Pfizer, in accordance with OVG SOP 001 Version 3 Receiving vaccine supplies. The vaccines were stored between +2 and +8°C within the OVG vaccine fridge at the CCVTM in accordance with the manufacturer's instructions and OVG SOP 002 'Clinical Trials Vaccine Storage'. All vaccine doses were accounted for within an accountability log.

Unused vaccines were disposed of in accordance with OVG SOP 45 'Disposal of Vaccines' at the end of the trial.

10.3. Compliance with dosing regime

All vaccines were to be administered by the Investigator, recorded in the CRF and verified by a second team member. The study medication was at no time in the possession of the participant and compliance therefore was not an issue.

11. Study Objectives

11.1. Primary Objective

To assess the proportion of participants, immunised with the 13-valent pneumococcal conjugate vaccine (PCV13) at 2, 4 and 12 months of age, who have IgG concentrations ≥ 0.35mcg/ml for PCV13 serotypes at the time when preschool booster vaccinations are due (at 40 months* of age).

11.2. Secondary Objectives

- To assess the proportion of participants, immunised with the 7-valent pneumococca conjugate vaccine (PCV7) at 2, 4 and 12 months of age, who have IgG concentrations ≥ 0.35mcg/ml for PCV13 serotypes at the time when preschool booster vaccinations are due (at 40 months* of age) and comparing these to the 12 proportion of participants achieving this threshold after infant immunisation with PCV13.

- To assess and compare PCV13 serotype-specific IgG geometric mean concentrations (GMCs), opsonophagocytic activity (OPA) geometric mean titres (GMTs) and the proportion of participants with PCV13 serotype-specific OPA titres ≥1:8 at 40 months* of age in children immunised in infancy with either PCV7 or PCV13.

- To assess and compare PCV serotype-specific IgG GMCs, OPA GMTs and the proportion of participants with IgG concentrations ≥ 0.35mcg/ml and OPA titres ≥ 1:8 one month following a booster dose of PCV13 at 40 months* of age in children previously immunised with PCV7 and PCV13 at 2, 4 and 12 months of age.

- To determine reactogenicity of the pre-school PCV13 booster in terms of rates of local and systemic reactions following vaccination.

- To investigate the influence of genetic polymorphisms on the above immunological markers following infant immunisation with PCV7 or PCV13 and following a booster dose of PCV13 at 40 months* of age and on the nature of adverse reactions observed after this booster immunisation.

- To measure the pneumococcal serotype-specific memory B cells frequencies before and 1 month after a dose of PCV13 at 40 months* of age in a subset of children previously immunised with PCV7 or PCV13 at 2, 4 and 12 months of age (serotype studies to include serotypes including 4, 14, 23F (present in PCV7) and, 1, 3, 19A).

12. Endpoints and Outcome Measures

12.1. Primary endpoint

The proportion of participants with PCV13 serotype-specific IgG concentrations ≥ 0.35mcg/ml at 40 months* of age following immunisation with PCV13 at 2, 4 and 12 months of age.

12.2. Secondary endpoints

- The proportion of participants with PCV13 serotype-specific IgG concentrations ≥ 0.35mcg/ml at 40 months* of age following immunisation with PCV7 at 2, 4 and 12 months of age.

- The PCV13 serotype-specific IgG GMCs, OPA GMTs and proportion of participants with OPA titres ≥ 1:8 at 40 months* of age in children primed with either PCV7 or PCV13.

- The PCV13 serotype-specific IgG GMCs, OPA GMTs and proportion of participants with IgG concentrations ≥ 0.35mcg/ and OPA titres ≥ 1:8 1 month following a dose of PCV13 at 40 months* of age in children who have received PCV7 or PCV13 at 2, 4 and 12 months of age.

- Rates of local and systemic reactions (reactogenicity) following vaccination with the pre-school PCV13 booster at 40 months*.

- The identification of genetic polymorphisms in influencing the above immunological markers and adverse reactions to vaccines. The frequency of PCV13 specific memory B cells before and after immunisation at 40 months* of age determined by B cell ELISPOT and, where sufficient B cells are available for analysis, phenotyping of these cells

13. Statistical Methods

Please see Appendix – Statistical Analysis plan.

14. Safety

No serious adverse events were recorded following the PCV-13 booster at approximately 3.5 years of age.

15. Protocol Deviations

There were a total of 18 protocol violations, related to either the age at vaccination (out of desired age range 39-46 months) or to Visit 2 being incorrectly timed, i.e. performed <28 or >42 days following vaccination. As specified in the protocol, participants were not excluded on account of these protocol violations. All analysis were made using ITT in accordance with the ICH Guidance on Statistical Principles for Clinical Trials.

16. Summary of scientific findings

Antibody persistence to preschool years

Approximately 2.5 years after completion of a 2+1 immunisation schedule with PCV-13, most children still had serotype-specific IgG antibodies above the protective threshold of 0.35µg/ml for most of the serotypes (mean number of serotypes "protected" against [95% CI]: 9.59 [9.01-10.17]). Interestingly, this was also true for children vaccinated with PCV-7 at 2, 4 and 12 months of age (PCV-7 group: mean number of serotypes "protected" against [95% CI]: 9.06 [8.45-9.67]), with no significant difference between the groups (Figure 2). In the PCV-13 primed group, 84%, 69% and 55% of the participants had protective antibody levels against 8, 9 and 10 serotypes, respectively. In the PCV-7 primed group, these numbers were just slightly lower at 77%, 58% and 42%.

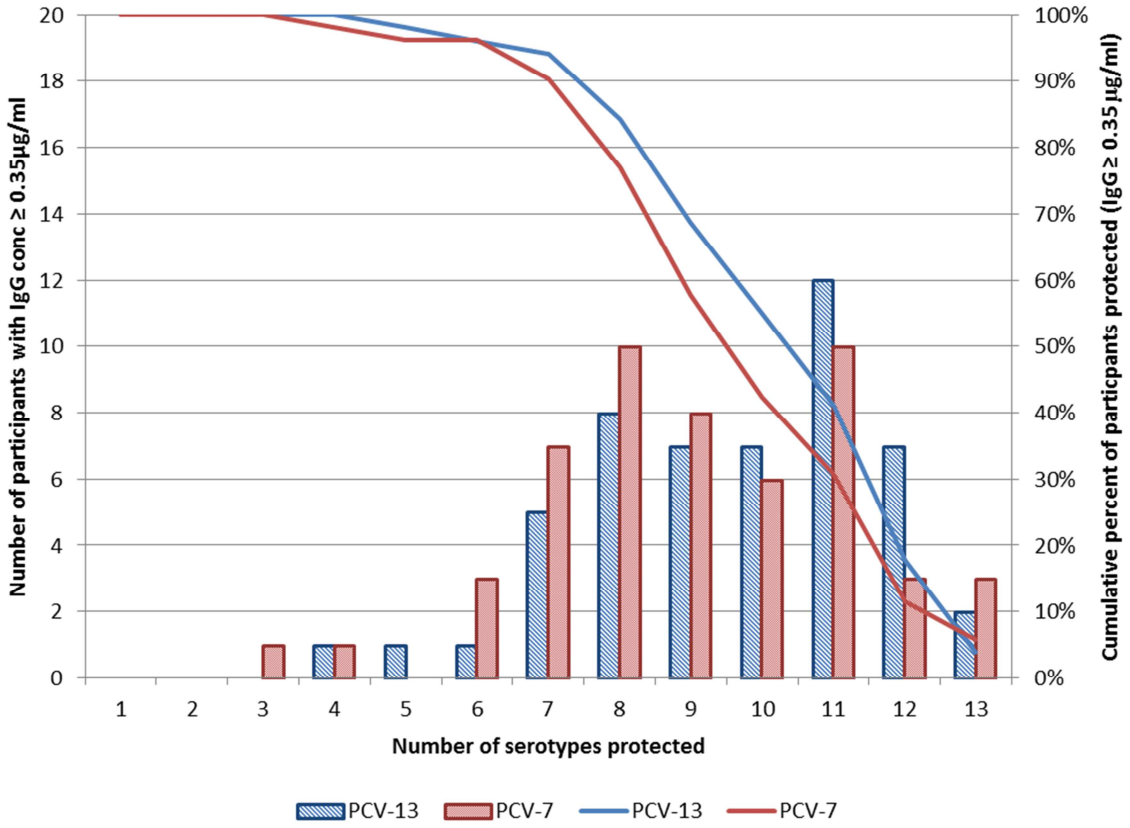

Figure 2 Number (%) participants protected (IgG ≥ 0.35µg/ml) at 3.5 years against number of serotypes

There were high proportions (>90%) of participants having IgG concentrations ≥0.35µg/ml against 5/7 of the PCV-13/7 serotypes (4, 6B, 9V, 14, 18C, 19F, 23F); the exceptions were serotype 4 (22% of PCV-13-recipients and 42% of PCV-7-recipients) and 18C (32% and 39%, respectively). For the 6 additional serotypes only included in PCV-13 (1, 3, 5, 6A, 7F, 19A) IgG concentrations ≥0.35µg/ml were seen in ≥80% of participants for serotypes 5, 6A and 19A in both groups. For serotype 1 these percentages were 51% and 14%, for serotype 3 52% and 59% and for 7F 75% and 39% in the PCV-13 and PCV-7 group, respectively (Figure 3).

Between the groups statistically significant differences in proportions of participants protected were only observed for serotypes 1, 4, 5 and 7F (with serotypes 1, 5 and 7F being significantly higher in the PCV-13 group and serotype 4 being significantly higher in the PCV-7 group).

Universally high antibody concentrations were detected against serotypes 6A and 19A in both groups although these serotypes are only present in PCV-13.

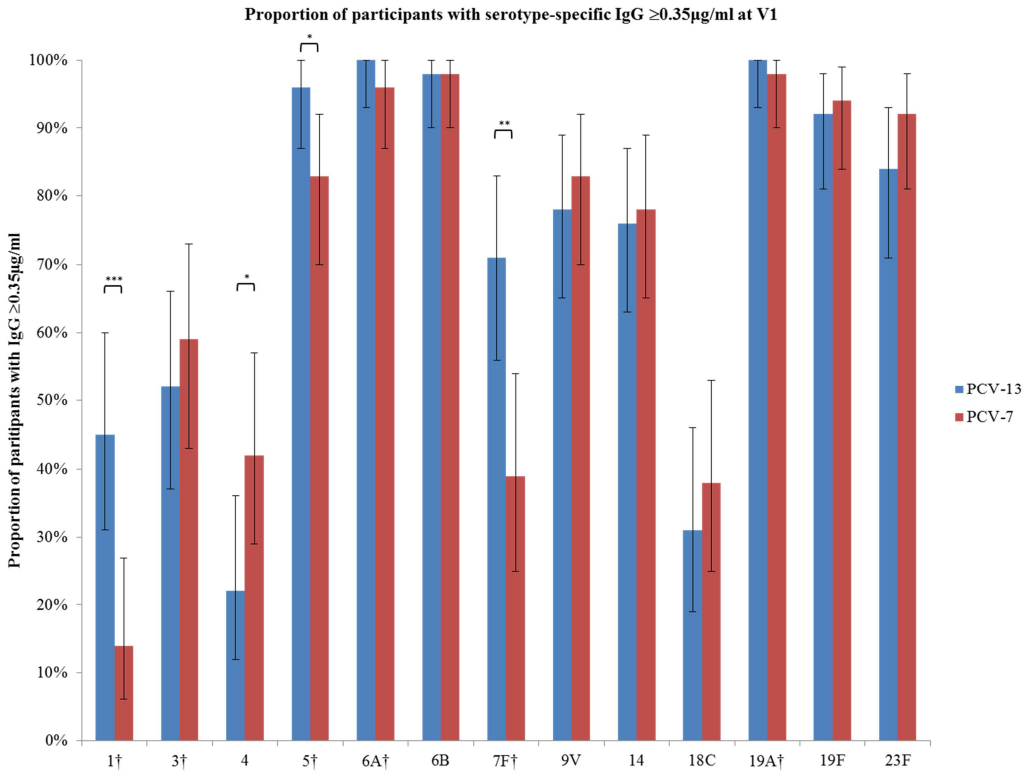

Figure 3 For 11 out of 13 serotypes, more than half of the children having received PCV-13 at 2, 4 and 12 months of age have antibody concentrations ≥0.35µg/ml. In the PCV-7 group the same was true for 9 out of 13 serotypes. Shown are mean values ±95% CI. [* p<0.05, ** p<0.01, *** p<0.001; † Serotypes only present in PCV-13].

Persistence of antibodies following primary vaccination was also assessed by determination of the geometric mean concentrations and comparing between groups before booster vaccination for each serotype. Antibodies against serotypes 1, 7F and 19A were higher in the PCV-13 group as expected, as these serotypes are only present in PCV-13. Antibody concentrations against the other 3 serotypes that are only included in PCV-13 did not significantly differ between the groups when assessed as GMC. Regarding the serotypes included in both vaccines, only serotype 4 was significantly higher in the PCV-7 group. Table 5 summarises the geometric mean concentration of IgG antibodies in each group including the ratio of groups.

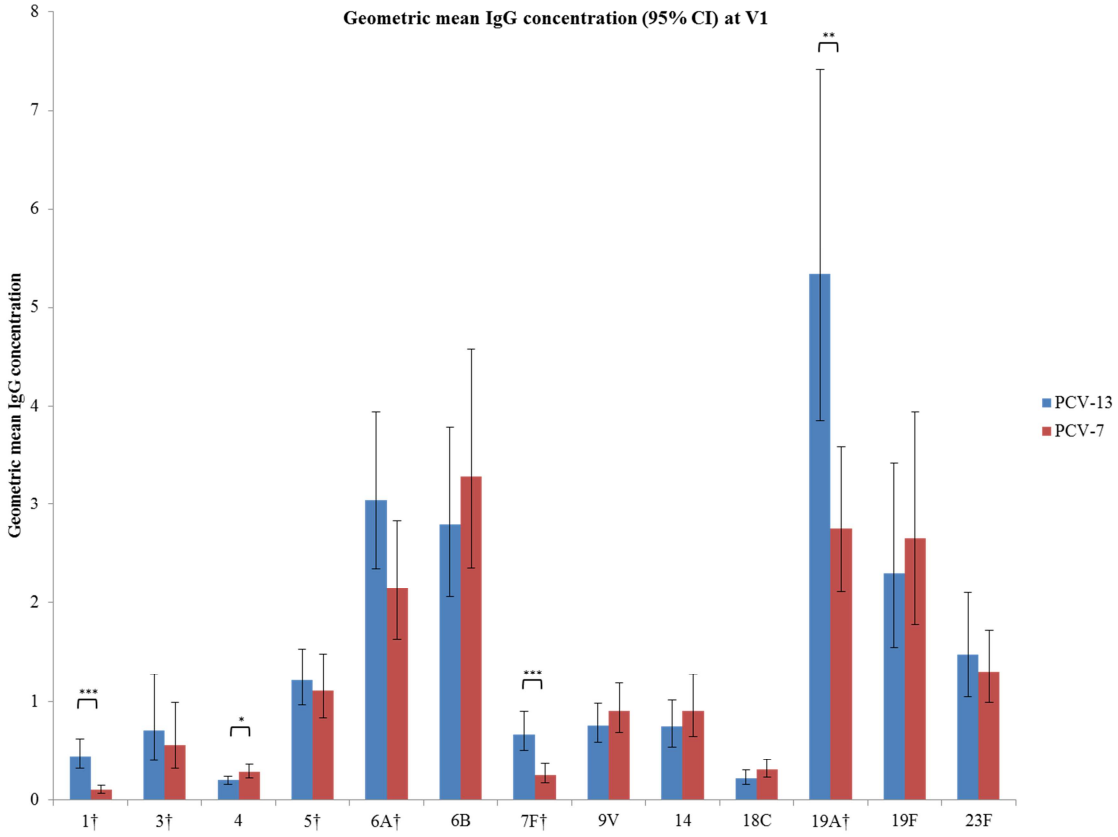

Figure 4 IgG geometric mean concentrations vary widely between the different serotypes. Significant differences between the 2 groups were observed for serotypes 1, 4, 7F and 19A. Shown are mean values ±95% CI. [* $p<0.05$, ** $p<0.01$, *** $p<0.001$; † Serotypes only present in PCV-13]

	/---------- GMC (95% CI) ----------/		GM Ratio (95 %CI)	
Serotype	***PCV-13***	***PCV-7***	***(PCV-13/PCV-7)***	***P value*****
1†	0.45 (0.32, 0.62)	0.10 (0.07, 0.15)	4.33 (2.69, 6.97)	**<.0001**
3†	0.71 (0.40, 1.28)	0.56 (0.32, 0.99)	1.27 (0.57, 2.86)	0.5552
4	0.20 (0.16, 0.24)	0.28 (0.22, 0.36)	0.69 (0.50, 0.96)	**0.0267**
5†	1.22 (0.97, 1.53)	1.11 (0.84, 1.48)	1.10 (0.76, 1.57)	0.6168
6A†	3.04 (2.34, 3.94)	2.14 (1.63, 2.83)	1.42 (0.97, 2.06)	0.0684
6B	2.79 (2.06, 3.78)	3.28 (2.35, 4.57)	0.85 (0.55, 1.33)	0.4779
7F†	0.67 (0.51, 0.90)	0.25 (0.17, 0.37)	2.72 (1.68, 4.42)	**<.0001**
9V	0.76 (0.59, 0.98)	0.91 (0.69, 1.19)	0.84 (0.58, 1.22)	0.3532
14	0.75 (0.54, 1.02)	0.91 (0.65, 1.28)	0.82 (0.52, 1.29)	0.3800
18C	0.22 (0.16, 0.30)	0.31 (0.23, 0.41)	0.72 (0.48, 1.07)	0.1051
19A†	5.34 (3.84, 7.42)	2.75 (2.11, 3.58)	1.94 (1.28, 2.94)	**0.0020**
19F	2.30 (1.55, 3.42)	2.65 (1.78, 3.94)	0.87 (0.50, 1.51)	0.6135
23F	1.48 (1.05, 2.10)	1.31 (0.99, 1.72)	1.14 (0.73, 1.76)	0.5646

***P value from independent samples t-test using Satterthwaites method for unequal variances where appropriate*

† Serotypes only present in PCV-13 vaccine

Table 5 Serotype-specific geometric mean concentrations and geometric mean ratios of IgG antibody concentrations (95% CI) at V1 with t-tests

Immunogenicity of the PCV-13 preschool booster

Following the PCV-13 booster all participants from both groups had IgG concentrations ≥0.35µg/ml for 12/13 serotypes except serotype 3 with 96% of participants in the PCV-13 group and 89% in the PCV-7 group (data not shown). Post-booster antibody levels were significantly higher in the PCV-13 group for 5/6 of the additional serotypes only included in PCV-13 except for serotype 3 indicating that PCV-13 does not prime efficiently for this serotype (Figure 5 and Figure 5).

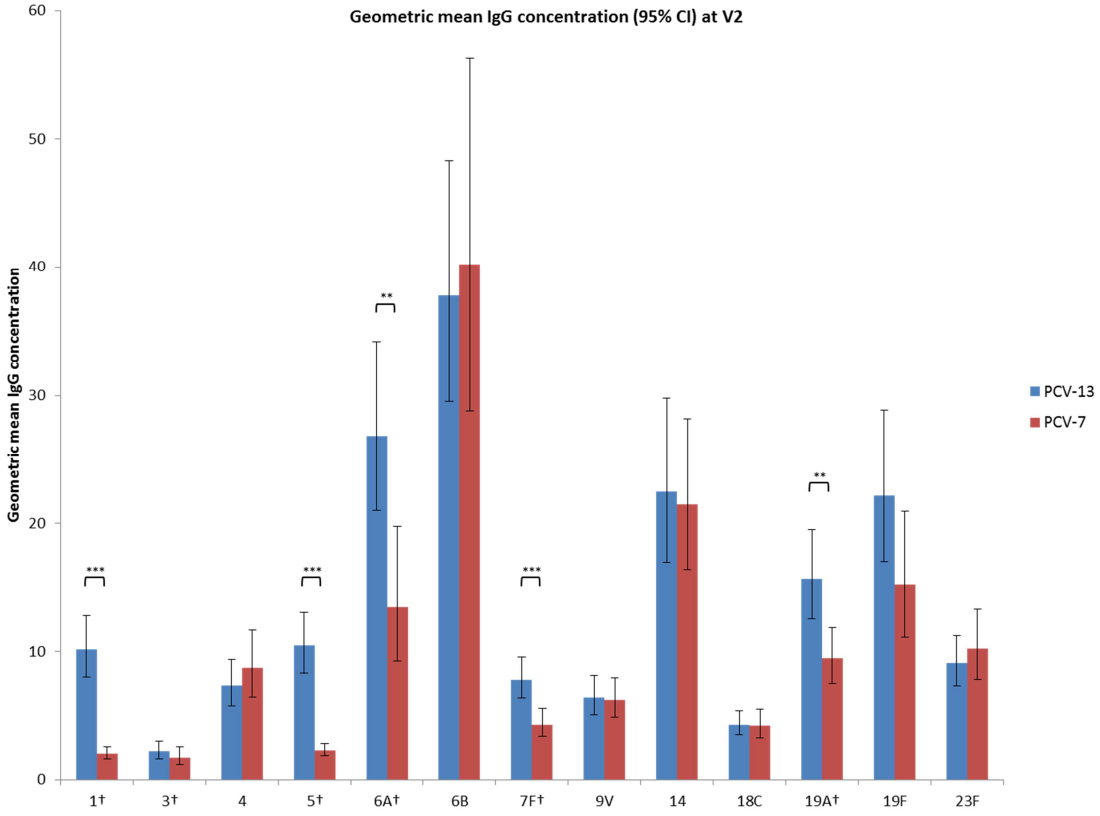

Figure 5 Geometric mean concentrations following the PCV-13 booster at 3.5 years of age are significantly higher in the PCV-13 group for 5/6 of the additional serotypes included in PCV-13, the exception being serotype 3. Shown are mean values ±95% CI. [* p<0.05, ** p<0.01, *** p<0.001; † Serotypes only present in PCV-13]

	/---------- GMC (95% CI) ----------/		GM Ratio (95 %CI)	
Serotype	***PCV-13***	***PCV-7***	***(PCV-13/PCV-7)***	***P value*****
1†	10.16 (8.04, 12.84)	2.03 (1.61, 2.56)	5.01 (3.62, 6.94)	**<.0001**
3†	2.18 (1.59, 2.98)	1.71 (1.16, 2.52)	1.27 (0.78, 2.07)	0.3357
4	7.37 (5.80, 9.37)	8.72 (6.48, 11.73)	0.85 (0.58, 1.23)	0.3744
5†	10.46 (8.34, 13.13)	2.28 (1.84, 2.81)	4.59 (3.38, 6.25)	**<.0001**
6A†	26.81 (21.01, 34.20)	13.55 (9.27, 19.80)	1.98 (1.27, 3.09)	**0.0032**
6B	37.82 (29.57, 48.37)	40.25 (28.78, 56.28)	0.94 (0.63, 1.41)	0.7616
7F†	7.82 (6.38, 9.59)	4.32 (3.33, 5.61)	1.81 (1.31, 2.50)	**0.0004**
9V	6.43 (5.06, 8.16)	6.23 (4.89, 7.94)	1.03 (0.74, 1.44)	0.8546
14	22.51 (17.00, 29.82)	21.51 (16.41, 28.18)	1.05 (0.71, 1.54)	0.8145
18C	4.33 (3.48, 5.41)	4.25 (3.25, 5.55)	1.02 (0.73, 1.43)	0.9054
19A†	15.70 (12.62, 19.54)	9.49 (7.54, 11.94)	1.65 (1.21, 2.26)	**0.0019**
19F	22.18 (17.05, 28.87)	15.29 (11.16, 20.96)	1.45 (0.97, 2.17)	0.0701
23F	9.10 (7.33, 11.32)	10.25 (7.84, 13.39)	0.89 (0.63, 1.25)	0.4897

***P Value from independent samples t-test using Satterthwaites method for unequal variances where appropriate*

† Serotypes only present in PCV-13 vaccine

Table 6 Serotype-specific IgG GMC 1 month following a pre-school PCV-13 booster

Reactogenicity

No serious adverse events were recorded following the PCV-13 booster at approximately 3.5 years of age. Local reactions such as redness, hardness and swelling were either absent or mild in most cases. However, moderate or severe tenderness was experienced by 18% of all participants (Table 7). Decreased appetite and irritability were recorded in 18% and 41% of participants respectively

with moderate or severe reactions being present in 4% and 13% of these children. Low-grade fever (38-39°C) was noted in 3% of the participants and none of the children had a temperature >39°C (Table 8). The reactogenicity profile of this PCV-13 booster was similar regardless of whether participants had previously been immunised with PCV-7 or PCV-13.

		PCV-13 (N=55)		*PCV-7 (N=49)*			*Both groups (N=104)*	
Reaction	***Level***	***N***	***Proportion***	***N***	***Proportion***	***P value*****	***N***	***Proportion***
Redness	Any	26	47%	20	41%	0.5566	46	44%
	Severe ≥ 5 cm	3	6%	1	2%		4	4%
Swelling	Any	8	15%	14	29%	0.0959	22	21%
	Severe ≥ 5 cm	1	2%	1	2%		2	2%
Hardness	Any	10	18%	16	33%	0.1136	26	25%
	Severe ≥ 5 cm	0	0%	0	0%		0	0%
Tenderness	Any	32	58%	28	57%	1.0000	60	58%
	Discomfort during routine activities	9	16%	6	12%		15	14%
	Interfering with limb movement	2	4%	2	4%		4	4%

*** Fisher's Exact Test*

Table 7 Local reactions following the pre-school PCV-13 booster

		PCV-13 (N=55)		*PCV-7 (N=49; 48 for T°)*			*Both groups (N=104; 103 for T°)*	
Reaction	***Level***	***N***	***Proportion***	***N***	***Proportion***	***P value*****	***N***	***Proportion***
Poor appetite	Appetite as usual	45	82%	40	82%	0.2322	19	18%
	Eating less/no effect on normal activity	6	11%	9	18%		15	14%
	Interfering with normal activity	3	6%	0	0%		3	3%
	Not eating at all	1	2%	0	0%		1	1%
Irritability	Behaviour as usual	27	49%	34	70%	0.1925	43	41%
	More irritable/no effect on normal activity	18	33%	11	22%		29	28%
	Interfering with normal activity	6	11%	2	4%		8	8%
	Preventing normal activity	4	7%	2	4%		6	6%
Axillary	<38.0°C	55	100%	45	94%	0.0978	100	97%

Temperature	38.0-38.4°C	0	0%	2	4%		2	2%
	38.5-38.9°C	0	0%	1	2%		1	1%

** *Fisher's Exact Test*

Table 8 Systemic reactions following the pre-school PCV-13 booster

17. Discussion & Conclusion

This is the first study to investigate the persistence of serotype-specific antibodies of any pneumococcal vaccine, given in an infant immunisation schedule, up to the age of 3.5 years. There are also no published data on the immunogenicity of a preschool booster in children who have been previously primed with a PCV in infancy.

We found that most children who had been immunised with PCV-13 at 2, 4 and 12 months had antibody levels above the presumed threshold of protection for most of the serotypes included in PCV-13. Exceptions are serotypes 4 and 18C of the PCV-13/7 common serotypes and serotypes 1 and 3 of the additional serotypes only included in PCV-13. The maintenance of antibody to preschool years is despite a significant decline from 13 months to pre-school years (data not shown).

In summary, these data suggest that children receiving PCV-7 or PCV-13 at 2, 4 and 12 months of age are unlikely to require a pre-school booster to maintain effectiveness against most vaccine serotypes through mid-childhood. A pre-school booster with the 13-valent pneumococcal conjugate vaccine is well tolerated with low rates of local and systemic side effects after priming with either PCV-7 or PCV-13.

18. Appendix

Statistical analysis plan