Final Study Report

A phase III randomised, open label clinical trial evaluating the immunogenicity of a 10-valent pneumococcal conjugate vaccine booster compared with the standard 13-valent pneumococcal conjugate vaccine booster given at 12 months of age to healthy children who have received the 13-valent pneumococcal conjugate vaccine at 2 and 4 months of age.

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Chief Investigator	Professor Andrew J Pollard
Investigators	Dr J. Trück, Dr D.F. Kelly, Dr M.D. Snape
Author of the report	Dr Johannes Trück

Investigator Agreement "I have read this protocol and agree to abide by all provisions set forth therein. I agree to comply with the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice."

Confidentiality Statement This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, host NHS Trust(s), regulatory authorities, and members of the Research Ethics Committee.

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1 AMENDMENT HISTORY OF PROTOCOL

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	2	06/01/2012	Dr Johannes Truck	Clarification of recruitment process. 6.4.1. Clarification of review process to collect SAEs at Visit 2. 6.6.3
2	2		Faye C Alexander	Clarification of names of participating sites (five PCTs included under Thames Valley Partnership)
3	3		Faye C Alexander	Change to 7.1 – clarification of site of injection
4	4		Dr Johannes Truck	Change to sample size calculation

2 SYNOPSIS AS PER PROTOCOL

Study Title	A phase III randomised, open label clinical trial evaluating the immunogenicity of a 10-valent pneumococcal conjugate vaccine booster compared with the standard 13-valent pneumococcal conjugate vaccine booster given at 12 months of age to healthy children who have received the 13-valent pneumococcal conjugate vaccine at 2 and 4 months of age.
Internal ref. no.	2011/05
Study Design	Phase III, 1:1 randomised, open label clinical trial
Study Participants	12 month old children
Eligibility criteria	Healthy children who have been vaccinated according to the routine immunisation schedule and received the 13-valent pneumococcal conjugate vaccine at 2 and 4 months of age.
Number of Participants	168 (84 per group) [The sample size is calculated based on non-inferiority (10% level) of a booster dose of PCV-10 compared with a booster dose of PCV-13 for proportion of participants who have serotype-specific IgG concentrations ≥0.35mcg/ml for the PCV-10 serotypes (1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F) 1 month following booster vaccination assuming 80% power and alpha 2.5% allowing for a 15% drop-out rate]. If, during the course of the study, the numbers required to meet the primary objective, at visit 2, are lower than anticipated, additional participants will be recruited to achieve sufficient numbers to meet the primary objective.
Follow-up duration	12 months
Planned Study Period	January 2012 – October 2013 (Recruitment 6 months, follow-up 12 months, sample processing and data analysis 4 months)
Primary Objective	To assess whether the 10-valent pneumococcal conjugate vaccine (PCV-10) is non-inferior to the 13-valent pneumococcal conjugate vaccine (PCV-13) in terms of proportion of participants who have IgG concentrations ≥0.35mcg/ml

	for 10 serotypes* one month following booster vaccination at 12 months of age with PCV-10 or PCV-13. [*serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F]
Secondary Objectives	To assess the proportion of participants who have IgG concentrations ≥0.35mcg/ml for serotypes 3, 6A, 6C and 19A one month following a booster vaccination with either PCV-10 or PCV-13. To assess serotype-specific IgG geometric mean concentrations (GMC) to 14 serotypes at baseline (before the booster is given) as well as 1 and 12 months following a booster with either PCV-10 or PCV-13. To assess serotype-specific opsonophagocytic activity (OPA) geometric mean titres (GMT) and the proportion of participants with serotype-specific OPA titres ≥1:8 at baseline as well as 1 and 12 months following a booster dose of either PCV-10 or PCV-13. To assess the proportion of participants at baseline, who have IgG concentrations ≥0.35mcg/ml for 14 serotypes. To assess reactogenicity of a booster at 12 months of age with either PCV-10 or PCV-13 in terms of rates of local and systemic reactions following vaccination. To assess immediate pain at time of injection of a booster at 12 months of age with either PCV-10 or PCV-13 determined by a validated pain assessment tool and crying time. To investigate the influence of genetic polymorphisms related to the immune response and reactogenicity to vaccination. To quantify and compare frequencies and phenotype of antigen-specific B cells in the peripheral blood before and following a booster dose of PCV-10 or PCV-13 at 12 months of age. To investigate and compare the immunoglobulin gene usage in response to vaccination.

Primary Endpoint	The proportion of participants with serotype-specific IgG concentrations ≥0.35mcg/ml to PCV-10 serotypes at 12 months of age one month following a booster with either PCV-10 or PCV-13.
Secondary Endpoints	Measurement of the proportion of participants with serotype-specific IgG concentrations ≥0.35mcg/ml to the serotypes 3, 6A, 6C and 19A at 12 months of age one month following a booster with either PCV-10 or PCV-13. Measurement of serotype-specific IgG GMC to 14 serotypes at baseline as well as 1 and 12 months following a booster dose of either PCV-10 or PCV-13 at 12 months of age. Measurement of serotype-specific OPA GMTs and proportion of participants with OPA titres ≥1:8 to 13 serotypes at baseline as well as 1 and 12 months following a booster dose of either PCV-10 or PCV-13 at 12 months of age. Calculation of the proportion of participants with serotype-specific IgG concentrations ≥0.35mcg/ml to 14 serotypes at 12 months of age before the booster with either PCV-10 or PCV-13. Rates of local and systemic reactions (reactogenicity) following booster vaccination with either PCV-10 or PCV-13 at 12 months of age. Measurement of immediate pain at time of injection of a booster at 12 months of age with either PCV-10 or PCV-13. Identification of genetic polymorphisms to the vaccine response and to adverse reactions to vaccines. Measurement of the frequency and phenotype of antigen-specific B cells in the peripheral blood before and following a booster dose of PCV-10 or PCV-13 at 12 months of age. Determination of the immunoglobulin gene usage in response to vaccination.
Intervention (s)	Immunisation with the 10-valent pneumococcal conjugate vaccine (Synflorix®, GSK Biologicals) or the 13-valent pneumococcal conjugate vaccine (Prevenar 13®, Pfizer) at 12 months of age in subjects primed with 2 dose vaccination schedule with PCV-13 at the age of 2 and 4 months.

Form	Injection
Dose	0.5 ml (both vaccines)
Route	Intramuscular

3 ABBREVIATIONS

AE	Adverse Event
ADR	Adverse Drug Reaction
AR	Adverse Reaction
CI	Chief Investigator
CRF	Case Report Form
CTRG	Clinical Trials & Research Governance, University of Oxford
GCP	Good Clinical Practice
GMC	Geometric Mean Concentration(s)
GMT	Geometric Mean Titre(s)
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
IgG	Immunoglobulin G
NIHR	National Institute of Health Research
NRES	National Research Ethics Service
OPA	Opsonophagocytic Assay
PCV-7	7-valent Pneumococcal Vaccine (Prevenar®)
PCV-10	10-valent Pneumococcal Vaccine (Synflorix®)
PCV-13	13-valent Pneumococcal Vaccine (Prevenar 13®)
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions

4 BACKGROUND AND RATIONALE

Infections caused by the encapsulated bacterium *Streptococcus pneumoniae* (pneumococcus) are important causes of childhood mortality and morbidity worldwide, especially in young children who suffer pneumococcal pneumonia, meningitis and septicaemia. More common but less severe manifestations of pneumococcal infection are otitis media, sinusitis and bronchitis [1, 2], which collectively place a huge burden on health services. More than 14 million episodes of serious pneumococcal disease and about 800 000 deaths in children under the age of five occur annually [1-3]. Worldwide the highest burden of disease is suffered by children under the age of five years and the elderly. Individuals with immune deficiency, especially HIV infection, have a high rate of pneumococcal disease. In the United Kingdom, pneumococcal protein-polysaccharide conjugate vaccines (PCV) were introduced into the infant immunisation schedule covering 7 serotypes of *S. pneumoniae* in 2006 successfully reducing the burden of invasive disease due to this pathogen [4]. The resistance of *S. pneumoniae* to commonly used antibiotics is a growing problem worldwide and emphasizes the importance of preventing pneumococcal disease through immunisation [5-8]. Pneumococci are frequently and asymptomatically carried in the nasopharynx of healthy individuals with the highest colonisation rates being among young children, who are thought to be the main transmitters in the population [9, 10]. Pneumococcal acquisition starts in the first few weeks of life [10]. Asymptomatic nasopharyngeal carriage is significant as it plays a big role in the spread of the organism to the elderly and vulnerable individuals in a population including those who are too young to be immunized, those with vaccine failure and those with underlying medical conditions putting them at risk for invasive pneumococcal disease.

The principal aim of immunisation against *S. pneumoniae* is to prevent the vaccinated individual from developing (invasive) disease caused by the organism. In the short term, this can be achieved by induction of antibodies in serum or on the mucosal surface that prevent colonisation or microbial invasion of the organism.

Pneumococcal conjugate vaccines were widely introduced as a four-dose regimen with three doses given in infancy together with a booster dose in the second year of life. This schedule has shown to be efficacious against invasive disease [11]. Shortage of vaccine in the US provided evidence that fewer than four doses are effective in preventing disease [12] although a booster at 12-15 months seems to confer additional protection [13]. Herd immunity provides protection for unvaccinated individuals through the immunisation of those members of the population who are normally responsible for transmission of the organism. To have the highest

impact, acquisition of the organisms by individuals in the population who are the main transmitters should be prevented by induction of high and sustained antibody levels at the mucosal surface [14]. For example, the introduction of pneumococcal conjugate vaccines for children under 2 years of age in the USA and UK resulted in a rapid decrease in disease caused by the vaccine serotypes among unvaccinated adults [4, 15, 16] and infants who were too young to be immunised [15]. The absolute number of cases prevented by this indirect effect of PCV-7 is actually estimated to be larger than the direct effect, which makes universal vaccination highly cost-effective [17, 18].

There are currently 3 licensed pneumococcal conjugate vaccines in the UK. The 7-valent pneumococcal conjugate vaccine (PCV-7) was developed by Wyeth® vaccines (now Pfizer®) and contains polysaccharides from pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F and 23F conjugated to non-toxic diphtheria toxin cross-reacting material (CRM_{197}). In April 2010, this vaccine has been replaced in the UK routine immunisation schedule by the 13-valent pneumococcal conjugate vaccine (PCV-13; Pfizer) covering the additional serotypes 1, 3, 5, 6A, 7F and 19A, all of which being conjugated to CRM_{197}. GSK's 10-valent pneumococcal conjugate vaccine (PCV-10) covering the serotypes 1,5 and 7F additional to those included in PCV-7 and conjugated to diphtheria toxoid carrier (serotype 19F), tetanus toxoid carrier protein (serotype 18C) and nontypeable *H. influenzae* Protein D (rest of the serotypes) was licensed in Europe in March 2009. However, due to an increase in serotype 19A disease and the availability of PCV-13 (licensed in December 2009) soon after its licensure, PCV-10 is currently not used in the UK. Further, the Joint Committee on Vaccination and Immunisation (JCVI) recommends use of PCV-13, therefore this vaccine rather than PCV-10 is used in the routine immunisation schedule.

In contrast to the currently used pneumococcal conjugate vaccine in the UK routine immunisation schedule (PCV-13), most of the pneumococcal serotypes contained in PCV-10 are conjugated to a different carrier protein, Protein D, which is derived from a bacterium called nontypeable *H. influenzae* (NTHi). The use of a novel carrier protein, which is not closely related to an antigen included in any concurrently or previously administered routine vaccine minimises the risk of interference related to the carrier protein, which might result in a better immune response against the contained pneumococcal serotypes. In addition, use of Protein D has the potential to protect children against NTHi, a common cause of acute otitis media, and possibly other nontypeable *H. influenzae* diseases through an immune response generated by the carrier protein. A large study looking at efficacy of a similar vaccine to prevent NTHi acute

otitis media showed that disease episodes caused by this pathogen were reduced by 35.3% [19]. There is, however, currently no known serological correlate of protection for antibodies directed against Protein D from nontypeable *H. influenzae* so it is difficult to draw a conclusion from increased antibody levels.

Unlike PCV-13, PCV-10 does not contain serotypes 3, 6A and 19A. However, immunisation with PCV-10 has been shown to induce cross-reactive antibodies and functional opsonophagocytic activity responses against serotypes 6A (from serotype 6B) and 19A (from serotype 19F) after primary as well as booster vaccination [20], which might be enough to protect children against disease caused by these two serotypes. In contrast to other vaccine serotypes, type 3 has been shown to have an atypical immune response [21] and vaccine efficacy against this serotype has not been demonstrated to date. Moreover, the only study looking at serotype 3 vaccine efficacy using a PCV that included this serotype, demonstrated no protection against serotype 3 acute otitis media following primary and booster vaccination [19].

Immunogenetics

An additional aspect to be assessed in this study is the impact of genetic factors influencing the response to immunisation. Host immunogenetics is likely to play a critical role in modulating the responses to paediatric vaccines. Twin studies on several vaccines including measles, mumps and rubella, have shown high heritability of vaccine antibody responses[29]. Some genetic associations have already been identified between genes of the adaptive and innate immune response and some vaccines, for example human leukocyte antigen (HLA) alleles and measles antibody responses [22, 23] and IL-1β polymorphisms and hepatitis B vaccine responses [24]. These studies have been small scale and based on single candidate genes and the extent to which genetic variation contributes to vaccine responses remains poorly understood. Insight into which genetic variants affect responses to specific vaccines will be of value for 2 main reasons:

1. it will help to identify the critical immune pathways leading to protection after vaccination and lead to the production of more effective vaccines
2. it will help to identify genes that may play important roles in wild-type infection and lead to better understanding of disease pathogenesis, which in turn may lead to the development of novel therapies

The blood samples obtained in this study provide an opportunity to extract DNA, which in turn will be added to a large biobank currently containing >3500 samples. These DNA samples can then be used for genome wide analysis of the genetic factors influencing the host response to the vaccines received in the relevant studies. This DNA extraction and storage will only occur with the specific consent of participants, and DNA will not be analysed for any other purpose than to assess factors influencing the immune response to vaccines.

In addition to the above described DNA biobank, other methods will be used to explore the genetic basis of the immune response. RNA expression profiles will be investigated pre and post booster vaccination in each group to elucidate genes that are differentially expressed in response to immunisation. This analysis could highlight genes of particular importance in vaccine responses. Furthermore, comparisons between RNA profiles and correlates of vaccine immunity may identify profiles which could be useful "biomarkers" of vaccine induced cellular and humoral immunity in future studies. Another important area of investigation is the evolution of the repertoire of B cell receptor immunoglobulin genes during the course of an immune response to a vaccine. A combination of a novel FACS based antigen-labelling method with the investigation of the immunoglobulin gene sequences of antigen-specific B cells will be used in this study.

Possible and desired outcomes

This study aims to investigate the potential of an alternative booster vaccine to be given to 12-month old children being primed with 2 doses of PCV-13 at 2 and 4 months of age. Therefore, non-inferiority of a PCV-10 booster dose compared with the currently used vaccine booster (PCV-13) will be tested for proportion of participants who have serotype-specific IgG concentrations ≥0.35mcg/ml for the PCV-10 serotypes 1 month following immunisation. Use of PCV-10 as a booster vaccine might improve the immune response generated against the included pneumococcal serotypes through prevention of immune interference by the carrier protein. In addition, use of this booster vaccine has the potential to protect against nontypeable *H. influenzae* acute otitis media.

Linked to antibody measurements frequencies, phenotype and immunoglobulin gene sequences of antigen-specific B cells will be investigated in order to help understanding the basis of antibody production by specific B cells and to test immune memory. RNA expression profile and the connection to a large paediatric biobank will be used to gain insights into the genes that are involved in response to immunisation.

5 OBJECTIVES

5.1 Primary Objective

To assess whether the 10-valent pneumococcal conjugate vaccine (PCV-10) is non-inferior to the 13-valent pneumococcal conjugate vaccine (PCV-13) in terms of proportion of participants who have IgG concentrations ≥0.35mcg/ml for 10 serotypes* one month following booster vaccination at 12 months of age with PCV-10 or PCV-13 (Figure 1).

[*serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F]

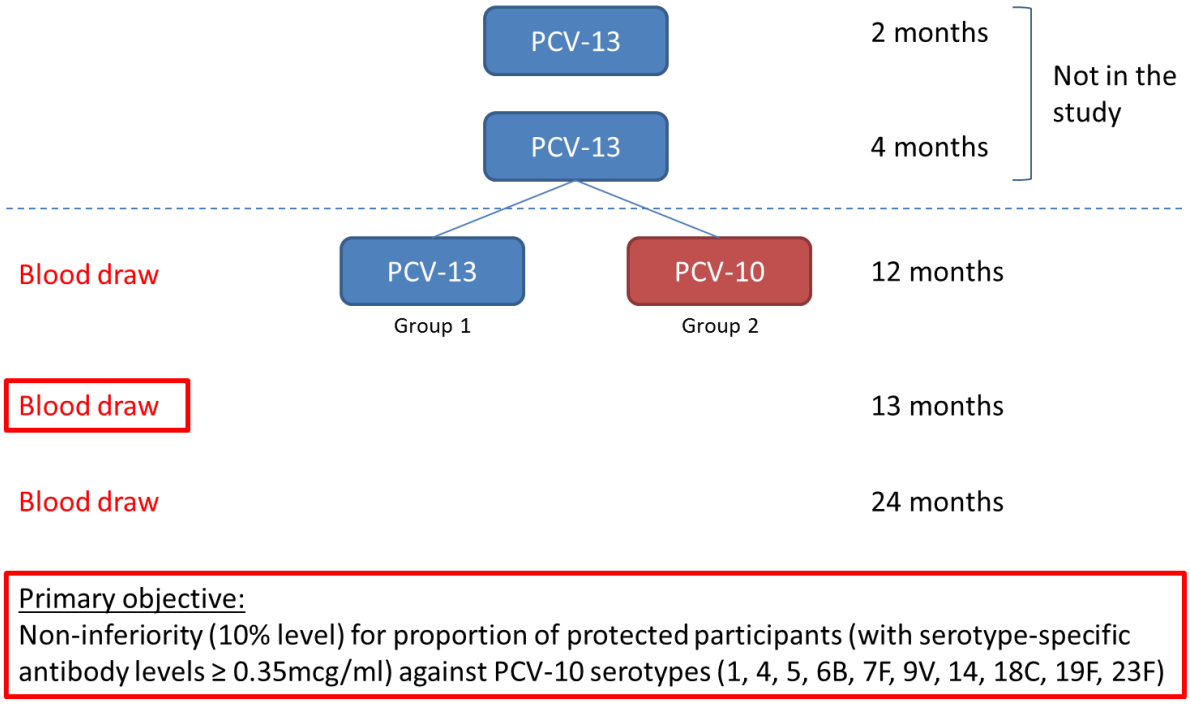

Figure 1 Study design with time points of study visits.

5.2 Secondary Objectives

To assess the proportion of participants with serotype-specific IgG concentrations ≥0.35mcg/ml to the serotypes 3, 6A, 6C and 19A at 12 months of age one month following a booster with either PCV-10 or PCV-13.

To assess the proportion of participants who have IgG concentrations ≥0.35mcg/ml for serotypes 3, 6A, 6C and 19A one month following a booster vaccination with either PCV-10 or PCV-13.

To assess serotype-specific IgG geometric mean concentrations (GMC) to 14 serotypes at baseline (before the booster is given) as well as 1 and 12 months following a booster with either PCV-10 or PCV-13.

To assess serotype-specific opsonophagocytic activity (OPA) geometric mean titres (GMT) and the proportion of participants with PCV-10 serotype-specific OPA titres ≥1:8 at baseline as well as 1 and 12 months following a booster dose of either PCV-10 or PCV-13.

To assess the proportion of participants at baseline, who have IgG concentrations ≥0.35mcg/ml for 14 serotypes.

To assess reactogenicity of a booster at 12 months of age with either PCV-10 or PCV-13 in terms of rates of local and systemic reactions following vaccination.

To assess immediate pain at time of injection of a booster at 12 months of age with either PCV-10 or PCV-13 determined by a validated pain assessment tool and crying time.

To investigate the influence of genetic polymorphisms related to the immune response and reactogenicity to vaccination.

To quantify and compare frequencies and phenotype of antigen-specific B cells in the peripheral blood before and following a booster dose of PCV-10 or PCV-13 at 12 months of age.

To investigate and compare the immunoglobulin gene usage in response to vaccination.

6 STUDY DESIGN

6.1 Summary of Study Design

The study will be a randomised controlled clinical trial. The study will be open labelled for participants and clinical trial staff, but blinded for laboratory staff. Participants will be recruited from children who were vaccinated according to the routine immunisation schedule with 2 immunisations of the 13-valent pneumococcal conjugate vaccine (PCV-13, Prevenar 13®, Pfizer) at 2 and 4 months of age. Following randomisation at recruitment, these children will be allocated to 2 groups according to the type of vaccine they will receive as a booster at 12 months of age:

Group 1 Booster vaccination with PCV-10 (Synflorix®, GSK Biologicals)

Group 2 Booster vaccination with PCV-13 (Prevenar 13®, Pfizer)

Participants must be healthy children and available for the entire study period.

The study will consist of 3 home visits (baseline, 1 month and 1 year). A blood test of 7.5 ml will be taken at each visit in addition to the booster immunisation at visit 1.

During visit 1, the parent/legal guardian will be provided with a diary card and a thermometer. Parents will be asked to record all reactions to the vaccine and will be specifically asked to keep a daily record of their child's temperature and any disturbed feeding, drowsiness, irritability and local redness, swelling and pain for the first 4 days after vaccination. This diary card will be collected at the second visit in order to establish the reactogenicity of both vaccines in this age group.

A total of 168 participants will be recruited to this study to reach the primary endpoint. 84 children in both groups would allow us to detect a 10% difference in the proportion of protected individuals (with serotype-specific IgG antibody concentration ≥0.35mcg/ml) to the 10 serotypes contained in PCV-10 using 80% power and alpha 2.5% allowing for a 15% drop-out rate.

If, during the course of the study, the numbers required to meet the primary objective at visit 2 are lower than anticipated, additional participants will be recruited to achieve sufficient numbers to meet the primary objective.

The B cell data will be expressed in a descriptive manner with no formal statistical analysis.

Recruitment for visit 1 will be over a period of 6 months with a follow-up of 12 month after enrolment into the study. The end of the study will be considered the end of processing samples for laboratory testing and data analysis resulting in a total study duration of 24 months.

6.2 Primary and Secondary Endpoints/Outcome Measures

6.2.1 Primary Endpoint

The proportion of participants with serotype-specific IgG concentrations ≥0.35mcg/ml to PCV-10 serotypes at 12 months of age one month following a booster with either PCV-10 or PCV-13.

6.2.2 Secondary Endpoints

Measurement of the proportion of participants with serotype-specific IgG concentrations ≥0.35mcg/ml to the serotypes 3, 6A, 6C and 19A at 12 months of age one month following a booster with either PCV-10 or PCV-13.

Measurement of serotype-specific IgG GMC to 14 serotypes at baseline as well as 1 and 12 months following a booster dose of either PCV-10 or PCV-13 at 12 months of age.

Measurement of serotype-specific OPA GMTs and proportion of participants with OPA titres ≥1:8 to 13 serotypes at baseline as well as 1 and 12 months following a booster dose of either PCV-10 or PCV-13 at 12 months of age.

Calculation of the proportion of participants with serotype-specific IgG concentrations ≥0.35mcg/ml to 14 serotypes at 12 months of age before the booster with either PCV-10 or PCV-13.

Rates of local and systemic reactions (reactogenicity) following booster vaccination with either PCV-10 or PCV-13 at 12 months of age.

Measurement of immediate pain at time of injection of a booster at 12 months of age with either PCV-10 or PCV-13.

Identification of genetic polymorphisms to the vaccine response and to adverse reactions to vaccines.

Measurement of the frequency and phenotype of antigen-specific B cells in the peripheral blood before and following a booster dose of PCV-10 or PCV-13 at 12 months of age.

Determination of the immunoglobulin gene usage in response to vaccination.

6.3 Study Participants

6.3.1 Overall Description of Study Participants

Healthy children aged 12 months who have been vaccinated with the 13-valent pneumococcal conjugate vaccine (PCV-13) according to the UK routine immunisation schedule.

6.3.2 Allocation to the 2 groups

After enrolment participants will be randomised to receive either PCV-10 or PCV-13 as a 12-months booster.

6.3.3 Inclusion Criteria

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

- Aged 12 months (-2 weeks to +6 weeks) at time of enrolment.

- Have received two doses of PCV-13 at less than 6 months of age with a gap of at least 6 weeks between the two vaccinations.
- Have received all primary vaccines according to the UK routine immunisation schedule (up to, but not including, 12 months of age).
- Available for the entire study period and whose parent/legal guardian can be reached by telephone.
- Healthy children as determined by medical history and 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.

6.3.4 Exclusion Criteria

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

- Previous receipt of pneumococcal vaccine other than the 13-valent pneumococcal conjugate vaccine (Prevenar 13®, Pfizer).
- Receipt of the routine 12 month immunisations (PCV-13 (3^{rd} dose), combined *Haemophilus influenzae* type b and serogroup C meningococcal glyco-conjugate vaccine (Hib-MenC) or measles, mumps and rubella vaccine (MMR)).
- 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; e.g., Synagis B).
- Parents who plan to move out of the geographical area where the study would be conducted.

6.3.5 Temporary exclusion criteria

- In the event of systemic illness or fever > 38.0°C at the time of the visit, immunisation will be deferred and rearranged as appropriate when the participant is recovered.
- Any live immunisation within 28 days prior to enrolment, or any other (non-live) vaccine within the 7 days prior to enrolment.
- Receipt of more than 2 weeks of immunosuppressants or immune modifying drugs, (e.g. prednisolone >0.5mg/kg/day) with 30 days of enrolment.

6.3.6 Expenses and Benefits

- All the study visits will be conducted at the participant's home or most convenient place to the participant's parent/legal guardian.
- None of the participants will receive economical reimbursement for their participation in the study.
- Participants will have the benefit of receiving their routine vaccines and the standard or the alternative pneumococcal conjugate vaccine at their homes at a convenient time for the family. In addition, participants will have investigated their immune response to the pneumococcal conjugate vaccine.

6.4 Study procedures

6.4.1 Screening for Eligibility and Study Discussion

An invitation letter, which describes the study and which includes a reply form, would be sent to parents by the study team. Parents who are interested in to taking part are requested to contact the study team and would then be given the opportunity to discuss the study. An appointment would then be set up for parents willing to enrol their child in the study. Eligible participants will be identified through the National Health Application and Infrastructure Services (NHAIS) who are responsible for the central NHS patient database. Children of parents interested in participating in the study would then be visited at their homes and recruited to the study after obtaining informed consent.

Inclusion and exclusion criteria will be checked on the first visit during which enrolment and randomisation will take place.

6.4.2 Informed Consent

Informed consent will be taken by members of the research team, either a doctor or nurse trained in taking informed consent. The parent/guardian must personally sign the latest approved version of the informed consent form before any study-specific procedures are performed.

Written and verbal versions of the participant information and informed consent will be presented to the participant's parent/guardian detailing no less than: the exact nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

The parent/guardian will be allowed as much time as they wish to consider the information, and the opportunity to question the Investigator, or other independent parties to decide whether to enrol their child in the study. Written Informed Consent will then be obtained by means of parent/guardian dated signature and dated signature of the person who presented and obtained the informed consent. A copy of the signed Informed Consent will be given to the parent/guardian. The original signed form will be retained at the study site.

In the event that the parent/guardian is illiterate then a third party may act as an impartial witness for the parent/guardian to attest that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the parent/guardian and that informed consent was freely given by the parent/guardian. In this event the impartial witness will also sign and date the consent form.

6.4.3 Baseline Assessments

During the initial visit informed consent will be obtained, following which a medical history would be elicited and full inclusion and exclusion criteria will be checked. If no exclusion criteria are identified the participant will be recruited into the study. Potential participants will be assessed by a study doctor or nurse.

- Demographics: The date of birth, gender, ethnicity* and address will be recorded.
- Medical History: Information regarding medical history, previous hospital admissions, and surgical interventions will be recorded.
- Concomitant Medication: All current prescription medication and any vaccines received will be recorded.
- Physical examination:

- A brief physical assessment of the child will be carried out on visit 1 by a study nurse or doctor. If specific concerns are raised by this assessment or the child's medical history, then a formal physical examination will be performed by a study doctor.
- Axillary temperature will be checked and recorded; this should be <38.0°C before any vaccine is administered.

The details of this assessment will be recorded in the CRF. If the inclusion/ exclusion criteria are satisfied and the informed written consent has been obtained the participant will be enrolled.

*Knowledge of ethnicity is necessary for the immunogenetics aspect of this study.

6.5 Randomisation

Eligible subjects will be prospectively randomised in a 1:1 ratio to receive PCV-13 or PCV-10 based upon a randomisation schedule prepared by a computer programme at the point of enrolment into the study. No separate randomisation visit is necessary. Due to the nature of the study, the study is unblinded, therefore no unblinding procedure is needed.

6.6 Subsequent Assessments

- Inclusion/Exclusion criteria will be checked on each subsequent visit following Visit 1.
- Axillary temperature will be checked on visit 1 and at visit 3 if a dose of PCV-13 is to be administered at this time (see section 10.3).
- The source document will be updated on each visit, recording any medical advice sought, any adverse event and concomitant medications started since the last visit.

6.6.1 Visit 1

- Study explanation provided.
- Obtain written informed consent from parent/legal guardian
- Obtain and record medical and vaccination history
- Check inclusion and exclusion criteria.
- Perform physical examination and record findings.
- Measure and record the participant's axillary temperature.
- If participant is suitable for inclusion in study, assign participant number
- Randomisation
- Collect a blood sample (up to 7.5 ml) after application of topical anaesthetic cream

- Administer a single 0.5-ml dose of PCV-10 or PCV-13 via intramuscular injection into the anterolateral aspect of either thigh. .
- Assessment of the crying time from the moment of needle insertion until all crying activity had ceased.
- Assessment of the observed pain as the vaccine is injected through the skin by
    a. Use of the Numerical Rating Scale (NRS) by parent/legal guardian
    b. Completion of the Modified Behavioural Pain Scale (MBPS) by the second member of the study team who did not administer the vaccine.
- Observe the participant for at least 15 minutes after vaccination for any significant acute reactions. Any AEs noted during the observation period should be recorded on the source documents and on the AE section of the case report form (CRF).
- Issue a ruler and a digital thermometer to the parent/legal guardian and provide instructions on their use.
- Issue a participant diary to the parent/legal guardian and provide instruction on its completion.
- Record vaccination details in the participant's red book.
- Ask the parent/legal guardian to contact the investigator immediately if any significant illness or hospitalization occurs during the study period, or if the participant experiences a large (> 14 mm) local reaction.
- Provision of topical anaesthetic cream for parent/guardian to apply prior to next visit (written instructions and appropriate dressings to be provided)
- Schedule visit 2 for 28-42 days after visit 1.
- The investigator or an authorised designee completes the CRF and updates the study vaccine accountability records.

6.6.2 Visit 2 (28 - 42 days after visit 1)

- Review the participant's diary data since the previous visit.
- Based on review of the participant's diary and clinical evaluation, determine whether any unsolicited AEs or serious adverse events (SAEs) have occurred since the last study visit and record them on the CRF.
- Ensure the participant continues to meet the criteria for participation in the trial.
- Measure and record the participant's axillary temperature.
- Collect a blood sample (7.5ml).

- Administer routine vaccines (MMR and Hib-MenC).
- Observe the participant for at least 15 minutes after vaccination for any significant acute reactions.
- Record vaccination details in the participant's red book.
- Schedule visit 3 for 11-12 months after visit 1.
- The investigator or an authorised designee completes the CRF and updates the study vaccine accountability records.

6.6.3 Visit 3 (11-12 months after visit 1)

- Review any serious adverse events (SAEs) that have occurred since the previous visit
- Ensure the participant continues to meet the criteria for continued participation in the trial.
- Collect a blood sample (7.5ml).
- The investigator or an authorised designee completes the CRF and updates the study vaccine accountability records.

The study procedures are summarised in the table below:

Study visits	V1 Recruitment	V2	V3
Immunisations	PCV-10 or PCV-13	MMR Hib-MenC	
Blood samples	√	√	√

6.6.4 Study Duration

The start date of this study will be January 2012, anticipating its end date in October 2013. Visit 1 will take place between January 2012 and July 2012. Visit 2 will take place 1 month and visit 3 12 months after Visit 1. Processing of the samples and data analysis will take another 6 months so that the total study duration is anticipated to be 24 months.

6.7 Definition of End of Study

The study will be considered ended when all the study visits have been completed and all the biological samples processed and analysed.

6.8 Discontinuation/Withdrawal of Participants from Study Treatment:

- The parent/legal guardian of the participating children has the right to withdraw their child from the study at any time, without having to provide any particular reason for doing so.
- Some children may be withdrawn from the study for the following reasons:
    a. Consent withdrawn
    b. Lost to follow up
- For any AE requiring withdrawal from a study or persisting at the end of it, appropriate follow up will be provided until its satisfactory resolution or stabilisation.
- The reason for withdrawal will be recorded in the source document/CRF.
- The investigator should aim to retain as many participants within the study as possible to respect the intention to treat analysis, but may consider excluding participants who meet the following criteria from the per-protocol analysis:
    a. An adverse event which requires discontinuation of the study or results in inability to comply with study procedures.
    b. Disease diagnosis or progression requiring discontinuation of the study treatment or results in inability to comply with study procedures.

6.9 Source Data

Source documents are original documents, data, and records from which the participant's CRF data are obtained. These include, but are not limited to hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory records and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g., there is no other written or electronic record of data). In this study the CRF will be used as the source document for assessments and measurements made at study visits. All documents will be stored safely in confidential conditions. On all study-specific documents or electronic case report forms, other than the initial response form from the participant and the signed consent, the participant will be referred to by the study participant number, not by name.

7 TREATMENT OF STUDY PARTICIPANTS

7.1 Interventions and study vaccines

Both pneumococcal conjugate vaccines (0.5 ml) will be administered intramuscularly using a 0.6 x 25 mm 23 gauge needle into the anterolateral aspect of either thigh, at visit 1.

Synflorix

The 10-valent pneumococcal conjugate vaccine (PCV-10, Synflorix®, GSK Biologicals) contains 1 µg of each capsular polysaccharide of the pneumococcal serotypes 1, 5, 6B, 7F, 9V, 14 and 23F, and 3 µg of serotype 4, conjugated individually to Protein D (total dose 13 µg), a recombinant non-lipidated form of a highly conserved 42-kDa cell-surface lipoprotein of non-typable *H. influenzae*; 3 µg of serotype 18C capsular polysaccharide conjugated to tetanus toxoid (8 µg); and 3 µg of serotype 19F capsular polysaccharide conjugated to diphtheria toxoid (5 µg). The pneumococcal conjugates are adsorbed onto 0.5 mg aluminium phosphate adjuvant per 0.5 ml dose.

Prevenar 13

The 13-valent pneumococcal conjugate vaccine (PCV-13, Prevenar 13®, Pfizer) contains saccharides from pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F individually conjugated to cross-reacting material (CRM_{197}). The vaccine is formulated to contain 2.2 µg of each saccharide, except for 4.4 µg of 6B, with a total nominal dose of 34 µg CRM_{197} per 0.5-mL dose. The final formulation contains 5mM succinate buffer, with 0.125 mg of aluminum as aluminum phosphate per 0.5ml dose.

7.2 Storage of study treatment

The vaccines will be stored at the study site following the manufacturer's recommendations. Both vaccines will be shipped at +2 centigrade to +8 centigrade to the study site. Upon receipt at the study site, vaccines should be immediately transferred to +2 to +8 centigrade temperature-monitored refrigerator for storage.

The refrigerator will be secure and have controlled access. The Investigator (or designee) will record daily refrigerator temperature readings, maintain a temperature log for the refrigerator, and alert the sponsor of any deviations. In case of temperature deviations, the study vaccines cannot be used and should be quarantined until authorisation to use the vaccine is received from GSK of Pfizer. Guidance in temperature monitoring and procedures for review or temperature deviations will be provided.

7.3 Compliance with study treatment

All vaccines will be administered by study stuff during the visits therefore compliance will not be an issue.

7.4 Accountability of study treatment

- All doses of Synflorix® will be supplied by GSK to the study site.
- Prevenar 13® will be supplied by Movianto or the local NHS pharmacy to the Oxford Vaccine group.
- A member of the Investigator's Team will collect the study treatment.
- All movements between GSK and the study site will be documented.
- The Investigator (or delegate) will be responsible of ordering a new supply of the vaccine when necessary.
- Unused Prevenar 13 vaccine will be given back to the local pharmacy or to General Practitioners at the end of the study and expired or damaged vaccines will be disposed of.
- Unused/expired/damaged Synflorix vaccine will be retrieved by the manufacturer at its agreed time/end of the study or destroyed locally, depending on the manufacturers instructions.
- A vaccine accountability log will be used in order to check that supplies, used and remaining vaccine numbers match at all times.
- A temperature log will be kept up to date.
- Cool boxes with attached thermometer will be used while transporting the study treatment during scheduled visits.
- Storage and transport temperature will follow the manufacturer's recommendations at all times

7.5 Concomitant medication

- All medications will be recorded on the source document/CRF together with the reason for starting it as well as start date and stop date if applicable.
- It is the investigator's responsibility to review the on-going eligibility of the participant during the duration of the study.

8 LABORATORY ANALYSIS

Blood samples of up to 7.5 ml will be collected at all three study visits. First, 2.5 ml will be collected into "red top" serum separator tubes. Following this, up to 5 ml will be collected in a heparinised tube for B cell analysis.

Blood samples in the serum separator tubes will be stored at room temperature for a sufficient time as to allow the red blood cells to clot and then stored between 2 to 8°C. Samples will be

centrifuged at 3000 rpm for 10 minutes within 24 hours at the study site and separated into 2 or 3 aliquots (volume of sample permitting) for storage at -20°C (range -15°C to -40°C) or below. DNA samples will be collected using blood clots remaining after serum collection. Shipping of the aliquots required for serological analysis from the study site to the relevant laboratory in London will be coordinated by the Oxford Vaccine Group.

8.1 Total and functional antibody concentrations

Both of the following antibody measurements will be performed at the WHO pneumococcal reference laboratory at the University College London (Director: Prof. D. Goldblatt):

Serum concentrations of total anticapsular immunoglobulin G (IgG) for all pneumococcal serotypes included in PCV-13 plus serotype 6C will be identified by Enzyme linked immunosorbant Assay (ELISA) method and expressed as µg/ml. The assay will employ 2 absorbents: a C polysaccharide-containing cell wall extract plus serotype 22F capsular polysaccharide.

Serum concentrations of functional anticapsular immunoglobulin G (IgG) for all pneumococcal serotypes included in PCV-13 plus serotype 6C will be measured by an opsonophagocytic activity assay (OPA).

If after the end of the trial any of the participants is found to have an IgG concentration <0.35 µg/ml specific for at least one of the serotypes included in PCV-13 in the serum obtained at visit 2 his/her parent/legal guardian will be notified and administration of a PCV-13 booster dose through the GP will be recommended to the participant.

8.2 DNA storage and analysis

With specific consent DNA will be extracted from blood clots and stored in the biobank of the Oxford Vaccine Centre laboratory at the Centre of Clinical Vaccinology and Tropical Medicine, University of Oxford for later analysis for genetic polymorphisms related to the immune response and reactogenicity to vaccines. Parents of study participants may decline consent for this and still participate in all other aspects of this study.

8.3 B cell analysis

Some of the blood taken at each visit will be placed in heparinised tubes for B cell analysis. These samples will be stored at room temperature until they are processed. Processing will

begin within 6 hours from the time of blood sampling whenever possible. The frequency of IgG antibody secreting cells (ASC) will be enumerated using an Enzyme Linked Immunosorbant Spot assay (ELISpot) either on uncultured cells or following 5 or 6 days of culture with combinations of Staphylococcus aureus Cowan strain (SAC,) pokeweed mitogen and CPG DNA. The ELISpot assay can detect IgG-ASC specific for tetanus and diphtheria toxoids or each of the pneumococcal capsular polysaccharides. For flow cytometry analysis, B cells will be separated by magnetic beads followed by labelling with various surface markers as well as fluorescently labelled antigens in order to detect antigen-binding B cells through flow cytometry. Gating on different combinations of surface markers will allow defining antigen-specific B cell subsets and their kinetics in the immune response to vaccines. Using flow cytometry, B cells of interest will be sorted and processed to reverse transcribe immunoglobulin-specific mRNA followed by PCR-amplification and sequencing of the relevant cDNA.

9 SAFETY

9.1 Adverse Event (AE)

9.1.1 An AE or adverse experience is

Any untoward medical occurrence in a patient or clinical investigation participant administered a medicinal product, which does not necessarily have to have a causal relationship with this treatment (the study medication).

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of the study medication, whether or not considered related to the study medication.

9.2 Adverse Reaction (AR)

All untoward and unintended responses to a medicinal product related to any dose. The phrase "responses to a medicinal product" means that a causal relationship between a study medication and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

9.3 Unexpected Adverse Reaction

An adverse reaction, the nature or severity of which is not consistent with the summary of product characteristics.

9.4 Serious or Severe Adverse Events

To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following note of clarification is provided:

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

9.5 Serious Adverse Event or Reaction

A serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose:

- Results in death,
- Is life-threatening,

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

- Requires inpatient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.

Medical and scientific judgment should be exercised in deciding whether an adverse event is serious in other situations. The time period for recording SAEs will be the duration of the study.

9.6 Expected Adverse Reactions

For all expected adverse reactions see summary of the product characteristics for each vaccine.

9.7 Suspected Unexpected Serious Adverse Reactions (SUSAR)

A serious adverse reaction, the nature or severity of which is not consistent with the applicable product information.

9.8 Causality assessment

The relationship of medically significant AEs to the study medication will be assessed by a medically qualified investigator according to the following criteria:

- Related: If the causal relationship between the vaccine and the SAE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.
- Not related: If there is no causal relationship between the vaccine and the SAE i.e. the event is caused by something other than the vaccine e.g. underlying disease, a concomitant medication.

9.9 Reporting procedures for serious adverse events

All Serious Adverse Events (SAEs) must be reported to the CI and sponsor (University of Oxford Clinical Trials and Research Governance Office) within one working day of discovery or notification of the event. All SAE information must be recorded on a SAE form and faxed or a scanned copy emailed to the sponsor.

In addition, SAEs that are experienced by participants taking a GSK product must also be reported to GSK within one working day of discovery or notification of the event. The SAE must be recorded on the SAE form belonging to the manufacturer to whom it will be faxed or scanned copy emailed.

Additional information received for a case reported to GSK (follow-up or corrections to the original case) need to be detailed on a new SAE form and faxed to the sponsor and to GSK if appropriate.

The CI will report suspected adverse reactions which are both serious and unexpected (SUSARs) experienced by participants taking a study vaccine to the Competent Authorities (MHRA) and the REC that gave a favourable opinion for the study.

Fatal or life-threatening SUSARs must be reported within 7 days and all other SUSARs within 15 days. In addition to the expedited reporting above, the CI shall submit once a year throughout the clinical trial or on request a safety report to the Competent Authority and Ethics Committee.

In addition to the above, the CI will be responsible for reporting any SUSARs to the relevant NHS Research and Development offices in their area.

The CTRG will ensure that all SAEs are reviewed by medical monitors on a weekly basis and at the next meeting of the University of Oxford Trials Safety Group (TSG), who will meet at regular intervals and consider:

- Occurrence and nature of adverse events
- Whether additional information on adverse events is required
- Consider taking appropriate action where necessary to halt trials
- Act/advise on incidents occurring between meetings that require rapid assessment (e.g. SUSARs).

9.10 Reporting procedures for adverse events

All AEs occurring in the first 4 days after booster immunisation, and all AEs resulting in an unscheduled visits to a physician or emergency department or withdrawal from the study occurring within 1 month after vaccination observed by the investigator or reported by the participant, whether or not attributed to study medication, will be reported on the CRF/source document, including the diary card. Reactions occurring in the first 4 days after immunisation will be divided up into solicited and unsolicited reactions.

Adverse events solicited in the diary card that are ongoing after day 4 (as recorded in the diary card provided) will similarly be recorded in the CRF/source document.

The following information will be recorded for medically significant AEs: description, date of onset and end date, severity, assessment of relatedness to study medication, other suspect drug or device and action taken. Follow up information should be provided as necessary.

9.11 Solicited Reactions

This refers to terms/symptoms that are pre-listed in the diary card. Solicited reactions are those that have previously been reported with the use of the study vaccines and, unless stated otherwise, will be considered related to the vaccine.

Solicited reactions will be recorded and monitored by the parent/legal guardian on the diary card provided by the study team from the day of immunisation until day 4 post-immunisation with the PCV-10 or PCV-13 booster vaccine.

9.11.1 Local Reactions

Local injection site reactions (redness, swelling and tenderness) at the site of PCV-10 or PCV-13 booster injection will be monitored daily for 4 days (day 0 to day 4) after each vaccination. An end date will be recorded for any reactions persisting after day 4.

9.11.2 Pain at time of injection

Immediate pain at vaccine administration will be measured in several ways:

- Duration of crying from the moment of needle insertion until all crying activity had ceased: a time in seconds or minutes will be recorded on the diary card
- Numerical Rating Scale (NRS) determined by the parent/carer of the child: a score (1-10) will be recorded on the diary card
- The Modified Behavioural Pain Scale (MBPS) determined by a member of the study team: a score (0-10) will be recorded on the diary card

9.11.3 Redness and Swelling

Redness and swelling will be measured and recorded on the diary card. They will be categorised as absent, mild, moderate and severe based on the scale given below. A ruler will be given to the parent/legal guardian with instructions for measuring any redness or swelling at the injection site. The parent/legal guardian will be asked to measure the largest diameter of a local reaction and record this in the diary given at visit 1.

- Absent No redness or swelling present (0mm)
- Mild 0 cm to 2.5 cm
- Moderate 2.5 cm to 5.0 cm
- Severe > 5.0 cm

9.11.4 Tenderness

The parent/legal guardian will be asked to assess and record in the diary card whether tenderness is present at the injection site and grade it on the following scale:

- Absent
- Tenderness present, does not interfere with routine activities
- Tenderness present, cries or shows discomfort while doing routine activities
- Tenderness interfering with limb movement

9.12 General Reactions

9.12.1 Temperature

Axillary temperature will be measured by the parent/legal guardian and recorded from the vaccination day (day 0) to day 4 post-vaccination. A digital thermometer will be provided by the study team on Visit 1. Temperature will be considered as fever if ≥38.0°C. In the event of fever, temperature will be collected daily until its resolution.

9.13 Unsolicited Reactions

These are any adverse events that are not pre-listed in the diary card but may be reported on the diary card by the participant or through interview with the participant. These will be reported in the CRF/source document adverse event form.

AEs considered related to the study medication by the investigator or the sponsor will be followed until resolution or the event is considered stable.

The following attributes must be assigned by the investigator to all adverse events occurring within 4 days of pneumococcal immunisation and all AEs resulting in an unscheduled visits to a physician or emergency department or withdrawal from the study: description, date of onset and resolution date, severity, assessment of relatedness to study medication, other suspected drug or device and action taken.

The investigator may be asked to provide follow-up information.

All related AEs that result in a participant's withdrawal from the study or are present at the end of the study, should be followed up until a satisfactory resolution occurs.

All deaths occurring during the study must be reported to the Sponsor/CI. These include deaths within 30 days of the final dose of study medication and deaths up to the last formal follow-up observational period, whichever is longer. For all deaths, available autopsy reports and relevant medical reports will be made available for reporting to the relevant authorities.

It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the participant's removal from treatment. A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the patient must undergo an end of study assessment and arrangements made for appropriate care until symptoms cease or the condition becomes stable. The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe. The relationship of AEs to the study medication will be assessed.

9.14 Other reportable information

The following events experienced by participants taking a GSK product will also be reported to GSK:

- Overdose
- Spontaneous reports of an unexpected therapeutic or clinical benefit associated with the use of a GSK product
- Abuse (for example, use for non-clinical reasons)
- Inadvertent or accidental exposure
- SIDS
- Apnoea
- Autism
- Hypotonic hyporesponsive episode (HHE)
- Metal poisoning
- Lack of effect

10 STATISTICS AND ANALYSIS

10.1 Description of statistical methods

Two sided binomial exact confidence intervals will be constructed around the proportion of subjects achieving specified antibody concentrations, and around rates of local and systemic reactions. The antibody concentrations will be logarithmically transformed for analysis, since the untransformed values are highly skewed. For each serotype separately, and at each time point, geometric means of the antibody concentrations from each of the blood draws will be calculated. Two-sided, 95% confidence intervals will be constructed by back transformation of the confidence intervals for the mean of the logarithmically transformed assay results computed using the Student t distribution. In addition, the geometric mean fold-rise for the serotype-specific concentrations will be derived from the exponent of the difference between the logarithmically transformed assay results before and after the booster dose. The corresponding 95% confidence intervals will be computed using the same method mentioned above, i.e. the confidence interval will be obtained on the log transformed scale, and then back transformed to give the result on the original scale.

A Chi-square test or Fisher's exact test will be used to compare the proportions between groups of participants. Two-sample t-test will be used to compare the logarithmically transformed

concentrations between the groups, or else analysis of variance, to adjust for baseline values, as appropriate. Differences between the two groups and the corresponding 95% confidence intervals will be calculated. Comparisons between the two groups will be carried out based on the allocated groups.

10.2 Level of Statistical Significance

A clinically significant difference would be shown if the lower bound of the confidence interval for the difference between vaccine groups (PCV-10 – PCV-13) for the proportion of participants with IgG antibody levels above 0.35 μg/ml 1 month following the booster dose is less than -10%. For this non-inferiority test, the level of statistical significance is 2.5% (as this is a one-sided test).

10.3 Analysis and Endpoints

For the primary objective (post-boost proportion of participants who have serotype-specific IgG concentrations ≥0.35 mcg/ml to the 10 serotypes included in PCV-10) the sample size of 84 participants in each of these groups will allow assessment of non-inferiority of PCV-10 compared with PCV-13 with a margin of 10% using 80% power and 2.5% significance level allowing for a 15% drop-out rate.

If, during the course of the study, the the numbers required to meet the primary objective at visit 2 are lower than anticipated, additional participants will be recruited to achieve sufficient numbers to meet the primary objective.

The Intention to treat (ITT) population for immunogenicity will consist of all participants receiving a dose of PCV-10 or PCV-13 and providing a blood sample at visit 2. Deviations from protocol resulting in participants being removed from the per-protocol population will be defined prior to data analysis. The primary endpoint analysis will be conducted on the per-protocol population as this is the more conservative analysis for a non-inferiority comparison. The ITT analysis will be conducted as a sensitivity analysis.

If after the end of the trial any of the participants is found to have an IgG concentration <0.35 μg/ml specific for at least one of the serotypes included in PCV-13 in the serum obtained at visit 2 his/her parent/legal guardian will be notified and administration of a PCV-13 booster dose through the GP will be recommended to the participant.

The rates of reactogenicity of the PCV booster (in terms of rates of each separate local and systemic reactions following vaccination) will be presented along with their 95% confidence intervals.

Reactogenicity and immediate pain at injection data will be summarised descriptively and the association between any genetic polymorphism and outcomes of booster does will be assessed using Fisher's exact test.

B cell data will be summarised descriptively.

A detailed statistical analysis plan will be prepared and agreed on prior to provision of data.

11 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Direct access to these will be granted to authorised and trained representatives from the sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

12 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures.

Regular monitoring will be performed according to the ICH GCP. Monitoring of this study will be conducted by the Oxford Vaccine Centre internal monitoring team according to a monitoring plan agreed by the sponsor.

Following written standard operating procedures and an approved monitoring plan, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

A trial steering group will be formed that will include, but not be limited to, the chief investigator, a statistician, a quality assurance manager and project manager.

13 ETHICS

13.1 Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the ethical principles of the current revision of the Declaration of Helsinki.

13.2 ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with the applicable regulatory requirements with the principles of the current revision of the ICH Guidelines for Good Clinical Practice.

13.3 Other Ethical Considerations

No study procedures or evaluations will occur until after informed consent is obtained.

14 PARTICIPANT CONFIDENTIALITY

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participants ID number on the CRF and any electronic case report form. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the Data Protection Act which requires data to be anonymised as soon as it is practical to do so. Any data or samples that relate to participants and that leave the study site will be identified by study number and or code only.

15 DATA HANDLING AND RECORD KEEPING

All study files (paper and electronic) with personal information, demographic and clinical details on the participants will be kept in a locked research office at the study centre. The study details will be entered on to a computer with an electronic database protected by a password. All blood samples will be identified by study number only and will have no personal identifiers.

Information on study participants will be recorded on hard copy case report forms (CRFs) held locally to be entered into a web based electronic CRF (eCRF, OpenClinica™ database stored on a secure University of Oxford server).

16 FINANCING AND INSURANCE

The study is funded by a grant from GSK and will be supported by staff funded by the NIHR Oxford Biomedical Research Centre. Insurance will be provided by the University of Oxford for staff and study participants. The respective vaccine manufacturers will be responsible for product liability.

17 CHARACTERISTICS OF STUDY POPULATION

17.1 Baseline characteristics of study participants

178 children aged 11-13 months, from the Oxfordshire region, were recruited and randomised into 2 groups to receive one dose of either PCV-10 or PCV-13 at 12 months of age. Figure 2 shows the number of participants and their flow through the study. Baseline demographics of randomised participants are shown in Table 1. The majority of participants were white males who appeared calm at the first visit.

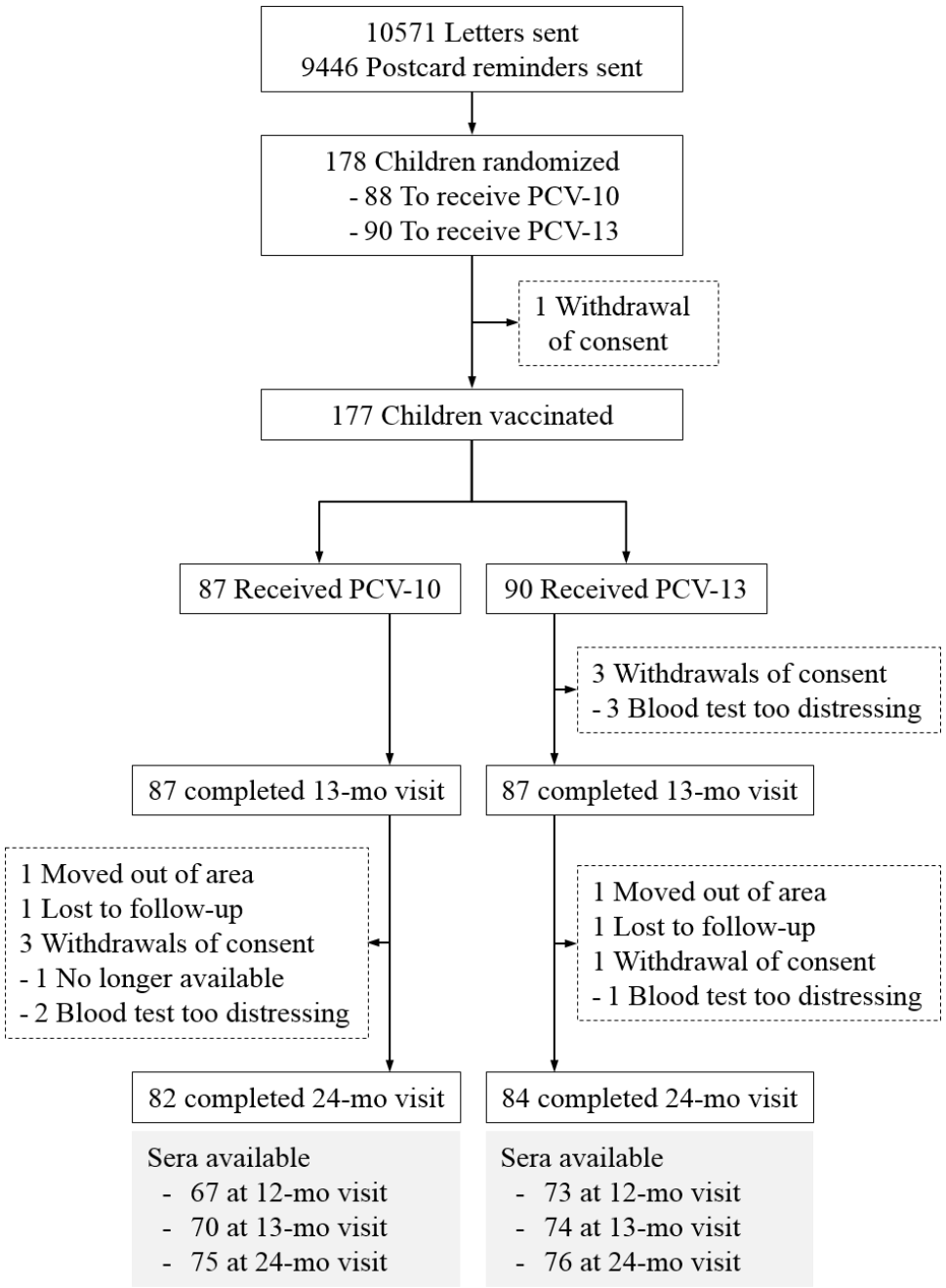

Figure 2 CONSORT diagram showing the flow through the study

Table 1 Baseline demographics by randomised group

	PCV-10 (n = 87)		PCV-13 (n = 90)	
	N	(%)	N	(%)
Age (Months)				
Mean (SD) {Min-Max}	12.6 (0.4) {11.1-13.3}		12.7 (0.4) {11.7-13.5}	
Sex				
Male	46	(52.9)	58	(64.4)
Female	41	(47.1)	32	(35.6)
Ethnicity				
White Caucasian/ European	78	(89.7)	74	(82.2)
Other	9	(10.3)	15	(16.7)
Unknown	0	0	1	(1.1)
Baseline Behaviour				
Calm	41	(47.1)	44	(48.9)
Distressed	15	(17.2)	15	(16.7)
Crying	11	(12.6)	13	(14.4)
Unknown	20	(23.0)	18	(20.0)

17.2 SAEs reported during the study

There were 5 SAEs reported during the study none of which were considered related to study vaccines (Table 2).

Table 2 Details of SAEs reported during the study.

Date of Report	Diagnosis	Outcome	Vaccine received	Relationship to Vaccine
12/Sep/2012	Febrile illness	Recovered	PCV-10	None
26/Apr/2013	Suspected Meningococcal septicaemia	Recovered	PCV-10	None
01/Mai/2013	Viral gastroenteritis	Recovered	PCV-13	None
12/Jun/2013	Viral induced wheeze	Recovered	PCV-10	None
09/Aug/2013	Febrile convulsion	Recovered	PCV-13	None

17.3 Withdrawal due to AEs or death

There were no deaths during the study. No child was withdrawn due to an adverse event.

17.4 Withdrawal of consent

Parents of 8 study participants withdrew consent (Figure 2).

17.5 Lost to follow-up

2 study participants were lost to follow-up and further 2 families moved out of the area (Figure 2).

17.6 Protocol deviations

There were 3 protocol deviations during the study related to processing of the blood samples. One of the blood samples taken at the 3rd visit was processed outside the 24 hour time window with 30 minutes over, which was not considered to affect the sample quality. Another B cell blood sample taken at 24 months was stored overnight in the fridge instead of it being stored at room temperature. Viability of the cells in this sample was similar to other samples and therefore the sample was processed normally. A further visit 3 blood sample was not processed due to a laboratory error and therefore data from this participant at this study time point was lost.

There were a number of protocol deviations related to the timing of visits and age at enrolment (Table 3 and Figure 3), none of which were thought to significantly affect any of the outcome measures.

Table 3 Protocol deviations related to the timing of visits.

Study participant	Type of deviation	Group	Value (days)	Range as indicated in the protocol
1	Too young at visit 1	PCV-10	340	351 – 407 days
7	Visit 2 too late	PCV-10	48	
10	Visit 2 too late	PCV-13	49	
12	Visit 2 too late	PCV-10	49	
13	Visit 2 too late	PCV-13	49	
25	Visit 2 too late	PCV-10	43	
29	Visit 2 too late	PCV-10	44	
38	Visit 2 too late	PCV-13	62	28 – 42 days
41	Visit 2 too late	PCV-13	48	
60	Visit 2 too early	PCV-13	20	
107	Visit 2 too late	PCV-13	51	
122	Visit 2 too early	PCV-13	21	
144	Visit 2 too late	PCV-13	44	
161	Visit 2 too late	PCV-13	48	
89	Visit 3 too late	PCV-10	386	
90	Visit 3 too late	PCV-13	373	334 – 365 days
129	Visit 3 too late	PCV-13	374	

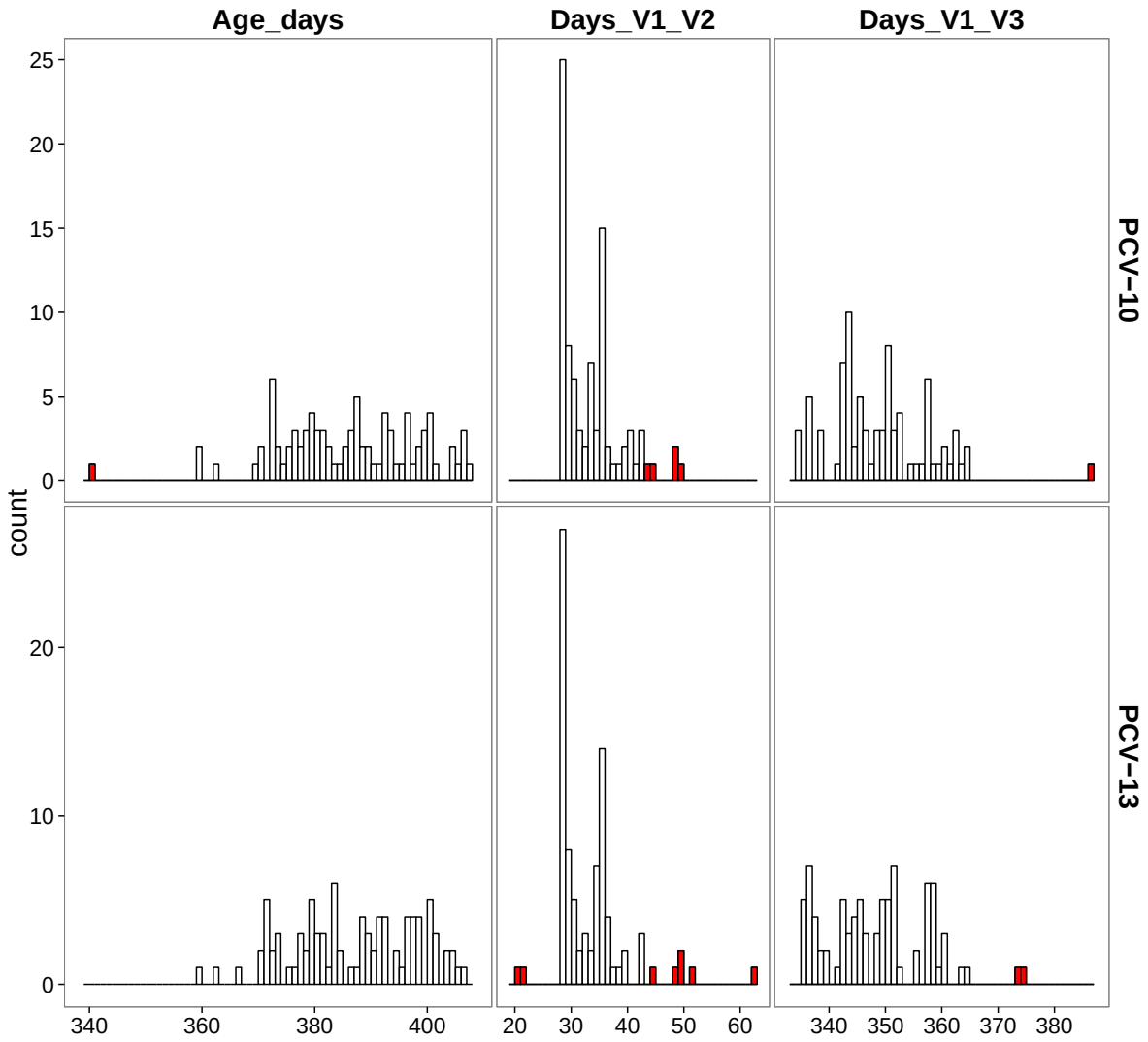

Figure 3 Histograms showing the distribution of age at visit 1, days between visit 1 and 2 and days between visit 1 and 3 with red bars indicating protocol violations.

17.7 Inclusion, exclusion, and withdrawal criteria

None of the 178 children enrolled in the study failed to comply with the inclusion criteria. There were a number of participants who had missed blood draws (Table 4) exceeding the anticipated 12.5% drop-out rate, which is why an additional 10 study participants were recruited to the study following amendment of the study protocol.

Table 4 Participants with no blood draw (missed blood)

Study visit (age)	PCV-10 group (n = 87)	PCV-13 group (n = 90)
V1 (12 mo)	n = 20	n = 17
V2 (13 mo)	n = 17	n = 16
V3 (24 mo)	n = 11	n = 15

17.8 Prohibited concomitant medications

No children were excluded for taking prohibited concomitant medicines.

18 IMMUNOGENICITY EVALUATION

ELISA lower limit of quantitation (LLOQ) as established during the validation are set as follows:

- All serotypes: <0.150 mcg/ml
- Values below the LLOQ were replaced with 0.075 mcg/ml

OPA lower LLOQ as established during the validation are set as follows:

- All serotypes: <8
- Values below the LLOQ will be replaced with 4

Memory B cell LLOQ:

- All antigens: 1 spot/4 wells = 0.25 spots/10^5 cultured lymphocytes → 1.25 spots/10^6 cultured lymphocytes
- Values below the LLOQ will be replaced with 0.625 spots/10^6 cultured lymphocytes

General note:

In the following tables, significant results ($p<0.05$) are displayed in bold and significant differences between groups are highlighted in colour with 'red' indicating superior results in the PCV-10 group and 'blue' indicating superior results in the PCV-13 group.

18.1 Primary endpoint

Table 5 below shows the results of the primary endpoint. Serotype 5 and 9V in the PCV-10 group were inferior to the PCV-13 group at 13 months.

Table 5 Proportion IgG antibody concentrations ≥0.35µg/ml by serotype and vaccine group at 13 months with a non-inferiority assessment.

	PCV-10 (n = 70)			**PCV-13 (n = 74)**			**PCV-10 - PCV-13**	
		Proportion IgG			Proportion IgG			
Serotype	N	≥0.35 µg/ml	95% CI	N	≥0.35 µg/ml	95% CI	Difference	95% CI
1	70	0.986	0.923, 1.000	74	0.987	0.927, 1.000	-0.001	-0.040, 0.038
4	70	1.000	0.949, 1.000	74	0.987	0.927, 1.000	0.014	-0.013, 0.040
5	**70**	**0.729**	**0.609, 0.828**	**74**	**0.960**	**0.886, 0.992**	**-0.231**	**-0.347, -0.115**

	PCV-10 (n = 70)			PCV-13 (n = 74)			PCV-10 - PCV-13	
		Proportion IgG			Proportion IgG			
Serotype	N	≥0.35 µg/ml	95% CI	N	≥0.35 µg/ml	95% CI	Difference	95% CI
6B	70	0.971	0.901, 0.997	73	1.000	0.951, 1.000	-0.029	-0.069, 0.011
7F	70	0.986	0.923, 1.000	74	1.000	0.951, 1.000	-0.014	-0.043, 0.014
9V	**70**	**0.871**	**0.770, 0.939**	**74**	**0.987**	**0.927, 1.000**	**-0.115**	**-0.200, -0.031**
14	70	1.000	0.949, 1.000	74	1.000	0.951, 1.000	0.000	
18C	70	1.000	0.949, 1.000	74	0.973	0.906, 0.997	0.027	-0.011, 0.065
19F	70	1.000	0.949, 1.000	74	0.987	0.927, 1.000	0.014	-0.013, 0.040
23F	64	1.000	0.944, 1.000	74	1.000	0.951, 1.000	0.000	

18.2 Secondary Endpoints

18.2.1 Serotype-specific IgG concentrations

18.2.1.1 Proportions of participants with IgG concentrations ≥0.35 µg/ml

Proportions of participants with serotype-specific IgG concentrations equal or above 0.35 µg/ml for both vaccine groups separately are shown for visit 1 (12 mo, Table 6), visit 2 (13 mo, Table 7), and visit 3 (24 mo, Table 8). At baseline, group differences were found for serotypes 6B and 19A with significantly higher proportions in the PCV-10 group for both serotypes (Table 6 and Figure 4). One month following the booster vaccine, the proportions of participants with serotype-specific IgG above the chosen threshold were significantly higher in the PCV-13 group for serotype 5 and 9V, which are serotypes common to both PCV-10 and PCV-13 (Table 7 and Figure 4). In addition, these proportions were also higher in the PCV-13 group for 2 out of the 3 serotypes only included in PCV-13, namely serotypes 3 and 6A but not serotype 19A (Table 7 and Figure 4). At 24 months, 12 months following the booster, significant higher proportions above the IgG threshold in the PCV-13 compared with the PCV-10 group were found for serotypes 6B, 7F, and 14 whereas proportions were significantly higher in the PCV-10 compared with the PCV-13 group for serotypes for serotype 4 and 19F (Table 8 and Figure 4). For serotypes only included in PCV-13 significantly higher proportions in the PCV-13 group remained only for serotype 6A while proportions above threshold had dropped to similar levels in both groups for serotypes 3 and 19A (Table 8 and Figure 4).

Table 6 Proportion of participants with IgG antibody concentrations ≥0.35 µg/ml by serotype and vaccine group at 12 months.

	PCV-10 (n = 67)			PCV-13 (n = 73)			
		Proportion IgG			Proportion IgG		Chi-Square
Serotype	N	≥0.35 µg/ml	95% CI	N	≥0.35 µg/ml	95% CI	Test P-value
1	67	0.224	0.131, 0.342	73	0.233	0.142, 0.346	0.899
4	67	0.075	0.025, 0.166	73	0.110	0.049, 0.205	0.476
5	67	0.179	0.096, 0.292	73	0.164	0.088, 0.270	0.817
6B	**67**	**0.313**	**0.206, 0.438**	**73**	**0.164**	**0.088, 0.270**	**0.038**
7F	66	0.561	0.433, 0.683	73	0.589	0.468, 0.703	0.735
9V	67	0.149	0.074, 0.257	73	0.137	0.068, 0.238	0.836
14	67	0.851	0.743, 0.926	73	0.904	0.812, 0.961	0.334
18C	66	0.152	0.075, 0.261	73	0.137	0.068, 0.238	0.807
19F	67	0.627	0.500, 0.742	73	0.480	0.361, 0.600	0.080
23F	67	0.194	0.108, 0.309	73	0.151	0.078, 0.254	0.497
3†	66	0.227	0.133, 0.347	70	0.200	0.114, 0.313	0.698
6A†	67	0.522	0.397, 0.646	73	0.548	0.427, 0.665	0.762
19A†	**63**	**0.603**	**0.472, 0.724**	**73**	**0.384**	**0.272, 0.505**	**0.011**

† Serotypes contained in PCV-13 only

Table 7 Proportion of participants with IgG antibody concentrations ≥0.35 µg/ml by serotype and vaccine group at 13 months.

	PCV-10 (n = 70)			PCV-13 (n = 74)			
		Proportion IgG			Proportion IgG		Chi-Square
Serotype	N	≥0.35 µg/ml	95% CI	N	≥0.35 µg/ml	95% CI	Test P-value
1	70	0.986	0.923, 1.000	74	0.987	0.927, 1.000	0.968
4	70	1.000	0.949, 1.000	74	0.987	0.927, 1.000	0.329
5	**70**	**0.729**	**0.609, 0.828**	**74**	**0.960**	**0.886, 0.992**	**0.000**
6B	70	0.971	0.901, 0.997	73	1.000	0.951, 1.000	0.146
7F	70	0.986	0.923, 1.000	74	1.000	0.951, 1.000	0.302
9V	**70**	**0.871**	**0.770, 0.939**	**74**	**0.987**	**0.927, 1.000**	**0.007**
14	70	1.000	0.949, 1.000	74	1.000	0.951, 1.000	.
18C	70	1.000	0.949, 1.000	74	0.973	0.906, 0.997	0.166
19F	70	1.000	0.949, 1.000	74	0.987	0.927, 1.000	0.329
23F	64	1.000	0.944, 1.000	74	1.000	0.951, 1.000	.
3†	**66**	**0.636**	**0.509, 0.751**	**74**	**0.946**	**0.867, 0.985**	**0.000**
6A†	**70**	**0.943**	**0.860, 0.984**	**74**	**1.000**	**0.951, 1.000**	**0.037**
19A†	67	0.985	0.920, 1.000	74	1.000	0.951, 1.000	0.292

† Serotypes contained in PCV-13 only.

Table 8 Proportion of participants with IgG antibody concentrations ≥0.35 µg/ml by serotype and vaccine group at 24 months.

	PCV-10 (n = 75)			PCV-13 (n = 76)			
		Proportion IgG			Proportion IgG		Chi-Square
Serotype	N	≥0.35 µg/ml	95% CI	N	≥0.35 µg/ml	95% CI	Test P-value
1	75	0.187	0.106, 0.293	76	0.276	0.180, 0.391	0.192
4	**75**	**0.427**	**0.313, 0.546**	**76**	**0.197**	**0.115, 0.305**	**0.002**
5	75	0.307	0.205, 0.424	76	0.421	0.309, 0.540	0.144
6B	**75**	**0.573**	**0.454, 0.687**	**76**	**0.776**	**0.666, 0.864**	**0.008**
7F	**75**	**0.400**	**0.289, 0.520**	**76**	**0.684**	**0.567, 0.786**	**0.000**
9V	75	0.200	0.116, 0.308	76	0.329	0.225, 0.446	0.073
14	**75**	**0.813**	**0.707, 0.894**	**76**	**0.934**	**0.853, 0.978**	**0.025**
18C	72	0.181	0.100, 0.289	75	0.147	0.076, 0.247	0.578
19F	**75**	**0.973**	**0.907, 0.997**	**76**	**0.868**	**0.771, 0.935**	**0.017**
23F	75	0.520	0.402, 0.637	76	0.618	0.500, 0.728	0.222
3†	68	0.324	0.215, 0.448	75	0.347	0.240, 0.465	0.770
6A†	**75**	**0.533**	**0.414, 0.649**	**76**	**0.921**	**0.836, 0.970**	**0.000**
19A†	73	0.822	0.715, 0.902	76	0.842	0.740, 0.916	0.742

† Serotypes contained in PCV-13 only.

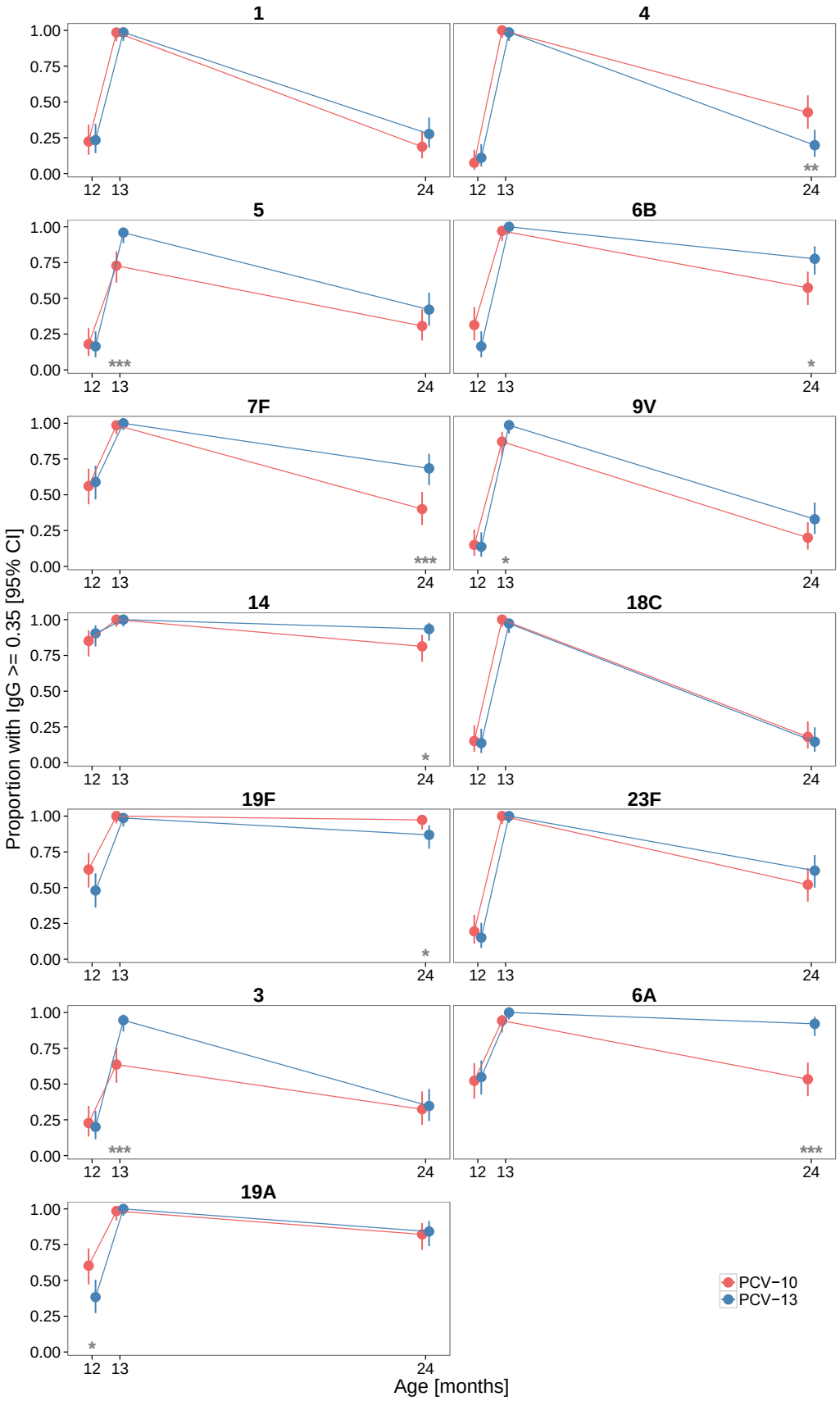

Figure 4 Proportion of participants with IgG antibody concentrations ≥0.35 µg/ml by serotype and vaccine group at all 3 study time points. Groups were compared using the Chi-square test and stars indicate the associated p-value (*** <.001; ** <.01; * <.05).

18.2.1.2 Serotype-specific IgG geometric mean concentrations (GMC) by serotype and vaccine group

Serotype-specific GMC are shown in in the tables below for all 3 visits when study participants were 12, 13 and 24 months old. At baseline (12 mo) there were significantly higher IgG GMC seen in the PCV-10 compared with the PCV-13 group for serotypes 19F and 19A (Table 9 and Figure 5). One month after booster vaccination at 13 months of age, significant differences between the groups were seen for all serotypes. Serotype-specific IgG GMC against serotypes 4, 18C and 19F were significantly higher in the PCV-10 group compared with the PCV-13 group (Table 10 and Figure 5) whereas GMCs were significantly higher in the PCV-13 compared with the PCV-10 group for the remaining serotypes common to both vaccines (1, 5, 6B, 7F, 9V, 13, and 23F) as well as for the serotypes only included in PCV-13 (3, 6A, and 19A; Table 10). When persistence of IgG concentrations were assessed at 2 years of age, significant differences remained for 6 serotypes. IgG GMCs for serotypes 4 and 19F were significantly higher in the PCV-10 group whereas GMCs for serotypes 6B, 7F, and 9V (serotypes common to both PCV-10 and PCV-13) and 6A (PCV-13 only serotype) were significantly higher in the PCV-13 compared with the PCV-10 group (Table 11 and Figure 5).

Table 9 Serotype-specific IgG geometric mean concentration and ratio at 12 months by vaccine group.

	PCV-10 (n = 67)			**PCV-13 (n = 73)**			**PCV-10 / PCV-13**		
		Geometric			Geometric		Geometric		
Serotype	N	Mean	95% CI	N	Mean	95% CI	Mean Ratio	95% CI	P-Value
1	67	0.197	0.160, 0.241	73	0.207	0.174, 0.248	0.948	0.725, 1.239	0.693
4	67	0.123	0.104, 0.146	73	0.125	0.106, 0.146	0.988	0.785, 1.243	0.918
5	67	0.155	0.127, 0.189	73	0.139	0.115, 0.166	1.117	0.854, 1.460	0.418
6B	67	0.206	0.169, 0.251	73	0.162	0.135, 0.195	1.268	0.969, 1.659	0.083
7F	66	0.386	0.324, 0.460	73	0.403	0.333, 0.488	0.958	0.741, 1.237	0.739
9V	67	0.135	0.111, 0.163	73	0.136	0.112, 0.165	0.991	0.758, 1.296	0.950
14	67	1.044	0.826, 1.319	73	1.086	0.862, 1.367	0.961	0.694, 1.331	0.811
18C	66	0.160	0.127, 0.201	73	0.142	0.116, 0.174	1.124	0.832, 1.520	0.443
19F	**67**	**0.562**	**0.411, 0.771**	**73**	**0.351**	**0.280, 0.440**	**1.602**	**1.091, 2.352**	**0.017**
23F	67	0.172	0.136, 0.218	73	0.156	0.129, 0.189	1.101	0.815, 1.488	0.529
3†	66	0.212	0.173, 0.261	70	0.183	0.154, 0.216	1.163	0.894, 1.513	0.258
6A†	67	0.400	0.319, 0.502	73	0.351	0.280, 0.439	1.142	0.832, 1.566	0.409
19A†	**63**	**0.478**	**0.325, 0.703**	**73**	**0.295**	**0.234, 0.373**	**1.620**	**1.036, 2.534**	**0.035**

† Serotypes contained in PCV-13 only

Table 10 Serotype-specific IgG geometric mean concentration and ratio at 13 months by vaccine group.

	PCV-10 (n = 70)			PCV-13 (n = 74)			PCV-10 / PCV-13		
		Geometric			Geometric		Geometric		
Serotype	N	Mean	95% CI	N	Mean	95% CI	Mean Ratio	95% CI	P-Value
1	**70**	**1.569**	**1.297, 1.898**	**74**	**2.574**	**2.118, 3.127**	**0.610**	**0.465, 0.799**	**0.000**
4	**70**	**3.328**	**2.740, 4.042**	**74**	**2.125**	**1.755, 2.572**	**1.566**	**1.195, 2.052**	**0.001**
5	**70**	**0.491**	**0.412, 0.586**	**74**	**1.180**	**0.999, 1.394**	**0.416**	**0.328, 0.529**	**0.000**
6B	**70**	**2.927**	**2.347, 3.650**	**73**	**5.765**	**4.656, 7.137**	**0.508**	**0.374, 0.688**	**0.000**
7F	**70**	**1.715**	**1.488, 1.976**	**74**	**3.395**	**2.883, 3.998**	**0.505**	**0.408, 0.626**	**0.000**
9V	**70**	**0.705**	**0.585, 0.851**	**74**	**1.800**	**1.534, 2.113**	**0.392**	**0.307, 0.500**	**0.000**
14	**70**	**5.392**	**4.531, 6.415**	**74**	**9.556**	**7.749, 11.79**	**0.564**	**0.431, 0.739**	**0.000**
18C	**70**	**2.941**	**2.487, 3.477**	**74**	**1.798**	**1.474, 2.192**	**1.636**	**1.264, 2.116**	**0.000**
19F	**70**	**16.76**	**13.55, 20.73**	**74**	**7.804**	**6.201, 9.822**	**2.148**	**1.574, 2.930**	**0.000**
23F	**64**	**3.474**	**2.806, 4.302**	**74**	**4.576**	**3.842, 5.451**	**0.759**	**0.577, 0.998**	**0.049**
3†	**66**	**0.456**	**0.373, 0.558**	**74**	**1.124**	**0.971, 1.300**	**0.406**	**0.317, 0.519**	**0.000**
6A†	**70**	**1.137**	**0.905, 1.428**	**74**	**10.16**	**8.267, 12.48**	**0.112**	**0.083, 0.152**	**0.000**
19A†	**67**	**3.327**	**2.453, 4.514**	**74**	**6.090**	**4.868, 7.618**	**0.546**	**0.375, 0.795**	**0.002**

† Serotypes contained in PCV-13 only

Table 11 Serotype-specific IgG geometric mean concentration and ratio at 24 months by vaccine group.

	PCV-10 (n = 75)			PCV-13 (n = 76)			PCV-10 / PCV-13		
		Geometric			Geometric		Geometric		
Serotype	N	Mean	95% CI	N	Mean	95% CI	Mean Ratio	95% CI	P-Value
1	75	0.177	0.147, 0.212	76	0.220	0.188, 0.258	0.802	0.631, 1.018	0.070
4	**75**	**0.298**	**0.246, 0.361**	**76**	**0.181**	**0.153, 0.214**	**1.647**	**1.280, 2.120**	**0.000**
5	75	0.260	0.217, 0.312	76	0.293	0.241, 0.355	0.890	0.684, 1.157	0.381
6B	**75**	**0.400**	**0.324, 0.493**	**76**	**0.630**	**0.522, 0.761**	**0.634**	**0.480, 0.839**	**0.002**
7F	**75**	**0.335**	**0.282, 0.397**	**76**	**0.498**	**0.431, 0.574**	**0.672**	**0.538, 0.839**	**0.001**
9V	**75**	**0.159**	**0.130, 0.194**	**76**	**0.231**	**0.191, 0.279**	**0.690**	**0.525, 0.907**	**0.008**
14	75	0.827	0.665, 1.029	76	1.007	0.840, 1.208	0.821	0.620, 1.089	0.170
18C	72	0.193	0.162, 0.231	75	0.156	0.130, 0.188	1.238	0.960, 1.597	0.099
19F	**75**	**2.186**	**1.711, 2.791**	**76**	**0.856**	**0.715, 1.024**	**2.554**	**1.890, 3.451**	**0.000**
23F	75	0.408	0.316, 0.525	76	0.510	0.398, 0.654	0.799	0.562, 1.136	0.210
3†	68	0.258	0.197, 0.338	75	0.281	0.229, 0.346	0.917	0.654, 1.286	0.613
6A†	**75**	**0.387**	**0.301, 0.497**	**76**	**0.885**	**0.742, 1.056**	**0.437**	**0.323, 0.593**	**0.000**
19A†	73	1.044	0.758, 1.437	76	0.909	0.698, 1.184	1.147	0.760, 1.732	0.510

† Serotypes contained in PCV-13 only

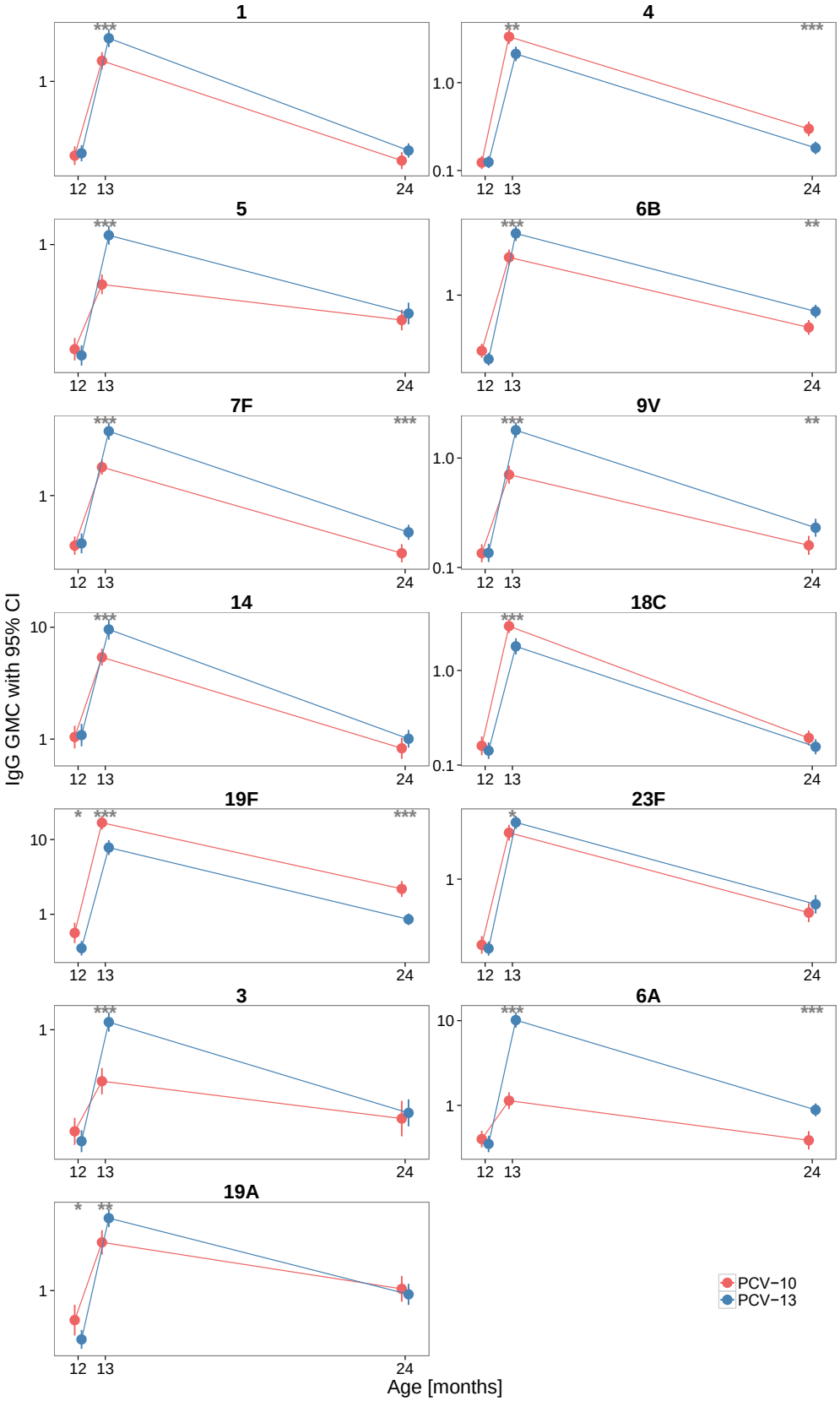

Figure 5 Serotype-specific IgG geometric mean concentrations by serotype and vaccine group at all 3 study time points. Groups were compared using independent samples t-tests using $\log_{10}$-transformed data with Satterthwaite's correction for unequal variances and stars indicate the associated p-value (*** <.001; ** <.01; * <.05).

18.2.1.3 IgG geometric mean fold change between study visits

Tables below show IgG geometric mean fold changes between study visits including unadjusted and adjusted treatment effects. For both study groups significant increases of IgG concentrations for all serotypes were seen between baseline (12 months) and 1 month post-booster at 13 months of age (Table 12). The effect of the type of vaccine adjusted for baseline IgG antibody concentrations, age, sex and ethnicity demonstrated significant differences between the groups for the increase in IgG antibody between 12 and 13 months of age for all serotypes. The 12 to 13 month increase in IgG antibody concentrations was significantly greater in the PCV-10 compared with the PCV-13 group for serotypes 4, 18C, and 19F whereas a greater increase was seen in the PCV-13 group compared with the PCV-10 for all other serotypes tested (Table 12). Significantly higher IgG concentrations were seen at 24 compared with 12 months of age for some but not all serotypes with some differences between the vaccine groups (Table 13). The increase in IgG concentrations between 12 and 24 months of age was significantly greater in the PCV-10 compared with the PCV-13 group for serotypes 4 and 19F whereas PCV-13 compared with PCV-10 recipients had a significantly greater increase between these 2 study time points for serotypes 1, 6B, 7F, 9B, and 6A (Table 13). The 24 months IgG concentrations were not different to the 12 months IgG concentrations for serotypes 1 and 14 in both groups, serotypes 7F, 9V, 3, and 6A in the PCV-10 group and serotypes 18C in the PCV-13 group (Table 13). A significant decline in IgG concentrations between 13 and 24 months of age was seen for most serotypes in both groups with some group differences (Table 14). The decline was significantly more pronounced in the PCV-13 compared with the PCV-10 group for serotypes 5, 9V, 14, 3, and 6A (Table 14).

Table 12 Serotype-specific IgG geometric mean fold change from 12 to 13 months by vaccine group

	PCV-10						**PCV-13**					
		N missing at months		Geometric Mean Fold				N missing at months		Geometric Mean Fold		
Serotype	N	12	13	Rise*	95% CI	P-Value	N	12	13	Rise*	95% CI	P-Value
1	**57**	**10**	**13**	**8.046**	**5.959,10.86**	**<0.001**	**61**	**12**	**13**	**12.35**	**9.241,16.50**	**<0.001**
4	**57**	**10**	**13**	**27.32**	**20.65,36.14**	**<0.001**	**61**	**12**	**13**	**16.65**	**12.70,21.82**	**<0.001**
5	**57**	**10**	**13**	**3.234**	**2.415,4.331**	**<0.001**	**61**	**12**	**13**	**8.457**	**6.447,11.09**	**<0.001**
6B	**57**	**10**	**13**	**14.58**	**10.66,19.95**	**<0.001**	**60**	**13**	**14**	**33.30**	**24.72,44.85**	**<0.001**
7F	**56**	**11**	**14**	**4.510**	**3.553,5.725**	**<0.001**	**61**	**12**	**13**	**9.191**	**6.972.12.12**	**<0.001**
9V	**57**	**10**	**13**	**5.548**	**4.310,7.141**	**<0.001**	**61**	**12**	**13**	**13.25**	**10.06,17.44**	**<0.001**
14	**57**	**10**	**13**	**5.311**	**3.851,7.324**	**<0.001**	**61**	**12**	**13**	**8.876**	**6.371,12.37**	**<0.001**

	PCV-10						PCV-13					
		N missing at months		Geometric Mean Fold				N missing at months		Geometric Mean Fold		
Serotype	N	12	13	Rise*	95% CI	P-Value	N	12	13	Rise*	95% CI	P-Value
18C	**56**	**11**	**14**	**19.29**	**14.24,26.12**	**<0.001**	**61**	**12**	**13**	**12.81**	**9.345,17.55**	**<0.001**
19F	**57**	**10**	**13**	**27.16**	**17.57,41.97**	**<0.001**	**61**	**12**	**13**	**23.85**	**17.15,33.17**	**<0.001**
23F	**51**	**16**	**19**	**17.50**	**11.97,25.58**	**<0.001**	**61**	**12**	**13**	**27.87**	**20.85,37.24**	**<0.001**
3†	**54**	**13**	**16**	**2.202**	**1.625,2.982**	**<0.001**	**59**	**14**	**15**	**6.302**	**4.905,8.096**	**<0.001**
6A†	**57**	**10**	**13**	**2.635**	**1.910,3.634**	**<0.001**	**61**	**12**	**13**	**25.55**	**18.33,35.59**	**<0.001**
19A†	**53**	**14**	**17**	**6.001**	**3.403,10.58**	**<0.001**	**61**	**12**	**13**	**19.12**	**13.55,26.98**	**<0.001**

* Geometric mean change from 12 months to 13 months
† Serotypes contained in PCV-13 only

Table 12 continued

	Unadjusted Treatment Effect**			Adjusted Treatment Effect***		
Serotype	PCV-10/ PCV-13	95% CI	P-Value	PCV-10/ PCV-13	95% CI	P-Value
1	**0.652**	**0.465,0.912**	**0.013**	**0.565**	**0.431,0.740**	**<0.001**
4	**1.641**	**1.117,2.410**	**0.012**	**1.560**	**1.129,2.153**	**0.007**
5	**0.382**	**0.294,0.497**	**<0.001**	**0.378**	**0.306,0.468**	**<0.001**
6B	**0.438**	**0.311,0.616**	**<0.001**	**0.452**	**0.336,0.608**	**<0.001**
7F	**0.491**	**0.356,0.677**	**<0.001**	**0.464**	**0.369,0.583**	**<0.001**
9V	**0.419**	**0.308,0.570**	**<0.001**	**0.371**	**0.294,0.468**	**<0.001**
14	**0.598**	**0.410,0.874**	**0.008**	**0.560**	**0.424,0.739**	**<0.001**
18C	**1.506**	**1.008,2.250**	**0.046**	**1.473**	**1.103,1.969**	**0.009**
19F	**1.139**	**0.690,1.880**	**0.609**	**1.720**	**1.243,2.381**	**0.001**
23F	**0.628**	**0.419,0.941**	**0.024**	**0.674**	**0.496,0.916**	**0.012**
3†	**0.349**	**0.270,0.452**	**<0.001**	**0.359**	**0.288,0.448**	**<0.001**
6A†	**0.103**	**0.071,0.151**	**<0.001**	**0.101**	**0.076,0.134**	**<0.001**
19A†	**0.314**	**0.205,0.480**	**<0.001**	**0.390**	**0.277,0.549**	**<0.001**

** Unadjusted estimate of the ratio of geometric means at 13 months (PCV-10/PCV-13)
*** Ratio of geometric means at 13 months (PCV-10/PCV-13), adjusted for 12 month values, age, sex and ethnicity
† Serotypes contained in PCV-13 only

Table 13 Serotype-specific IgG geometric mean fold change from 12 to 24 months by vaccine group

	PCV-10						PCV-13					
		N missing at months		Geometric Mean Fold				N missing at months		Geometric Mean Fold		
Serotype	N	12	13	Rise*	95% CI	P-Value	N	12	13	Rise*	95% CI	P-Value
1	58	9	17	0.888	0.654,1.205	0.441	64	9	12	1.156	0.898,1.487	0.257

	PCV-10						PCV-13					
		N missing at months		Geometric Mean Fold				N missing at months		Geometric Mean Fold		
Serotype	N	12	13	Rise*	95% CI	P-Value	N	12	13	Rise*	95% CI	P-Value
4	**58**	**9**	**17**	**2.440**	**1.828,3.257**	**<0.001**	**64**	**9**	**12**	**1.488**	**1.164,1.901**	**0.002**
5	**58**	**9**	**17**	**1.646**	**1.217,2.224**	**0.001**	**64**	**9**	**12**	**2.017**	**1.509,2.696**	**<0.001**
6B	**58**	**9**	**17**	**1.765**	**1.291,2.413**	**<0.001**	**64**	**9**	**12**	**3.930**	**2.934,5.264**	**<0.001**
7F	57	10	18	0.812	0.629,1.047	0.108	**64**	**9**	**12**	**1.307**	**1.006,1.697**	**0.045**
9V	58	9	17	1.114	0.824,1.508	0.479	**64**	**9**	**12**	**1.814**	**1.357,2.424**	**0.000**
14	58	9	17	0.790	0.557,1.120	0.184	64	9	12	0.960	0.694,1.327	0.803
18C	**56**	**11**	**19**	**1.367**	**1.019,1.835**	**0.037**	63	10	13	1.166	0.864,1.574	0.313
19F	**58**	**9**	**17**	**4.054**	**2.612,6.291**	**<0.001**	**64**	**9**	**12**	**2.497**	**1.824,3.419**	**<0.001**
23F	**58**	**9**	**17**	**2.492**	**1.693,3.666**	**<0.001**	**64**	**9**	**12**	**3.324**	**2.366,4.669**	**<0.001**
3†	51	16	24	1.124	0.772,1.635	0.539	**60**	**13**	**16**	**1.505**	**1.149,1.972**	**0.003**
6A†	58	9	17	0.811	0.560,1.175	0.265	**64**	**9**	**12**	**2.470**	**1.795,3.399**	**<0.001**
19A†	**54**	**13**	**21**	**2.526**	**1.437,4.441**	**0.002**	**64**	**9**	**12**	**3.004**	**2.118,4.261**	**<0.001**

* Geometric mean change from 12 months to 24 months
† Serotypes contained in PCV-13 only

Table 13 continued

	Unadjusted Treatment Effect**			Adjusted Treatment Effect***		
Serotype	PCV-10/ PCV-13	95% CI	P-Value	PCV-10/ PCV-13	95% CI	P-Value
1	**0.768**	**0.592,0.996**	**0.047**	**0.771**	**0.620,0.957**	**0.019**
4	**1.640**	**1.180,2.280**	**0.004**	**1.653**	**1.239,2.206**	**0.001**
5	0.816	0.625,1.065	0.133	0.827	0.649,1.054	0.124
6B	**0.449**	**0.328,0.615**	**<0.001**	**0.513**	**0.387,0.681**	**<0.001**
7F	**0.621**	**0.492,0.784**	**<0.001**	**0.631**	**0.520,0.765**	**<0.001**
9V	**0.614**	**0.443,0.853**	**0.004**	**0.639**	**0.480,0.850**	**0.002**
14	0.823	0.591,1.146	0.245	0.847	0.638,1.125	0.249
18C	1.173	0.845,1.628	0.338	1.199	0.918,1.565	0.180
19F	**1.624**	**1.047,2.517**	**0.031**	**2.316**	**1.675,3.203**	**<0.001**
23F	0.750	0.521,1.079	0.119	0.738	0.521,1.047	0.088
3†	0.746	0.543,1.025	0.071	0.821	0.601,1.121	0.211
6A†	**0.328**	**0.230,0.469**	**<0.001**	**0.365**	**0.270,0.495**	**<0.001**
19A†	0.841	0.512,1.381	0.490	1.039	0.683,1.582	0.856

** Unadjusted estimate of the ratio of geometric means at 13 months (PCV-10/PCV-13)
*** Ratio of geometric means at 24 months (PCV-10/PCV-13), adjusted for 12 month values, age, sex and ethnicity
† Serotypes contained in PCV-13 only

Table 14 Serotype-specific IgG geometric mean fold change from 13 to 24 months by vaccine group

	PCV-10						PCV-13					
		N missing at months		Geometric Mean Fold				N missing at months		Geometric Mean Fold		
Serotype	N	12	13	Rise*	95% CI	P-Value	N	12	13	Rise*	95% CI	P-Value
1	58	9	17	0.888	0.654,1.205	0.441	64	9	12	1.156	0.898,1.487	0.257
4	**58**	**9**	**17**	**2.440**	**1.828,3.257**	**<0.001**	**64**	**9**	**12**	**1.488**	**1.164,1.901**	**0.002**
5	**58**	**9**	**17**	**1.646**	**1.217,2.224**	**0.001**	**64**	**9**	**12**	**2.017**	**1.509,2.696**	**<0.001**
6B	**58**	**9**	**17**	**1.765**	**1.291,2.413**	**<0.001**	**64**	**9**	**12**	**3.930**	**2.934,5.264**	**<0.001**
7F	57	10	18	0.812	0.629,1.047	0.108	**64**	**9**	**12**	**1.307**	**1.006,1.697**	**0.045**
9V	58	9	17	1.114	0.824,1.508	0.479	**64**	**9**	**12**	**1.814**	**1.357,2.424**	**<0.001**
14	58	9	17	0.790	0.557,1.120	0.184	64	9	12	0.960	0.694,1.327	0.803
18C	**56**	**11**	**19**	**1.367**	**1.019,1.835**	**0.037**	63	10	13	1.166	0.864,1.574	0.313
19F	**58**	**9**	**17**	**4.054**	**2.612,6.291**	**<0.001**	**64**	**9**	**12**	**2.497**	**1.824,3.419**	**<0.001**
23F	**58**	**9**	**17**	**2.492**	**1.693,3.666**	**<0.001**	**64**	**9**	**12**	**3.324**	**2.366,4.669**	**<0.001**
3†	51	16	24	1.124	0.772,1.635	0.539	**60**	**13**	**16**	**1.505**	**1.149,1.972**	**0.003**
6A†	58	9	17	0.811	0.560,1.175	0.265	**64**	**9**	**12**	**2.470**	**1.795,3.399**	**<0.001**
19A†	**54**	**13**	**21**	**2.526**	**1.437,4.441**	**0.002**	**64**	**9**	**12**	**3.004**	**2.118,4.261**	**<0.001**

* Geometric mean change from 12 months to 13 months
† Serotypes contained in PCV-13 only

Table 14 continued

	Unadjusted Treatment Effect**			Adjusted Treatment Effect***		
Serotype	PCV-10/ PCV-13	95% CI	P-Value	PCV-10/ PCV-13	95% CI	P-Value
1	1.359	1.017,1.815	0.038	1.349	0.981,1.857	0.065
4	1.126	0.854,1.484	0.398	1.089	0.793,1.495	0.595
5	**2.103**	**1.600,2.764**	**0.000**	**2.096**	**1.527,2.877**	**0.000**
6B	1.136	0.817,1.580	0.446	1.090	0.759,1.566	0.638
7F	1.176	0.923,1.498	0.188	1.188	0.902,1.564	0.218
9V	**1.729**	**1.277,2.340**	**0.000**	**1.663**	**1.193,2.317**	**0.003**
14	**1.451**	**1.101,1.912**	**0.009**	**1.446**	**1.076,1.942**	**0.015**
18C	0.724	0.533,0.984	0.039	0.782	0.552,1.109	0.166
19F	1.302	0.886,1.914	0.177	1.371	0.915,2.053	0.124
23F	1.056	0.733,1.521	0.769	1.082	0.746,1.570	0.674
3†	**2.012**	**1.417,2.857**	**0.000**	**2.232**	**1.522,3.273**	**0.000**
6A†	**3.698**	**2.622,5.214**	**0.000**	**3.412**	**2.336,4.983**	**0.000**
19A†	**2.340**	**1.490,3.673**	**0.000**	**2.658**	**1.597,4.423**	**0.000**

** Unadjusted estimate of the ratio of geometric means at 13 months (PCV-10/PCV-13)

*** Ratio of geometric means at 24 months (PCV-10/PCV-13), adjusted for 13 month values, age, sex and ethnicity

	Unadjusted Treatment Effect**			Adjusted Treatment Effect***		
Serotype	PCV-10/ PCV-13	95% CI	P-Value	PCV-10/ PCV-13	95% CI	P-Value

† Serotypes contained in PCV-13 only

18.2.2 Serotype-specific opsonophagocytic activity (OPA) titres

18.2.2.1 Proportion of participants with OPA titres ≥8

At baseline, there were no significant differences between proportions of study participants with OPA titres ≥8 for all 13 serotypes with generally low proportions with the exception of serotypes 7F and 14 (Table 15 and Figure 6). One month following the booster, the proportions of participants with OPA ≥8 were high for most serotypes children were vaccinated against but there were significantly higher proportions in the PCV-13 compared with the PCV-10 group for serotypes 1, 5, and 9V and all 3 serotypes only contained in PCV-13 (3, 6A, 19A) (Table 16 and Figure 6). These proportions dropped again when assessed at 24 months of age (Table 17 and Figure 6). Significant differences between the groups were seen for serotypes 6B, 7F, 9B and 6A (higher in the PCV-13 group) and serotype 19F (higher in the PCV-10 group) (Table 17 and Figure 6).

Table 15 Proportions of participants with OPA titres ≥8 by serotype and vaccine group at 12 months.

	PCV-10 (n = 67)			**PCV-13 (n = 73)**				
Serotype	N	Proportion OPA ≥8	95% CI	N	Proportion OPA ≥8	95% CI	Fisher's exact test p-value	Chi-Square Test p-value
1	66	0.015	0.000, 0.082	73	0.014	0.000, 0.074	1.0000	0.9427
4	61	0.295	0.185, 0.426	71	0.296	0.193, 0.416	1.0000	0.9931
5	61	0.213	0.119, 0.337	71	0.268	0.169, 0.386	0.5433	0.4664
6B	62	0.323	0.209, 0.453	69	0.304	0.199, 0.427	0.8521	0.8222
7F	62	0.903	0.801, 0.964	72	0.861	0.759, 0.931		0.4535
9V	65	0.215	0.123, 0.335	69	0.174	0.093, 0.284	0.6629	0.5440
14	63	0.889	0.784, 0.954	70	0.943	0.860, 0.984	0.3485	0.2592
18C	66	0.364	0.249, 0.491	66	0.318	0.209, 0.444	0.7137	0.5817
19F	66	0.439	0.317, 0.567	68	0.397	0.280, 0.523	0.7264	0.6194
23F	63	0.556	0.425, 0.681	69	0.449	0.329, 0.574		0.2225
3†	62	0.161	0.080, 0.277	67	0.060	0.017, 0.146	0.0891	0.0638
6A†	63	0.381	0.261, 0.512	66	0.455	0.331, 0.582		0.3970
19A†	63	0.270	0.166, 0.397	73	0.206	0.120, 0.316	0.4214	0.3776

† Serotypes contained in PCV-13 only

Table 16 Proportions of participants with OPA titres ≥8 by serotype and vaccine group at 13 months.

	PCV-10 (n = 69)			PCV-13 (n = 74)				
Serotype	N	Proportion OPA ≥8	95% CI	N	Proportion OPA ≥8	95% CI	Fisher's exact test p-value	Chi-Square Test p-value
1	**67**	**0.687**	**0.562, 0.794**	**73**	**0.849**	**0.746, 0.922**	**0.0270**	**0.0220**
4	68	1.000	0.947, 1.000	73	1.000	0.951, 1.000	-	.
5	**66**	**0.939**	**0.852, 0.983**	**74**	**1.000**	**0.951, 1.000**	**0.0470**	**0.0317**
6B	69	1.000	0.948, 1.000	74	1.000	0.951, 1.000	-	.
7F	69	1.000	0.948, 1.000	74	1.000	0.951, 1.000	-	.
9V	**68**	**0.912**	**0.818, 0.967**	**74**	**1.000**	**0.951, 1.000**	**0.0107**	**0.0090**
14	69	1.000	0.948, 1.000	74	1.000	0.951, 1.000	-	.
18C	69	1.000	0.948, 1.000	74	1.000	0.951, 1.000	-	.
19F	69	1.000	0.948, 1.000	74	0.987	0.927, 1.000	1.0000	0.3325
23F	69	0.986	0.922, 1.000	74	1.000	0.951, 1.000	0.4825	0.2987
3†	**69**	**0.116**	**0.051, 0.216**	**72**	**0.833**	**0.727, 0.911**	**<.0001**	**<.0001**
6A†	**64**	**0.859**	**0.750, 0.934**	**74**	**1.000**	**0.951, 1.000**	**<.0001**	**0.0008**
19A†	**68**	**0.941**	**0.856, 0.984**	**74**	**1.000**	**0.951, 1.000**	**0.0502**	**0.0343**

† Serotypes contained in PCV-13 only

Table 17 Proportions of participants with OPA titres ≥8 by serotype and vaccine group at 24 months.

	PCV-10 (n = 75)			PCV-13 (n = 76)				
Serotype	N	Proportion OPA ≥8	95% CI	N	Proportion OPA ≥8	95% CI	Fisher's exact test p-value	Chi-Square Test p-value
1	75	0.000	0.000, 0.048	76	0.013	0.000, 0.071	1.0000	0.3189
4	72	0.514	0.393, 0.633	73	0.425	0.310, 0.546	0.3199	0.2817
5	73	0.110	0.049, 0.205	71	0.141	0.070, 0.244	0.6213	0.5707
6B	**74**	**0.351**	**0.244, 0.471**	**74**	**0.797**	**0.688, 0.882**	**<.0001**	**<.0001**
7F	**74**	**0.905**	**0.815, 0.961**	**76**	**1.000**	**0.953, 1.000**	**0.0061**	**0.0060**
9V	**74**	**0.176**	**0.097, 0.282**	**74**	**0.581**	**0.461, 0.695**	**<.0001**	**<.0001**
14	73	0.973	0.905, 0.997	75	0.973	0.907, 0.997	1.0000	0.9781
18C	72	0.694	0.575, 0.798	71	0.620	0.497, 0.732	0.3816	0.3465
19F	**73**	**0.863**	**0.762, 0.932**	**75**	**0.600**	**0.480, 0.711**	**<.0001**	**0.0003**
23F	**69**	**0.681**	**0.558, 0.788**	**75**	**0.880**	**0.784, 0.944**	**0.0045**	**0.0037**
3†	73	0.082	0.031, 0.170	76	0.145	0.075, 0.244	0.3046	0.2300
6A†	**70**	**0.271**	**0.172, 0.391**	**73**	**0.877**	**0.779, 0.942**	**<.0001**	**<.0001**
19A†	69	0.623	0.498, 0.737	71	0.761	0.645, 0.854	0.0994	0.0781

† Serotypes contained in PCV-13 only

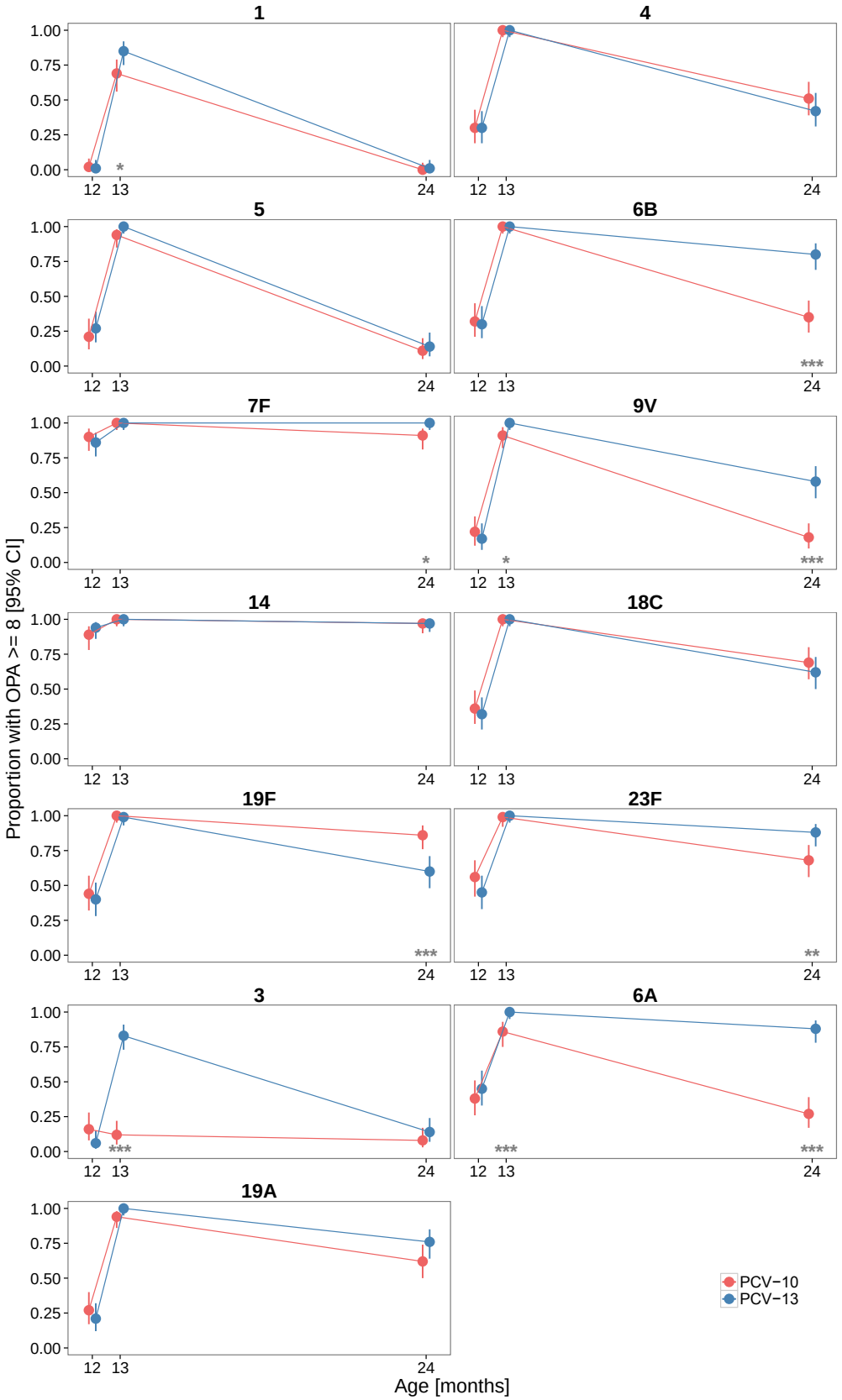

Figure 6 Proportion of participants with OPA titres ≥8 by serotype and vaccine group at all 3 study time points. Groups were compared using the Chi-square test and stars indicate the associated p-value (*** <.001; ** <.01; * <.05).

18.2.2.2 Serotype-specific geometric mean OPA titre (GMT) by serotype and vaccine group

Serotype-specific OPA GMT are shown in the tables below for all 3 visits when study participants were 12, 13 and 24 months old. At baseline, significantly higher OPA GMT were seen in the PCV-10 compared with the PCV-13 group only for serotype 3 (Table 18 and Figure 7). One month following the booster, significantly higher GMTs were seen in the PCV-13 compared with the PCV-10 group for serotypes 1, 5, 6B, 7F, 9V, 14, and 23F as well as for all 3 serotypes only included in PCV-13 (3, 6A, 19A) (Table 19 and Figure 7). For serotypes 4 and 18C there were no significant differences between the groups whereas the OPA response to serotype 19F was significantly higher in the PCV-10 compared with the PCV-13 group (Table 19 and Figure 7). At 24 months, significant differences between the groups remained for 7 out of the 11 serotypes that were significantly different at 13 months with statistically higher responses in the PCV-13 compared with the PCV-10 group for serotypes 6B, 7F, 9V, 23F, 6A, and 19A and statistically lower responses in PCV-13 compared with PCV-10 recipients for serotype 19F (Table 20 and Figure 7).

Table 18 Geometric mean OPA titres and ratios at 12 months by vaccine group

	PCV-10 (n = 67)			**PCV-13 (n = 73)**			**PCV-10 / PCV-13**		
		Geometric			Geometric		Geometric		
Serotype	N	Mean	95% CI	N	Mean	95% CI	Mean Ratio	95% CI	P-Value
1	66	4.042	3.958, 4.128	73	4.157	3.849, 4.490	0.972	0.898, 1.053	0.484
4	61	8.840	6.075, 12.86	71	7.913	5.913, 10.59	1.117	0.698, 1.789	0.642
5	61	5.790	4.642, 7.221	71	5.777	4.947, 6.745	1.002	0.767, 1.310	0.987
6B	62	8.813	6.328, 12.27	69	8.353	6.082, 11.47	1.055	0.670, 1.662	0.816
7F	62	365.9	233.7, 572.9	72	315.5	195.4, 509.3	1.160	0.606, 2.222	0.652
9V	65	9.085	5.828, 14.16	69	8.101	5.449, 12.04	1.121	0.622, 2.023	0.701
14	63	234.6	154.1, 357.0	70	252.2	183.6, 346.5	0.930	0.552, 1.568	0.784
18C	66	12.65	8.020, 19.95	66	8.989	6.216, 13.00	1.407	0.787, 2.516	0.247
19F	66	17.28	10.75, 27.76	68	12.27	8.040, 18.73	1.408	0.750, 2.643	0.284
23F	63	34.56	19.59, 60.96	69	21.20	12.70, 35.38	1.630	0.764, 3.477	0.204
3†	**62**	**5.137**	**4.282, 6.163**	**67**	**4.203**	**4.002, 4.414**	**1.222**	**1.013, 1.475**	**0.037**
6A†	63	19.50	11.12, 34.17	66	26.48	15.41, 45.50	0.736	0.340, 1.594	0.434
19A†	63	10.95	6.452, 18.57	73	6.843	5.037, 9.296	1.600	0.872, 2.934	0.128

† Serotypes contained in PCV-13 only

Table 19 Geometric mean OPA titres and ratios at 13 months by vaccine group

	PCV-10 (n = 69)			PCV-13 (n = 74)			PCV-10 / PCV-13		
		Geometric			Geometric		Geometric		
Serotype	N	Mean	95% CI	N	Mean	95% CI	Mean Ratio	95% CI	P-Value
1	**67**	**27.31**	**17.86, 41.77**	**73**	**66.93**	**43.90, 102.03**	**0.408**	**0.225, 0.739**	**0.003**
4	68	1306	1071, 1593	73	1497	1213, 1848	0.872	0.655, 1.162	0.348
5	**66**	**81.80**	**57.23, 116.9**	**74**	**311.8**	**243.5, 399.3**	**0.262**	**0.170, 0.404**	**0.000**
6B	**69**	**636.1**	**505.4, 800.7**	**74**	**2148**	**1717, 2686**	**0.296**	**0.215, 0.407**	**0.000**
7F	**69**	**1106**	**878.1, 1393**	**74**	**5254**	**3904, 7070**	**0.211**	**0.145, 0.306**	**0.000**
9V	**68**	**285.6**	**196.7, 414.6**	**74**	**2373**	**1794, 3139**	**0.120**	**0.076, 0.191**	**0.000**
14	**69**	**1118**	**872.4, 1432**	**74**	**3137**	**2418, 4069**	**0.356**	**0.250, 0.509**	**0.000**
18C	69	2501	1989, 3145	74	2539	1921, 3356	0.985	0.689, 1.409	0.934
19F	**69**	**3056**	**2395, 3898**	**74**	**1994**	**1442, 2757**	**1.532**	**1.025, 2.291**	**0.038**
23F	**69**	**609.3**	**458.6, 809.5**	**74**	**3694**	**2794, 4883**	**0.165**	**0.111, 0.245**	**0.000**
3†	**69**	**4.691**	**4.081, 5.393**	**72**	**34.61**	**25.48, 47.03**	**0.136**	**0.097, 0.189**	**0.000**
6A†	**64**	**264.2**	**165.4, 421.9**	**74**	**7010**	**5363, 9164**	**0.038**	**0.022, 0.064**	**0.000**
19A†	**68**	**328.0**	**208.4, 516.3**	**74**	**1389**	**999.1, 1932**	**0.236**	**0.135, 0.412**	**0.000**

† Serotypes contained in PCV-13 only

Table 20 Geometric mean OPA titres and ratios at 24 months by vaccine group

	PCV-10 (n = 75)			PCV-13 (n = 76)			PCV-10 / PCV-13		
		Geometric			Geometric		Geometric		
Serotype	N	Mean	95% CI	N	Mean	95% CI	Mean Ratio	95% CI	P-Value
1	75	4.000	4.000, 4.000	76	4.088	3.914, 4.270	0.978	0.937, 1.022	0.321
4	72	31.67	19.13, 52.45	73	28.48	16.39, 49.51	1.112	0.529, 2.335	0.778
5	73	4.985	4.272, 5.817	71	5.928	4.634, 7.584	0.841	0.630, 1.122	0.237
6B	**74**	**18.90**	**11.46, 31.18**	**74**	**201.3**	**123.3, 328.6**	**0.094**	**0.047, 0.188**	**0.000**
7F	**74**	**421.0**	**282.3, 627.8**	**76**	**1368**	**1082, 1731**	**0.308**	**0.194, 0.488**	**0.000**
9V	**74**	**9.821**	**6.223, 15.50**	**74**	**96.78**	**50.61, 185.1**	**0.101**	**0.046, 0.223**	**0.000**
14	73	556.7	427.1, 725.7	75	606.4	448.2, 820.5	0.918	0.616, 1.368	0.672
18C	72	150.8	82.74, 274.8	71	112.9	59.58, 213.8	1.336	0.560, 3.186	0.511
19F	**73**	**237.6**	**148.8, 379.4**	**75**	**60.01**	**34.83, 103.4**	**3.959**	**1.943, 8.067**	**0.000**
23F	**69**	**142.6**	**76.34, 266.5**	**75**	**624.0**	**380.1, 1025**	**0.229**	**0.104, 0.504**	**0.000**
3†	73	5.016	4.005, 6.281	76	4.971	4.227, 5.847	1.009	0.766, 1.329	0.949
6A†	**70**	**14.76**	**8.711, 24.99**	**73**	**469.6**	**287.2, 767.9**	**0.031**	**0.015, 0.064**	**0.000**
19A†	**69**	**41.25**	**24.01, 70.87**	**71**	**109.6**	**64.78, 185.4**	**0.376**	**0.178, 0.795**	**0.011**

† Serotypes contained in PCV-13 only

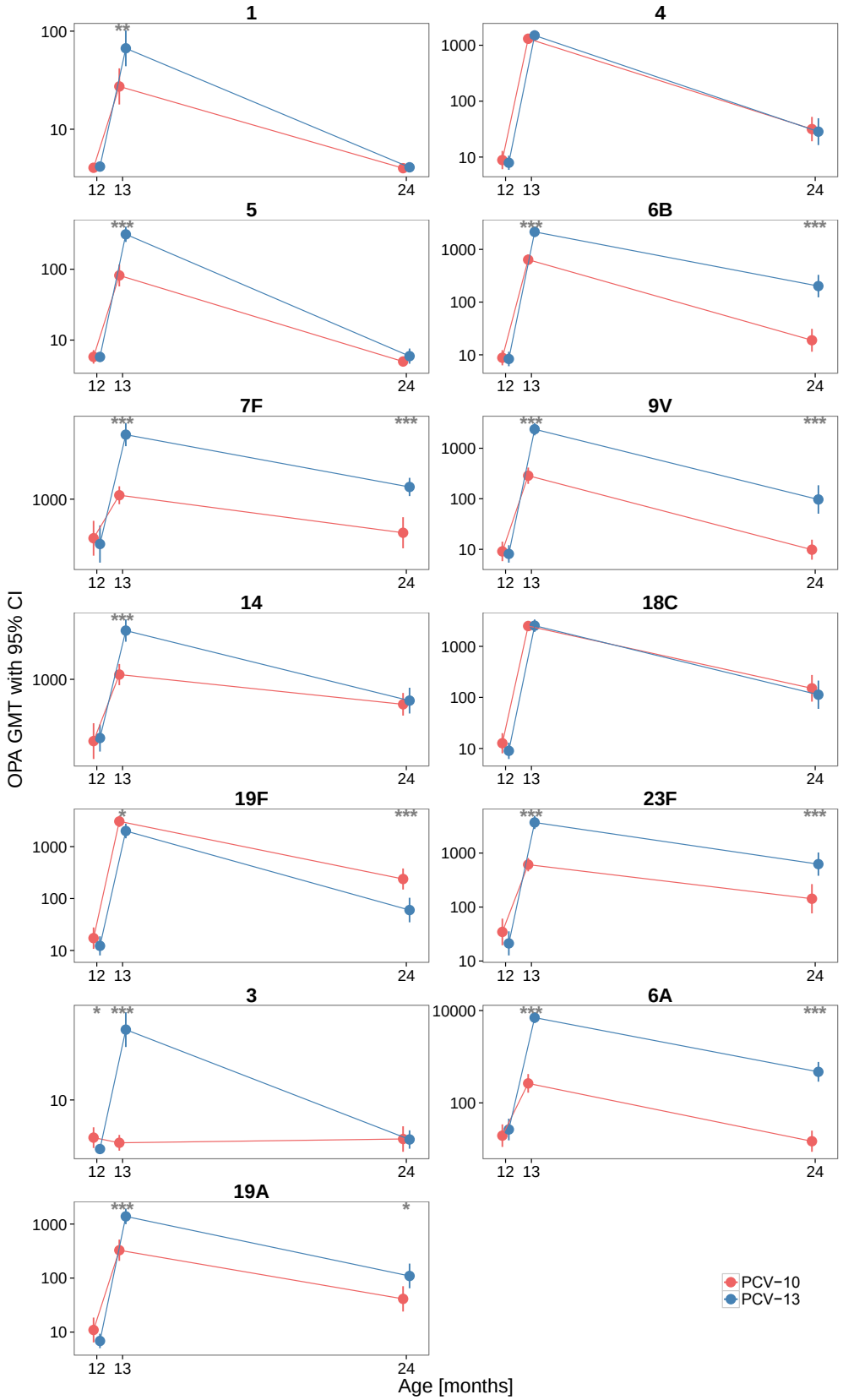

Figure 7 Serotype-specific geometric mean OPA titres by serotype and vaccine group at all 3 study time points. Groups were compared using independent samples t-tests using log_{10}-transformed data with Satterthwaite's correction for unequal variances and stars indicate the associated p-value (*** <.001; ** <.01; * <.05).

18.2.2.3 OPA geometric mean fold change between study visits

Tables below show OPA geometric mean fold changes between study visits including unadjusted and adjusted treatment effects. For both study groups significant increases of OPA titres for all serotypes with the exception of serotype 3 in the PCV-10 group were seen between baseline (12 months) and 1 month post-booster at 13 months of age (Table 21). The effect of the type of vaccine adjusted for baseline OPA titres, age, sex and ethnicity demonstrated significant differences between the groups for the increase in OPA titres between 12 and 13 months of age for 6 out of the 10 serotypes common to PCV-10 and PCV-13 and all 3 serotypes only included in PCV-13 (Table 21). The 12 to 13 month increase in OPA titres was significantly greater in the PCV-13 compared with the PCV-10 group for serotypes 5, 6B, 7F, 9V, 14, 23F, 3, 6A, and 19A. For the serotypes common to PCV-10 and PCV-13, significantly higher OPA titres were seen at 24 compared with 12 months of age for 5 out of 10 serotypes in the PCV-10 group and 8 out of 10 serotypes in the PCV-13 group (Table 22). The increase in OPA titres between 12 and 24 months of age was significantly greater in PCV-13 compared with PCV-10 recipients for serotypes 6B, 7F, 9V, 23F, and 6A whereas for serotype 19F a statistically greater increase between these 2 study time points was seen in the PCV-10 compared with the PCV-13 group (Table 22). A significant decline in OPA titres between 13 and 24 months of age was seen for most serotypes in both groups with some group differences (Table 23). The decline was significantly more pronounced in the PCV-13 compared with the PCV-10 group for serotypes 5, 14, and 3 whereas it was significantly greater in PCV-10 compared with PCV-13 recipients for serotype 6B (Table 23).

Table 21 Serotype-specific OPA geometric mean fold change from 12 to 13 months by vaccine group

	PCV-10						**PCV-13**					
		N missing at months		Geometric Mean Fold				N missing at months		Geometric Mean Fold		
Serotype	N	12	13	Rise*	95% CI	P-Value	N	12	13	Rise*	95% CI	P-Value
1	**55**	**12**	**14**	**3.417**	**2.626,4.447**	**<0.01**	**60**	**13**	**14**	**4.245**	**3.279,5.494**	**<0.01**
4	**50**	**17**	**19**	**150.4**	**93.59,241.7**	**<0.01**	**58**	**15**	**16**	**181.5**	**124.6,264.3**	**<0.01**
5	**49**	**18**	**20**	**11.83**	**7.163,19.54**	**<0.01**	**59**	**14**	**15**	**56.54**	**41.27,77.46**	**<0.01**
6B	**52**	**15**	**17**	**65.44**	**41.87,102.3**	**<0.01**	**58**	**15**	**16**	**237.6**	**153.1,368.6**	**<0.01**
7F	**52**	**15**	**17**	**3.505**	**1.941,6.327**	**<0.01**	**60**	**13**	**14**	**20.02**	**10.74,37.32**	**<0.01**
9V	**55**	**12**	**14**	**32.85**	**17.61,61.28**	**<0.01**	**59**	**14**	**15**	**253.0**	**144.5,442.8**	**<0.01**
14	**53**	**14**	**16**	**5.277**	**2.985,9.330**	**<0.01**	**59**	**14**	**15**	**12.93**	**8.102,20.64**	**<0.01**
18C	**56**	**11**	**13**	**238.5**	**141.2,402.6**	**<0.01**	**57**	**16**	**17**	**284.4**	**173.9,465.1**	**<0.01**
19F	**55**	**12**	**14**	**180.8**	**99.53,328.6**	**<0.01**	**58**	**15**	**16**	**158.8**	**91.13,276.7**	**<0.01**

	PCV-10						PCV-13					
		N missing at months		Geometric Mean Fold				N missing at months		Geometric Mean Fold		
Serotype	N	12	13	Rise*	95% CI	P-Value	N	12	13	Rise*	95% CI	P-Value
23F	**53**	**14**	**16**	**15.15**	**7.400,31.03**	**<0.01**	**59**	**14**	**15**	**166.7**	**86.02,323.2**	**<0.01**
3†	52	15	17	0.874	0.662,1.153	0.336	**57**	**16**	**17**	**9.085**	**6.373,12.95**	**<0.01**
6A†	**48**	**19**	**21**	**11.24**	**4.793,26.38**	**<0.01**	**56**	**17**	**18**	**186.9**	**95.28,366.5**	**<0.01**
19A†	**53**	**14**	**16**	**27.54**	**12.38,61.30**	**<0.01**	**61**	**12**	**13**	**183.1**	**110.8,302.5**	**<0.01**

* Geometric mean change from 12 months to 13 months
† Serotypes contained in PCV-13 only

Table 21 continued

	Unadjusted Treatment Effect**			Adjusted Treatment Effect***		
Serotype	PCV-10/ PCV-13	95% CI	P-Value	PCV-10/ PCV-13	95% CI	P-Value
1	0.805	0.558,1.162	0.244	0.740	0.519,1.053	0.094
4	0.829	0.462,1.487	0.525	0.933	0.653,1.332	0.699
5	**0.209**	**0.127,0.344**	**0.000**	**0.187**	**0.120,0.290**	**<0.01**
6B	**0.275**	**0.156,0.487**	**0.000**	**0.268**	**0.189,0.379**	**<0.01**
7F	**0.175**	**0.085,0.359**	**0.000**	**0.194**	**0.131,0.287**	**<0.01**
9V	**0.130**	**0.061,0.274**	**0.000**	**0.122**	**0.075,0.198**	**<0.01**
14	**0.408**	**0.230,0.724**	**0.002**	**0.403**	**0.273,0.596**	**<0.01**
18C	0.838	0.451,1.560	0.575	0.998	0.657,1.517	0.992
19F	1.139	0.513,2.527	0.747	1.513	0.989,2.316	0.056
23F	**0.091**	**0.041,0.203**	**0.000**	**0.133**	**0.085,0.209**	**<0.01**
3†	**0.096**	**0.066,0.140**	**0.000**	**0.101**	**0.069,0.149**	**<0.01**
6A†	**0.060**	**0.025,0.144**	**0.000**	**0.033**	**0.019,0.057**	**<0.01**
19A†	**0.150**	**0.071,0.320**	**0.000**	**0.182**	**0.101,0.329**	**<0.01**

** Unadjusted estimate of the ratio of geometric means at 13 months (PCV-10/PCV-13)
*** Ratio of geometric means at 13 months (PCV-10/PCV-13), adjusted for 12 month values, age, sex and ethnicity
† Serotypes contained in PCV-13 only

Table 22 Serotype-specific OPA geometric mean fold change from 12 to 24 months by vaccine group

	PCV-10						PCV-13					
		N missing at months		Geometric Mean Fold				N missing at months		Geometric Mean Fold		
Serotype	N	12	13	Rise*	95% CI	P-Value	N	12	13	Rise*	95% CI	P-Value
1	58	9	17	0.988	0.965,1.012	0.322	64	9	12	0.982	0.887,1.087	0.723
4	**53**	**14**	**22**	**3.652**	**1.785,7.474**	**0.001**	**59**	**14**	**17**	**3.786**	**1.949,7.356**	**0.000**
5	52	15	23	0.788	0.587,1.056	0.109	58	15	18	1.082	0.767,1.528	0.649

	PCV-10						PCV-13					
		N missing at months		Geometric Mean Fold				N missing at months		Geometric Mean Fold		
Serotype	N	12	13	Rise*	95% CI	P-Value	N	12	13	Rise*	95% CI	P-Value
6B	53	14	22	1.648	0.844,3.217	0.142	**59**	**14**	**17**	**24.50**	**12.56,47.80**	**0.000**
7F	54	13	21	0.940	0.523.1.689	0.835	**63**	**10**	**13**	**4.569**	**2.519,8.289**	**0.000**
9V	56	11	19	1.053	0.520,2.133	0.885	**59**	**14**	**17**	**15.07**	**6.610,34.36**	**0.000**
14	**54**	**13**	**21**	**1.985**	**1.153,3.419**	**0.014**	**60**	**13**	**16**	**2.533**	**1.572,4.080**	**0.000**
18C	**55**	**12**	**20**	**12.78**	**5.576,29.30**	**0.000**	**53**	**20**	**23**	**14.05**	**6.125,32.25**	**0.000**
19F	**55**	**12**	**20**	**15.89**	**7.793,32.40**	**0.000**	**59**	**14**	**17**	**6.775**	**3.179,14.44**	**0.000**
23F	**51**	**16**	**24**	**4.506**	**1.760,11.53**	**0.002**	**59**	**14**	**17**	**28.86**	**13.06,63.78**	**0.000**
3†	52	15	23	0.920	0.679,1.248	0.590	59	14	17	1.127	0.983,1.291	0.085
6A†	**49**	**18**	**26**	**0.404**	**0.168,0.972**	**0.043**	**56**	**17**	**20**	**18.43**	**8.270,41.08**	**0.000**
19A†	**49**	**18**	**26**	**3.656**	**1.470,9.092**	**0.006**	**59**	**14**	**17**	**12.41**	**6.303,24.45**	**0.000**

* Geometric mean change from 12 months to 24 months
† Serotypes contained in PCV-13 only

Table 22 continued

	Unadjusted Treatment Effect**			Adjusted Treatment Effect***		
Serotype	PCV-10/ PCV-13	95% CI	P-Value	PCV-10/ PCV-13	95% CI	P-Value
1	1.006	0.902,1.123	0.911	0.974	0.921,1.030	0.345
4	0.965	0.387,2.406	0.938	1.082	0.456,2.570	0.857
5	0.728	0.490,1.079	0.113	0.702	0.499,0.988	0.043
6B	**0.067**	**0.028,0.159**	**0.000**	**0.066**	**0.030,0.146**	**0.000**
7F	**0.206**	**0.107,0.398**	**0.000**	**0.275**	**0.175,0.432**	**0.000**
9V	**0.070**	**0.025,0.192**	**0.000**	**0.078**	**0.031,0.197**	**0.000**
14	0.784	0.431,1.427	0.422	0.871	0.552,1.376	0.551
18C	0.909	0.343,2.410	0.847	0.989	0.380,2.574	0.981
19F	**2.346**	**0.890,6.183**	**0.084**	**3.198**	**1.439,7.106**	**0.005**
23F	**0.156**	**0.057,0.426**	**0.000**	**0.189**	**0.081,0.440**	**0.000**
3†	0.817	0.599,1.113	0.198	0.976	0.754,1.263	0.853
6A†	**0.022**	**0.008,0.063**	**0.000**	**0.020**	**0.009,0.044**	**0.000**
19A†	0.294	0.117,0.739	0.010	0.349	0.152,0.801	0.013

** Unadjusted estimate of the ratio of geometric means at 13 months (PCV-10/PCV-13)
*** Ratio of geometric means at 24 months (PCV-10/PCV-13), adjusted for 12 month values, age, sex and ethnicity
† Serotypes contained in PCV-13 only

Table 23 Serotype-specific OPA geometric mean fold change from 13 to 24 months by vaccine group

	PCV-10						PCV-13					
		N missing at months		Geometric Mean Fold				N missing at months		Geometric Mean Fold		
Serotype	N	12	13	Rise*	95% CI	P-Value	N	12	13	Rise*	95% CI	P-Value
1	**58**	**11**	**17**	**0.317**	**0.248,0.405**	**0.000**	**64**	**10**	**12**	**0.232**	**0.182,0.294**	**0.000**
4	**57**	**12**	**18**	**0.029**	**0.016,0.053**	**0.000**	**61**	**13**	**15**	**0.024**	**0.013,0.047**	**0.000**
5	**56**	**13**	**19**	**0.066**	**0.042,0.102**	**0.000**	**60**	**14**	**16**	**0.020**	**0.014,0.029**	**0.000**
6B	**59**	**10**	**16**	**0.036**	**0.019,0.066**	**0.000**	**63**	**11**	**13**	**0.093**	**0.052,0.165**	**0.000**
7F	**59**	**10**	**16**	**0.355**	**0.219,0.574**	**0.000**	**65**	**9**	**11**	**0.265**	**0.177,0.396**	**0.000**
9V	**58**	**11**	**17**	**0.034**	**0.018,0.066**	**0.000**	**63**	**11**	**13**	**0.039**	**0.018,0.083**	**0.000**
14	**60**	**9**	**15**	**0.457**	**0.305,0.686**	**0.000**	**64**	**10**	**12**	**0.212**	**0.142,0.315**	**0.000**
18C	**57**	**12**	**18**	**0.053**	**0.026,0.107**	**0.000**	**61**	**13**	**15**	**0.043**	**0.020,0.091**	**0.000**
19F	**60**	**9**	**15**	**0.082**	**0.048,0.142**	**0.000**	**64**	**10**	**12**	**0.034**	**0.017,0.067**	**0.000**
23F	**54**	**15**	**21**	**0.232**	**0.110,0.489**	**0.000**	**64**	**10**	**12**	**0.215**	**0.123,0.374**	**0.000**
3†	58	11	17	0.993	0.768,1.285	0.958	**64**	**10**	**12**	**0.149**	**0.102,0.217**	**0.000**
6A†	**53**	**16**	**22**	**0.055**	**0.026,0.120**	**0.000**	**62**	**12**	**14**	**0.069**	**0.038,0.127**	**0.000**
19A†	**56**	**13**	**19**	**0.168**	**0.075,0.374**	**0.000**	**61**	**13**	**15**	**0.084**	**0.043,0.165**	**0.000**

* Geometric mean change from 13 months to 24 months
† Serotypes contained in PCV-13 only

Table 23 continued

	Unadjusted Treatment Effect**			Adjusted Treatment Effect***		
Serotype	PCV-10/ PCV-13	95% CI	P-Value	PCV-10/ PCV-13	95% CI	P-Value
1	1.370	0.971,1.933	0.072	1.325	0.898,1.954	0.155
4	1.200	0.573,2.509	0.626	1.261	0.539,2.951	0.590
5	**3.294**	**2.089,5.192**	**0.000**	**3.681**	**2.249,6.026**	**0.000**
6B	**0.383**	**0.194,0.759**	**0.006**	**0.247**	**0.112,0.543**	**0.001**
7F	1.338	0.839,2.132	0.219	1.340	0.813.2.210	0.248
9V	0.888	0.384,2.054	0.780	0.846	0.334,2.145	0.722
14	**2.159**	**1.394,3.343**	**0.001**	**1.870**	**1.122,3.116**	**0.017**
18C	1.220	0.532,2.797	0.636	1.105	0.438,2.789	0.831
19F	2.412	1.267,4.590	0.008	1.884	0.925,3.841	0.081
23F	1.082	0.529,2.210	0.828	1.239	0.568,2.702	0.586
3†	**6.676**	**4.295,10.38**	**0.000**	**10.17**	**6.242,16.58**	**0.000**
6A†	0.798	0.370,1.719	0.562	0.670	0.284,1.582	0.357
19A†	1.989	0.832,4.755	0.121	2.428	0.871,6.771	0.089

** Unadjusted estimate of the ratio of geometric means at 13 months (PCV-10/PCV-13)
*** Ratio of geometric means at 24 months (PCV-10/PCV-13), adjusted for 13 month values, age, sex and ethnicity
† Serotypes contained in PCV-13 only

18.2.3 Antigen-specific memory B cell frequencies

18.2.3.1 Antigen-specific memory B cell (B_{MEM}) geometric mean frequencies (GMF) by antigen and vaccine group

At baseline, no significant differences between the groups were seen in B_{MEM} GMF for all antigens tested (Table 24, Figure 8 and Figure 9). One month following the booster vaccination, significantly higher B_{MEM} GMF were found for serotypes 1, 4, 9V and 3 in the PCV-13 compared with the PCV-10 group whereas B_{MEM} responses to tetanus toxoid were statistically higher in PCV-10 compared with PCV-13 recipients (Table 25, Figure 8 and Figure 9). At 24 months of age, no significant differences were detected between the groups for any of the antigens tested (Table 26, Figure 8 and Figure 9).

Table 24 Geometric mean B_{MEM} frequencies and ratios at 12 months by vaccine group

	PCV-10 (n = 59)			**PCV-13 (n = 63)**			**PCV-10 / PCV-13**		
		Geometric			Geometric		Geometric		
Serotype	N	Mean	95% CI	N	Mean	95% CI	Mean Ratio	95% CI	P-Value
1	59	3.519	2.615, 4.737	63	3.413	2.533, 4.599	1.031	0.680, 1.564	0.885
4	59	3.084	2.324, 4.092	63	2.812	2.088, 3.786	1.097	0.731, 1.647	0.653
9V	59	3.261	2.355, 4.514	63	2.942	2.124, 4.075	1.108	0.703, 1.748	0.656
14	59	1.908	1.432, 2.542	63	2.418	1.832, 3.190	0.789	0.532, 1.171	0.238
3†	59	1.493	1.179, 1.892	63	1.611	1.251, 2.074	0.927	0.658, 1.306	0.662
19A†	59	2.661	1.933, 3.664	62	2.809	2.095, 3.767	0.947	0.616, 1.456	0.803
Dip*	57	12.16	8.765, 16.86	62	10.18	7.166, 14.46	1.194	0.743, 1.920	0.461
Tet**	58	5.233	3.511, 7.798	62	4.041	2.760, 5.917	1.295	0.750, 2.235	0.351

† Serotypes contained in PCV-13 only
*represents carrier protein CRM_{197} (PCV-13) or diphtheria toxoid (PCV-10)
**represents carrier protein tetanus toxoid (contained in PCV-10 only)

Table 25 Geometric mean B_{MEM} frequencies and ratios at 13 months by vaccine group

	PCV-10 (n = 56)			**PCV-13 (n = 69)**			**PCV-10 / PCV-13**		
		Geometric			Geometric		Geometric		
Serotype	N	Mean	95% CI	N	Mean	95% CI	Mean Ratio	95% CI	P-Value
1	**56**	**4.411**	**3.199, 6.083**	**69**	**7.609**	**5.653, 10.24**	**0.580**	**0.376, 0.894**	**0.014**
4	**56**	**3.174**	**2.273, 4.432**	**69**	**7.700**	**5.812, 10.20**	**0.412**	**0.268, 0.635**	**0.000**
9V	**55**	**3.229**	**2.262, 4.611**	**69**	**8.139**	**5.945, 11.14**	**0.397**	**0.248, 0.635**	**0.000**
14	55	2.531	1.775, 3.609	69	3.350	2.516, 4.462	0.756	0.481, 1.187	0.221
3†	**56**	**1.406**	**1.121, 1.764**	**69**	**17.06**	**12.64, 23.03**	**0.082**	**0.057, 0.120**	**0.000**

	PCV-10 (n = 56)			PCV-13 (n = 69)			PCV-10 / PCV-13		
		Geometric			Geometric		Geometric		
Serotype	N	Mean	95% CI	N	Mean	95% CI	Mean Ratio	95% CI	P-Value
19A†	55	4.861	3.347, 7.059	68	8.184	5.633, 11.89	0.594	0.352, 1.002	0.051
Dip*	51	28.72	20.68, 39.91	64	25.08	18.58, 33.86	1.145	0.737, 1.779	0.543
Tet**	**53**	**10.89**	**7.156, 16.56**	**67**	**4.163**	**2.945, 5.886**	**2.615**	**1.526, 4.480**	**0.001**

† Serotypes contained in PCV-13 only
*represents carrier protein CRM_{197} (PCV-13) or diphtheria toxoid (PCV-10)
**represents carrier protein tetanus toxoid (contained in PCV-10 only)

Table 26 Geometric mean B_{MEM} frequencies and ratios at 24 months by vaccine group

	PCV-10 (n = 66)			PCV-13 (n = 64)			PCV-10 / PCV-13		
		Geometric			Geometric		Geometric		
Serotype	N	Mean	95% CI	N	Mean	95% CI	Mean Ratio	95% CI	P-Value
1	66	4.528	3.381, 6.063	63	3.827	2.925, 5.006	1.183	0.799, 1.753	0.398
4	66	4.434	3.277, 6.000	64	5.210	4.060, 6.686	0.851	0.577, 1.255	0.413
9V	65	4.608	3.440, 6.170	64	4.730	3.695, 6.054	0.974	0.667, 1.423	0.892
14	65	3.825	2.809, 5.208	64	3.990	2.955, 5.387	0.959	0.626, 1.468	0.845
3†	66	2.227	1.705, 2.910	64	2.882	2.209, 3.761	0.773	0.532, 1.123	0.174
19A†	63	4.764	3.533, 6.424	63	5.079	3.835, 6.727	0.938	0.625, 1.408	0.755
Dip*	62	12.68	9.327, 17.25	60	11.43	8.517, 15.33	1.110	0.729, 1.691	0.624
Tet**	61	7.601	5.378, 10.74	61	6.431	4.650, 8.893	1.182	0.739, 1.890	0.482

† Serotypes contained in PCV-13 only
*represents carrier protein CRM_{197} (PCV-13) or diphtheria toxoid (PCV-10)
**represents carrier protein tetanus toxoid (contained in PCV-10 only)

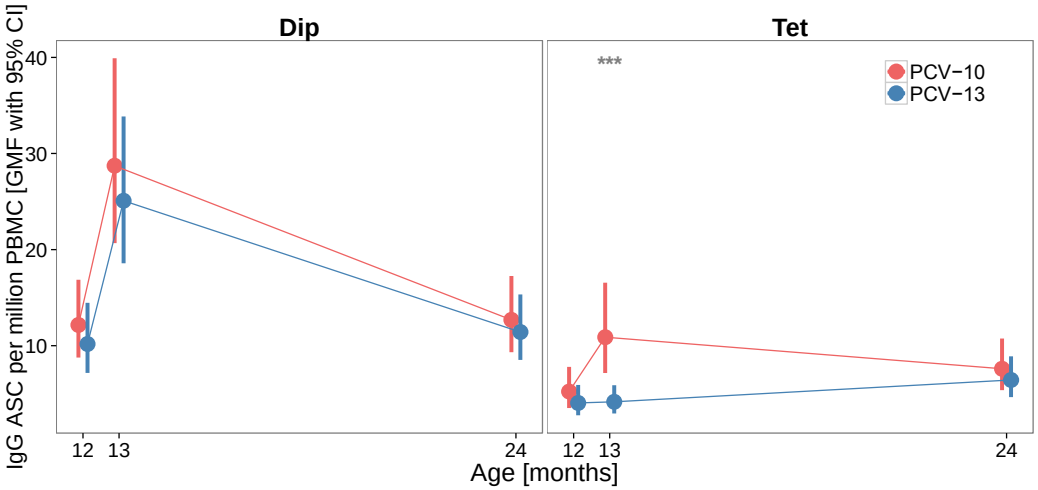

Figure 8 Geometric mean frequencies of (along with 95% CI) of B_{MEM} specific for diphtheria and tetanus toxoid. Groups were compared using independent samples t-tests using log_{10}-transformed data with

Satterthwaite's correction for unequal variances and stars indicate the associated p-value (*** <.001; ** <.01; * <.05).

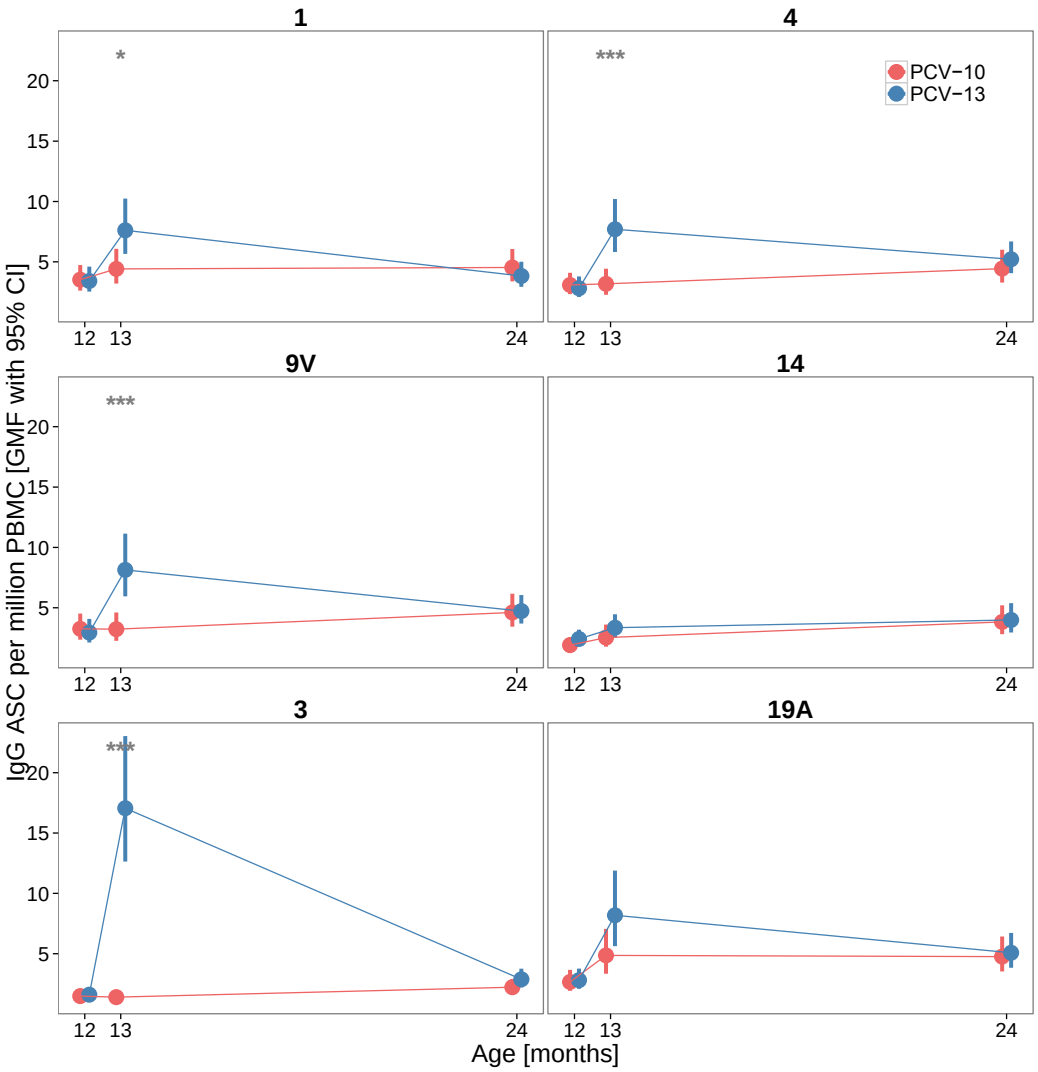

Figure 9 Geometric mean frequencies of (along with 95% CI) of B_{MEM} specific for pneumococcal serotypes. Groups were compared using independent samples t-tests using log_{10}-transformed data with Satterthwaite's correction for unequal variances and stars indicate the associated p-value (*** <.001; ** <.01; * <.05).

18.2.3.2 Antigen-specific B_{MEM} frequency fold change

Significant increases between B_{MEM} measured at baseline and 1 month post-booster were seen for all pneumococcal serotypes in the PCV-13 group and none in the PCV-10 group (Table 27). For antigens representing carrier proteins, a significant rise was seen for both diphtheria and tetanus toxoid in the PCV-10 group but only diphtheria toxoid in the PCV-13 group (Table 27). Adjusted changes in B_{MEM} frequencies between 12 and 13 months of age were significantly greater in the PCV-13 compared with the PCV-10 group for all pneumococcal serotypes and

statistically superior in PCV-10 compared with PCV-13 recipients only for tetanus toxoid (Table 27). Frequencies of B_{MEM} were not significantly different between 24 and 12 months for most serotypes in both groups. In the PCV-10 group, significantly higher B_{MEM} frequencies were seen for serotypes 14 and 19A whereas in the PCV-13 group a significant fold increase was only seen for serotype 3 (Table 28). No group differences were seen for these changes when adjusted for baseline values, age, sex and ethnicity (Table 28). Significant geometric mean fold changes between 13 and 24 months of age were seen for all serotypes (with the exception of serotype 14) and diphtheria toxoid in the PCV-13 group but only for diphtheria toxoid in the PCV-10 group (Table 29).

Table 27 Antigen-specific B_{MEM} frequency geometric mean fold change from 12 to 13 months by vaccine group

	PCV-10						**PCV-13**					
		N missing at months		Geometric Mean Fold				N missing at months		Geometric Mean Fold		
Serotype	N	12	13	Rise‡	95% CI	P-Value	N	12	13	Rise‡	95% CI	P-Value
1	39			1.160	0.714,1.886	0.544	**54**			**2.436**	**1.530,3.877**	**0.000**
4	39			0.929	0.563,1.532	0.770	**54**			**2.917**	**1.855,4.588**	**0.000**
9V	38			0.880	0.504,1.537	0.650	**54**			**3.091**	**1.872,5.105**	**0.000**
14	38			1.025	0.613,1.713	0.925	**54**			**1.556**	**1.000,2.420**	**0.050**
3†	39			0.809	0.549,1.191	0.278	**54**			**12.90**	**8.587,19.37**	**0.000**
19A†	38			1.775	0.996,3.161	0.051	**53**			**3.238**	**1.951,5.373**	**0.000**
Dip*	**33**			**2.157**	**1.231,3.780**	**0.008**	**51**			**2.660**	**1.568,4.510**	**0.000**
Tet**	**35**			**2.249**	**1.089,4.643**	**0.029**	51			1.084	0.604,1.944	0.785

‡ Geometric mean change from 12 months to 13 months
† Serotypes contained in PCV-13 only
* represents carrier protein CRM_{197} (PCV-13) or diphtheria toxoid (PCV-10)
** represents carrier protein tetanus toxoid (contained in PCV-10 only)

Table 27 continued

	Unadjusted Treatment Effect***			Adjusted Treatment Effect****		
Serotype	PCV-10/ PCV-13	95% CI	P-Value	PCV-10/ PCV-13	95% CI	P-Value
1	**0.476**	**0.269,0.843**		**0.469**	**0.284,0.773**	**0.003**
4	**0.318**	**0.175,0.578**		**0.340**	**0.210,0.550**	**0.000**
9V	**0.285**	**0.151,0.537**		**0.295**	**0.172,0.506**	**0.000**
14	**0.659**	**0.366,1.186**		**0.582**	**0.352,0.964**	**0.036**
3†	**0.063**	**0.037,0.106**		**0.057**	**0.037,0.086**	**0.000**
19A†	**0.548**	**0.286,1.050**		**0.429**	**0.240,0.766**	**0.005**

	Unadjusted Treatment Effect***			Adjusted Treatment Effect****		
Serotype	PCV-10/ PCV-13	95% CI	P-Value	PCV-10/ PCV-13	95% CI	P-Value
Dip*	0.811	0.439,1.498		0.865	0.525,1.426	0.566
Tet**	**2.075**	**1.105,3.898**		**2.188**	**1.229.3.894**	**0.008**

* represents carrier protein CRM_{197} (PCV-13) or diphtheria toxoid (PCV-10)

** represents carrier protein tetanus toxoid (contained in PCV-10 only)

*** Unadjusted estimate of the ratio of geometric means at 13 months (PCV-10/PCV-13)

**** Ratio of geometric means at 13 months (PCV-10/PCV-13), adjusted for 12 month values, age, sex and ethnicity

† Serotypes contained in PCV-13 only

Table 28 Antigen-specific B_{MEM} frequency geometric mean fold change from 12 to 24 months by vaccine group

	PCV-10						**PCV-13**					
		N missing at months		Geometric Mean Fold				N missing at months		Geometric Mean Fold		
Serotype	N	12	13	Rise‡	95% CI	P-Value	N	12	13	Rise‡	95% CI	P-Value
1	44			1.374	0.823,2.292	0.221	45			0.830	0.512,1.344	0.445
4	44			1.494	0.934,2.390	0.093	46			1.518	0.961,2.397	0.073
9V	43			1.326	0.765,2.299	0.310	46			1.353	0.841,2.177	0.209
14	**43**			**2.109**	**1.263,3.521**	**0.005**	46			1.324	0.815,2.150	0.253
3†	44			1.501	0.978,2.306	0.063	**46**			**1.614**	**1.055,2.468**	**0.028**
19A†	**42**			**1.803**	**1.054,3.087**	**0.032**	44			1.314	0.821,2.103	0.252
Dip*	40			1.046	0.609,1.796	0.869	42			0.926	0.532,1.614	0.785
Tet**	39			1.273	0.683,2.374	0.443	43			1.140	0.608,2.138	0.679

‡ Geometric mean change from 12 months to 13 months

† Serotypes contained in PCV-13 only

* represents carrier protein CRM_{197} (PCV-13) or diphtheria toxoid (PCV-10)

** represents carrier protein tetanus toxoid (contained in PCV-10 only)

Table 28 continued

	Unadjusted Treatment Effect***			Adjusted Treatment Effect****		
Serotype	PCV-10/ PCV-13	95% CI	P-Value	PCV-10/ PCV-13	95% CI	P-Value
1	1.655	0.844,3.244		1.272	0.763,2.121	0.351
4	0.985	0.517,1.876		0.883	0.556,1.402	0.594
9V	0.980	0.528,1.819		0.948	0.589,1.526	0.825
14	1.592	0.850,2.984		1.215	0.703,2.099	0.481
3†	0.930	0.539,1.605		0.843	0.537,1.322	0.452
19A†	1.372	0.735,2.562		1.074	0.655,1.762	0.774

	Unadjusted Treatment Effect***			Adjusted Treatment Effect****		
Serotype	PCV-10/ PCV-13	95% CI	P-Value	PCV-10/ PCV-13	95% CI	P-Value
Dip*	1.129	0.592,2.155		1.209	0.729,2.006	0.457
Tet**	1.116	0.528,2.360		1.189	0.665,2.127	0.554

* represents carrier protein CRM_{197} (PCV-13) or diphtheria toxoid (PCV-10)

** represents carrier protein tetanus toxoid (contained in PCV-10 only)

*** Unadjusted estimate of the ratio of geometric means at 13 months (PCV-10/PCV-13)

**** Ratio of geometric means at 24 months (PCV-10/PCV-13), adjusted for 12 month values, age, sex and ethnicity

† Serotypes contained in PCV-13 only

Table 29 Antigen-specific B_{MEM} frequency geometric mean fold change from 13 to 24 months by vaccine group

	PCV-10						**PCV-13**					
		N missing at months		Geometric Mean Fold				N missing at months		Geometric Mean Fold		
Serotype	N	12	13	Rise‡	95% CI	P-Value	N	12	13	Rise‡	95% CI	P-Value
1	45			0.979	0.609,1.573	0.928	**51**			**0.445**	**0.278,0.712**	**0.001**
4	45			1.099	0.670,1.800	0.706	**51**			**0.607**	**0.401,0.918**	**0.019**
9V	45			1.188	0.719,1.961	0.497	**51**			**0.518**	**0.327,0.821**	**0.006**
14	45			1.342	0.792,2.272	0.271	51			1.064	0.657,1.724	0.800
3†	45			1.360	0.917,2.017	0.125	**51**			**0.155**	**0.097,0.247**	**0.000**
19A†	44			1.036	0.610,1.761	0.893	**50**			**0.514**	**0.300,0.881**	**0.016**
Dip*	**40**			**0.427**	**0.256,0.714**	**0.001**	**44**			**0.418**	**0.252,0.692**	**0.001**
Tet**	41			0.634	0.355,1.134	0.123	48			1.672	0.961,2.910	0.068

‡ Geometric mean change from 12 months to 13 months

† Serotypes contained in PCV-13 only

* represents carrier protein CRM_{197} (PCV-13) or diphtheria toxoid (PCV-10)

** represents carrier protein tetanus toxoid (contained in PCV-10 only)

Table 29 continued

	Unadjusted Treatment Effect***			Adjusted Treatment Effect****		
Serotype	PCV-10/ PCV-13	95% CI	P-Value	PCV-10/ PCV-13	95% CI	P-Value
1	1.655	0.844,3.244		1.272	0.763,2.121	0.351
4	0.930	0.539,1.605		0.843	0.537,1.322	0.452
9V	0.985	0.517,1.876		0.883	0.556,1.402	0.594
14	1.592	0.850,2.984		1.215	0.703,2.099	0.481
3†	0.930	0.539,1.605		0.843	0.537,1.322	0.452
19A†	1.372	0.735,2.562		1.074	0.655,1.762	0.774

Serotype	Unadjusted Treatment Effect*** PCV-10/ PCV-13	95% CI	P-Value	Adjusted Treatment Effect**** PCV-10/ PCV-13	95% CI	P-Value
Dip*	1.129	0.592,2.155		1.209	0.729,2.006	0.457
Tet**	1.116	0.528,2.360		1.189	0.665,2.127	0.554

* represents carrier protein CRM_{197} (PCV-13) or diphtheria toxoid (PCV-10)

** represents carrier protein tetanus toxoid (contained in PCV-10 only)

*** Unadjusted estimate of the ratio of geometric means at 13 months (PCV-10/PCV-13)

**** Ratio of geometric means at 24 months (PCV-10/PCV-13), adjusted for 13 month values, age, sex and ethnicity

† Serotypes contained in PCV-13 only

19 IMMEDIATE PAIN AT TIME OF VACCINE INJECTION

The comparison of vaccine group using ANCOVA, Table 30, shows the vaccine group effect adjusted for sex, age, ethnicity, baseline behaviour, site of injection, vaccinator and the observer for MBPS. Model diagnostics were explored, and the assumption of linearity confirmed for MBPS and NRS. Plots of the residuals of the model for crying time (not shown) showed a slight deviation from the assumption of normality.

For the measures of immediate pain, group differences were seen for the MBPS with significantly higher scores in the PCV-13 compared with the PCV-10 group, but not for crying time or the NRS (Table 30).

Table 30 Summary table for immediate pain at time of infection by vaccine group

	PCV-13		**PCV-10**		**ANCOVA Adjusted Group Effect***	**95% Confidence Interval**	**P-Value**
	N	(%)	N	(%)			
Crying Time (Seconds)					4.945	-4.070, 13.961	0.280
N (Mean) {SD}	78 (41.0) {29.2}		74 (36.4) {21.5}				
[Min-Max]	[0-152]		[0-97]				
Modified Behavioural Pain Scale (MBPS)					**0.5934**	**0.115, 1.072**	**0.016**
0	0	(0)	0	(0)			
1	0	(0)	0	(0)			
2	0	(0)	0	(0)			
3	1	(1.1)	2	(2.3)			
4	2	(2.2)	1	(1.1)			
5	1	(1.1)	4	(4.6)			
6	11	(12.2)	15	(17.2)			
7	18	(20.0)	23	(26.4)			

	PCV-13		PCV-10		ANCOVA Adjusted Group Effect*	95% Confidence Interval	P-Value
	N	(%)	N	(%)			
8	21	(23.3)	15	(17.2)			
9	17	(18.9)	8	(9.2)			
10	8	(8.9)	6	(6.9)			
Missing	11	(12.2)	14	(15.9)			
Numerical Rating Scale (NRS)					0.478	-0.164, 1.121	0.143
0	0	(0)	0	(0)			
1	0	(0)	3	(3.4)			
2	4	(4.4)	5	(5.7)			
3	9	(10.0)	9	(10.3)			
4	9	(10.0)	9	(10.3)			
5	12	(13.3)	13	(14.9)			
6	14	(15.6)	12	(13.8)			
7	23	(25.6)	17	(19.5)			
8	7	(7.8)	8	(9.2)			
9	2	(2.2)	0	0			
10	0	0	1	(1.1)			
Missing	10	(11.1)	11	(12.5)			

*Adjusted for sex, age, ethnicity, baseline behaviour, site of injection, vaccinator (and the observer for MBPS only)

20 REACTOGENICITY

During the study, there were 5 SAEs reported, none of which was considered related to vaccination (see 17.2). The reactogenicity profile of the booster vaccination was similar regardless of whether participants had received PCV-10 or PCV-13 (Table 31).

Local reactions such as redness, hardness, and swelling were either absent or mild in most cases. Moderate or severe localised pain was reported by almost 13% of parents of study participants (Table 32). Irritability, drowsiness and decreased appetite were recorded in 53%, 29% and 29%, respectively, which were moderate or severe in 21%, 7% and 7% of children (Table 32). Low-grade fever (38-39°C) was noted in 4% of participants and 4 children (2%) had a temperature of >39°C in the first 4 days following booster vaccination (Table 32). The majority of these reported adverse effects of vaccination were short-lived lasting 1-3 days (Table 33).

Table 31 Reactogenicity of the booster vaccine by vaccine group

Reaction	Group	N	Proportion	95% Exact Confidence Limits		P-value*
				Lower	Upper	
Redness	PCV-13	40	0.460	0.352	0.570	0.289
	PCV-10	47	0.540	0.430	0.648	.
Swelling	PCV-13	21	0.241	0.156	0.345	0.858
	PCV-10	20	0.230	0.146	0.333	.
Hardness	PCV-13	27	0.310	0.216	0.419	0.741
	PCV-10	25	0.287	0.195	0.394	.
Pain	PCV-13	25	0.287	0.195	0.394	0.741
	PCV-10	27	0.310	0.216	0.419	.
Irritability	PCV-13	47	0.540	0.430	0.648	1.000
	PCV-10	47	0.540	0.430	0.648	.
Drowsiness	PCV-13	26	0.300	0.205	0.407	0.868
	PCV-10	25	0.287	0.195	0.394	.
Loss of Appetite	PCV-13	26	0.299	0.205	0.407	0.868
	PCV-10	25	0.287	0.195	0.394	.
Fever	PCV-13	5	0.059	0.019	0.132	0.786
	PCV-10	6	0.069	0.026	0.144	.

*Chi-Square test with no Yates Continuity Correction

Table 32 Summary of the severity of side effects of the booster vaccine by vaccine group.

	PCV-13		**PCV-10**		**All**	
	N	%	N	%	N	%
Redness						
Absent	47	52.22	40	45.98	87	49.15
Mild (<2.5cm)	38	42.22	46	52.87	84	47.46
Mod (>=2.5cm to <5cm)	2	2.22	1	1.15	3	1.69
Severe (>=5cm)	0	0	0	0	0	0
Missing	3	3.33	0	0	3	1.69
Swelling						
Absent	66	73.33	67	77.01	133	75.14
Mild (<2.5cm)	18	20.00	20	22.99	38	21.47
Mod (>=2.5cm to <5cm)	2	2.22	0	0	2	1.13
Severe (>=5cm)	1	1.11	0	0	1	0.56
Missing	3	3.33	0	0	3	1.69
Hardness						
Absent	60	66.67	62	71.26	122	68.93
Mild (<2.5cm)	24	26.67	24	27.59	48	27.12
Mod (>=2.5cm to <5cm)	2	2.22	1	1.15	3	1.69
Severe (>=5cm)	1	1.11	0	0	1	0.56

	PCV-13		PCV-10		All	
	N	%	N	%	N	%
Missing	3	3.33	0	0	3	1.69
Pain						
Absent	62	68.89	60	68.97	122	68.93
Mild	11	12.22	18	20.69	29	16.38
Mod	12	13.33	6	6.90	18	10.17
Severe	2	2.22	3	3.45	5	2.82
Missing	3	3.33	0	0	3	1.69
Irritability						
Absent	40	44.44	40	45.98	80	45.20
Mild	28	31.11	28	32.18	56	31.64
Mod	16	17.78	14	16.09	30	16.95
Severe	3	3.33	5	5.75	8	4.52
Missing	3	3.33	0	0	3	1.69
Drowsiness						
Absent	61	67.78	62	71.26	123	69.49
Mild	20	22.22	19	21.84	39	22.03
Mod	4	4.44	6	6.90	10	5.65
Severe	2	2.22	0	0	2	1.13
Missing	3	3.33	0	0	3	1.69
Loss of Appetite						
Absent	61	67.78	62	71.26	123	69.49
Mild	20	22.22	19	21.84	39	22.03
Mod	6	6.67	5	5.75	11	6.21
Severe	0	0	1	1.15	1	0.56
Missing	3	3.33	0	0	3	1.69
Fever (°C)						
< 38.0	80	88.89	81	93.10	161	90.96
38.0-38.4	2	2.22	2	2.30	4	2.26
38.5-38.9	1	1.11	2	2.30	3	1.69
39.0-39.4	1	1.11	2	2.30	3	1.69
39.5-39.9	1	1.11	0	0	1	0.56
40.0-40.4	0	0	0	0	0	0
40.5-40.9	0	0	0	0	0	0
>41	0	0	0	0	0	0
Missing	5	5.56	0	0	5	2.82

Table 33 Summary of duration of side effects of the booster vaccine by vaccine group.

	PCV-13		PCV-10		All	
	N	%	N	%	N	%
Redness						
0 days	46	51.11	40	45.98	86	48.59
1 day	9	10.00	14	16.09	23	12.99
2 days	11	12.22	14	16.09	25	14.12
3 days	10	11.11	10	11.49	20	11.30
4 days	7	7.78	9	10.34	16	9.04
Missing	7	7.78	0	0	7	3.95
Swelling						
0 days	65	72.22	67	77.01	132	74.58
1 day	5	5.56	10	11.49	15	8.47
2 days	10	11.11	6	6.90	16	9.04
3 days	4	4.44	1	1.15	5	2.82
4 days	1	1.11	3	3.45	4	2.26
Missing	5	5.56	0	0	5	2.82
Hardness						
0 days	59	65.56	62	71.26	121	68.36
1 day	7	7.78	5	5.75	12	6.78
2 days	10	11.11	6	6.90	16	9.04
3 days	4	4.44	8	9.20	12	6.78
4 days	4	4.44	6	6.90	10	5.65
Missing	6	6.67	0	0	6	3.39
Pain						
0 days	62	68.89	60	68.97	122	68.93
1 day	14	15.56	14	16.09	28	15.82
2 days	5	5.56	9	10.34	14	7.91
3 days	2	2.22	2	2.30	4	2.26
4 days	2	2.22	2	2.30	4	2.26
Missing	5	5.56	0	0	5	2.82
Irritability						
0 days	39	43.33	39	44.83	78	44.07
1 day	20	22.22	27	31.03	47	26.55
2 days	13	14.44	9	10.34	22	12.43
3 days	9	10.00	6	6.90	15	8.47
4 days	4	4.44	6	6.90	10	5.65
Missing	5	5.56	0	0	5	2.82
Drowsiness						
0 days	60	66.67	62	71.26	122	68.93
1 day	14	15.56	17	19.54	31	17.51

	PCV-13		PCV-10		All	
	N	%	N	%	N	%
2 days	8	8.89	6	6.90	14	7.91
3 days	2	2.22	1	1.15	3	1.69
4 days	1	1.11	1	1.15	2	1.13
Missing	5	5.56	0	0	5	2.82
Loss of Appetite						
0 days	58	64.44	62	71.26	120	67.80
1 day	16	17.78	15	17.24	31	17.51
2 days	6	6.67	5	5.75	11	6.21
3 days	3	3.33	3	3.45	6	3.39
4 days	2	2.22	2	2.30	4	2.26
Missing	5	5.56	0	0	5	2.82
Fever						
0 days	71	78.89	76	87.36	147	83.05
1 day	3	3.33	3	3.45	6	3.39
2 days	1	1.11	2	2.30	3	1.69
3 days	1	1.11	0	0	1	0.56
4 days	0	0	1	1.15	1	0.56
Missing	14	15.56	5	5.75	19	10.73

21 DISCUSSION

In the UK, children receive the 13-valent pneumococcal conjugate vaccine (PCV-13) at 2, 4 and 12 months of age. This study aimed to assess the potential of PCV-10 as an alternative booster vaccine to be given to 12-month old children who had already been primed with 2 doses of PCV-13. In the study, 178 children who had previously been vaccinated with PCV-13 at 2 and 4 months were randomised 1:1 to receive a booster dose of either PCV-13 or PCV-10 at 12 months of age and the vaccines were given to 87 (PCV-10) and 90 (PCV-13) healthy children. Blood was taken before, at 1 and 12 months following vaccination. Serum IgG concentrations and OPA titres were quantified for PCV-13 serotypes and memory B cell frequencies were calculated for 6 pneumococcal serotypes and 2 protein antigens (representing 2 out of the 3 carrier proteins contained in either PCV-10 or PCV-13). The reactogenicity of the 12-month booster was assessed using diary cards containing parental reports of local and systemic reactions following vaccination. In addition, following vaccination, the immediate pain at time of injection with either PCV-10 or PCV-13 was determined using validated pain assessment tools.

There were no significant differences in baseline demographics between randomised groups (Table 1). There were 5 SAEs reported during the study, none of which were considered related to vaccination (Table 2). There were 8 withdrawals of consent and 4 participants were either lost to follow-up or moved out of the area (Figure 2). A number of protocol deviations were found, mainly relating to the timing of study visits (Table 3); none of these deviations were considered to affect the immunogenicity results. A higher than expected number of participants had missed bloods (Table 4), which is why – during the course of the study – the protocol was amended and an additional 10 study participants were enrolled into the study.

As the primary endpoint, post-booster proportions of participants with IgG $\geq$0.35 µg/ml for PCV-10 serotypes were compared between groups in a non-inferiority analysis. A high proportion of participants (>97%) in both groups had IgG $\geq$0.35 µg/ml for 8 out of 10 serotypes; inferior responses were seen for serotypes 5 and 9V in PCV-10 (73%, 87%) compared with PCV-13 recipients (96%, 99%) (Table 5).

Secondary endpoints included a range of different measures of immunogenicity. A robust immune response was induced by both vaccines when given as a booster dose at 12 months of age. Overall, PCV-13 was more immunogenic for most serotypes when IgG GMC and OPA GMT were considered with the exception of serotypes 4, 18C and 19F, which showed similar or statistically superior 13-month responses in the PCV-10 compared with the PCV-13 group.

Interestingly, serotypes 4, 18C and 19F are contained in PCV-10 in a higher concentration and/or conjugated to different carrier protein when compared with the other serotypes. Group differences at 13 months were usually less pronounced when proportions of participants above IgG ≥0.35 µg/ml or OPA titre ≥8 were considered. There was a rapid decline in antibody following the booster leading to the return to baseline levels for antibody against a number of serotypes in both groups.

Low-level memory B cells could be detected at baseline specific for most antigens with no significant differences between the groups (Table 24). One month following the booster vaccination, B_{MEM} frequencies were significantly higher in the PCV-13 compared with the PCV-10 group for 3 out of 4 of the assessed pneumococcal serotypes common to both PCV-13 and PCV-10 (Table 25). B_{MEM} directed against diphtheria toxoid representing the carrier proteins CRM_{197} (PCV-13) and diphtheria toxoid (PCV-10), respectively, rose significantly in both groups post-booster with no group differences (Table 27). B_{MEM} directed against tetanus toxoid representing the carrier protein tetanus toxoid only contained in PCV-10 increased significantly only in PCV-10 recipients whereas it remained low for samples taken from PCV-13 recipients.

A (booster) dose of PCV-10 given to 12 month old children shows a trend toward less immediate pain at time of vaccine injection as measured with validated pain tools. PCV-10 recipients had significantly lower scores on the modified behavioural pain scale but non-significant numerical rating scale scores and similar crying times.

A booster dose of either PCV-10 or PCV-13 given at 12 months of age is well tolerated with low rates of local and systemic side effects.

22 CONCLUSION

A booster dose of PCV-10 induces a robust antibody response when given as a booster vaccine to children previously vaccinated with PCV-13 at 2 and 4 months of age. However, IgG responses are non-inferior to PCV-13 for only 8 out of 10 serotypes contained in PCV-10.

23 REFERENCES

1. *Pneumococcal conjugate vaccine for childhood immunization--WHO position paper.* Wkly Epidemiol Rec, 2007. **82**(12): p. 93-104.
2. O'Brien, K.L., et al., *Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates.* Lancet, 2009. **374**(9693): p. 893-902.
3. Scott, J.A., *The preventable burden of pneumococcal disease in the developing world.* Vaccine, 2007. **25**(13): p. 2398-405.
4. Kaye, P., et al., *Invasive Pneumococcal Disease (IPD) in England & Wales after 7-valent conjugate vaccine (PCV7); potential impact of 10 and 13-valent vaccines: Health Protection Agency*, in *ESPID*. 2009: Brussels, Belgium.
5. Reinert, R.R., *The antimicrobial resistance profile of Streptococcus pneumoniae.* Clin Microbiol Infect, 2009. **15 Suppl 3**: p. 7-11.
6. Dagan, R., et al., *Introduction and proliferation of multidrug-resistant Streptococcus pneumoniae serotype 19A clones that cause acute otitis media in an unvaccinated population.* J Infect Dis, 2009. **199**(6): p. 776-85.
7. Kaplan, S.L., et al., *Six year multicenter surveillance of invasive pneumococcal infections in children.* Pediatr Infect Dis J, 2002. **21**(2): p. 141-7.
8. Appelbaum, P.C., *Resistance among Streptococcus pneumoniae: Implications for drug selection.* Clin Infect Dis, 2002. **34**(12): p. 1613-20.
9. Garcia-Rodriguez, J.A. and M.J. Fresnadillo Martinez, *Dynamics of nasopharyngeal colonization by potential respiratory pathogens.* J Antimicrob Chemother, 2002. **50 Suppl S2**: p. 59-73.
10. Sleeman, K.L., et al., *Acquisition of Streptococcus pneumoniae and nonspecific morbidity in infants and their families: a cohort study.* Pediatr Infect Dis J, 2005. **24**(2): p. 121-7.
11. Black, S., et al., *Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group.* Pediatr Infect Dis J, 2000. **19**(3): p. 187-95.
12. Mahon, B.E., et al., *Effectiveness of abbreviated and delayed 7-valent pneumococcal conjugate vaccine dosing regimens.* Vaccine, 2006. **24**(14): p. 2514-20.
13. Whitney, C.G., et al., *Effectiveness of seven-valent pneumococcal conjugate vaccine against invasive pneumococcal disease: a matched case-control study.* Lancet, 2006. **368**(9546): p. 1495-502.
14. Pollard, A.J., K.P. Perrett, and P.C. Beverley, *Maintaining protection against invasive bacteria with protein-polysaccharide conjugate vaccines.* Nat Rev Immunol, 2009. **9**(3): p. 213-20.
15. Pilishvili, T., et al., *Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine.* J Infect Dis, 2010. **201**(1): p. 32-41.
16. Lexau, C.A., et al., *Changing epidemiology of invasive pneumococcal disease among older adults in the era of pediatric pneumococcal conjugate vaccine.* JAMA, 2005. **294**(16): p. 2043-51.
17. *Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease--United States, 1998-2003.* MMWR Morb Mortal Wkly Rep, 2005. **54**(36): p. 893-7.
18. McIntosh, E.D., et al., *Pneumococcal pneumonia in the UK--how herd immunity affects the cost-effectiveness of 7-valent pneumococcal conjugate vaccine (PCV).* Vaccine, 2005. **23**(14): p. 1739-45.
19. Prymula, R., et al., *Pneumococcal capsular polysaccharides conjugated to protein D for prevention of acute otitis media caused by both Streptococcus pneumoniae and non-*

typable Haemophilus influenzae: a randomised double-blind efficacy study. Lancet, 2006. **367**(9512): p. 740-8.

20. Prymula, R. and L. Schuerman, *10-valent pneumococcal nontypeable Haemophilus influenzae PD conjugate vaccine: Synflorix.* Expert Rev Vaccines, 2009. **8**(11): p. 1479-500.
21. Poolman, J., et al., *Pneumococcal serotype 3 otitis media, limited effect of polysaccharide conjugate immunisation and strain characteristics.* Vaccine, 2009. **27**(24): p. 3213-22.
22. Newport, M.J., et al., *Genetic regulation of immune responses to vaccines in early life.* Genes Immun, 2004. **5**(2): p. 122-9.
23. Ovsyannikova, I.G., et al., *Human leukocyte antigen haplotypes in the genetic control of immune response to measles-mumps-rubella vaccine.* J Infect Dis, 2006. **193**(5): p. 655-63.
24. Yucesoy, B., et al., *IL-1beta gene polymorphisms influence hepatitis B vaccination.* Vaccine, 2002. **20**(25-26): p. 3193-6.