An open-labeled, randomized controlled trial evaluating for non-inferiority of 1+1 compared to 2+1 dosing schedules of 10-valent and 13-valent Pneumococcal Conjugate Vaccine (PCV) in South African children.

Abbreviated title: Reduced PCV dosing schedules.

Sponsored by the Bill & Melinda Gates Foundation (Grant number: OPP1152352)

Protocol Chair & Principal investigator: Shabir A. Madhi

Protocol Collaborator: David Goldblatt (University College, London)

Co-investigators: Clare L. Cutland

Sub-Investigators: Michelle Groome, Anthonet Koen, Carmen Briner, Lisa Jose, Adeebah Adams, Stephanie Jones, Welekazi Boyce

Protocol Chair & Principal investigator
Shabir A. Madhi
University of Witwatersrand
Respiratory and Meningeal Pathogens
Research Unit & DST/NRF Vaccine
Preventable Diseases
Chris Hani Road, Chris Hani-Baragwanath
Hospital; New Nurses Residence-11th Floor,
Bertsham 2013
Johannesburg
South Africa
Phone: +27 11 386 6137
FAX: +27 11 882 1872
E-mail: ShabirM@nicd.ac.za

Protocol Collaborator
David Goldblatt
University Colege, London

Co- investigator
Clare Cutland
University of Witwatersrand
Respiratory and Meningeal Pathogens
Research Unit & DST/NRF Vaccine
Preventable Diseases
Chris Hani Road, Chris Hani-Baragwanath
Hospital; New Nurses Residence-11th Floor,
Bertsham 2013, Johannesburg, South Africa
Phone: +27 11 983 4277
FAX: +27 86 674 0564
E-mail: cutlandc@rmpru.co.za

Protocol Laboratory Scientist
Elize De Bryn
University of Witwatersrand
Respiratory and Meningeal Pathogens
Research Unit & DST/NRF vaccine
Preventable Diseases
Chris Hani Road, Chris Hani-Baragwanath
Hospital; New Nurses Residence-11th Floor,
Bertsham 2013, Johannesburg
South Africa
Phone+27 11 983 4283
E-mail: debryne@rmpru.co.za

Sub-investigators:
Michelle Groome
Anthonet Koen
Carmen Briner
Lisa Jose
Adeebah Adams
Stephanie Jones
Welekazi Boyce

University of Witwatersrand
Respiratory and Meningeal Pathogens
Research Unit & DST/NRF vaccine
Preventable Diseases
Chris Hani Road, Chris Hani-Baragwanath
Hospital; New Nurses Residence-11th Floor,
Bertsham 2013, Johannesburg
South Africa
Phone: +27 11 983 4283

Protocol Statistician
Alane Izu
University of Witwatersrand
Respiratory and Meningeal Pathogens
Research Unit & DST/NRF vaccine
Preventable Diseases
Chris Hani Road, Chris Hani-Baragwanath
Hospital; New Nurses Residence-11th Floor,
Bertsham 2013, Johannesburg
South Africa
Phone: +27 11 983 4283
E-mail: izua@rmpru.co.za

Table of contents:

Abbreviations

CHBAH	Chris Hani Baragwanath Academic Hospital
GAVI	Global Alliance for Vaccines and Immunisation
IPD	Invasive pneumococcal disease
PCV	Pneumococcal conjugate vaccine
RMPRU	Respiratory and Meningeal Pathogens Research Unit

Protocol version 2.1, 6th February 2017 (revised from version 2.0, 15th September 2016)

Location of change	Change/ modification in version 2.1	Page #
Trial visit details	Clarification of participant ages and visit windows for all trial visits	19-20
Trial visit details	Addition of blood draw at enrolment for testing of antibodies against vaccine preventable diseases	19-20
Tables 2a, 2b and 2c	Clarification of ages and visit windows for all visits	21-24
Tables 2a, 2b and 2c	Addition of blood draw at baseline for antibody testing against vaccine preventable diseases	21-24
Tables 2a, 2b and 2c	Addition of 9-month blood draw for PCV ELISA to align with what was described in text	21-24
Laboratory methods	Addition of details of additional tests for antibodies against vaccine preventable diseases	25-26

Introduction and background

Pneumococcal conjugate vaccines (PCV) have shown great success in reducing the burden of under-5 childhood morbidity, including invasive pneumococcal disease (IPD) and all-cause pneumonia hospitalization in high and some middle income countries [1-4]. Furthermore, vaccination of young children has been associated with reduction in IPD and pneumonia among unvaccinated individuals due to an indirect effect of childhood PCV immunisation [4, 5]. Currently there are two PCV formulations, which include either 13 serotypes (PCV13) or 10 serotypes (PCV10) that are used globally. Both of these vaccine formulations have now been established to show efficacy and effectiveness against vaccine serotype IPD and all-cause pneumonia in high and middle-low income settings [6]. Although initially licensed as a three dose primary series with a booster dose in the second year of life (3+1 dosing), the high cost of these vaccines coupled with immunogenicity and epidemiological data has resulted in many countries, including South Africa, adopting a two dose primary series followed by a booster dose (2+1 dosing schedule) [7]. Recent meta-analyses concluded that there was no difference in protection between a 2+1 compared to 3+1 dosing schedule against vaccine-serotype IPD, pneumonia or vaccine-serotype colonization [1, 8, 9]. Furthermore, the antibody concentrations following the third dose of a 2+1 schedule in South Africa was significantly greater than after a third dose as part of a three dose primary series (3+0) schedule [10]. Despite this reduced dosing schedule, PCV immunisation remains unaffordable and sustainability of funding remains questionable for many low and middle income countries.

In South Africa, the cost of procurement of PCV ($20 per dose) totals almost 50% of the total cost of all vaccines purchased for the national immunisation program. Similarly, PCV is the most expensive vaccine purchased by the Global Alliance for Vaccines and Immunisation (GAVI), which heavily funds vaccine procurement for low income countries [11]. The sustainability of continued procurement of this vaccine at the current pricing in low-middle income countries remains uncertain. Furthermore, the high cost of PCV in middle income countries such as South Africa, could work against the introduction of other vaccines into the public immunisation program, including expanding the use of Human Papilloma Virus vaccine. Similarly, countries which are progressing economically and will no longer be eligible for GAVI funding of vaccine procurement, are likely to be challenged when required to increase their co-funding/funding of PCV, even at the GAVI subsidized price ($3.5 per dose). Accordingly, other strategies need to be considered to further reduce the cost of PCV in immunisation programs, without necessarily compromising the public-health benefits of vaccination. In the absence of reduced pricing from industry, the only option is for further reductions in the number of doses of PCV used in immunisation programs.

A number of factors favor the possibility of being able to reduce the PCV dosing schedule to a single primary dose, followed by a booster dose later in infancy or in the second year of life (1+1 dosing schedule).

1. Foremost, following the widespread routine use of PCV in the childhood immunisation program, there have been dramatic reductions in exposure and colonization (a pre-requisite to developing pneumococcal disease) to the commonly colonizing vaccine-serotypes among PCV-vaccinated and -unvaccinated members of the community [12, 13]. This effect, results from PCV reducing the risk of vaccine-serotype nasopharyngeal acquisition among vaccinated children. As children are the main source of transmission of pneumococci in the community, the reduction in colonization among vaccinated children has been associated with declining prevalence of colonization among unvaccinated individuals, including high risk groups. In South Africa, the prevalence of PCV13 serotype colonization declined by 68% in children <5 years and 60% in HIV-infected and HIV-uninfected women between 2010 and 2012 (i.e. only one year after transitioning to PCV in the immunisation program) [13]. The clinical impact of this interruption in community transmission of PCV is evident in South Africa, as well as in many other settings, from the reduction in vaccine-serotype IPD observed in adults, as well as children <10 weeks who either received no or only a single recent dose of PCV [4]. Consequently, the reduced circulation of the vaccine-serotype pneumococci brought about by childhood PCV immunisation, has in itself reduced the overall community exposure and risk of developing pneumococcal disease due to these serotypes among PCV-vaccinated and PCV-unvaccinated individuals.
2. A further potential benefit of lesser circulation of the vaccine serotypes is that it could heighten the antibody response to the primary series of PCV. This is corroborated by immunogenicity studies in South Africa and elsewhere, which demonstrated that colonization by the vaccine serotype prior to or at the time of vaccination with the primary series was associated with a dampened immune response to the homotypic serotypes [14-16]. With the decline in circulation of the vaccine-serotype, there is now a lower likelihood of early colonization by the common colonizing serotypes, and consequently this is less likely to contribute to an attenuated immune response to the primary series of PCV.
3. Although the proportion of children who develop antibody concentrations above the WHO defined putative threshold of protection against IPD (≥0.35 ug/ml) following the first dose of PCV is lower than after the second dose given at 14 weeks of age, a high proportion of children develop antibody concentrations above the putative threshold of protection after a single dose at 6 weeks of age (range 22% for serotype 6B to 94% for serotype 19F), which would provide protection over and above the indirect protection

from reduced exposure to the vaccine serotypes [10]. Similarly, a single dose of PCV at 6 weeks of age, was also associated with a high proportion of children with opsonophagocytic activity (OPA) titers ≥8 to five of the seven PCV7 serotypes (range 63% to 94%), whilst being 46% for serotype 23F and 14% for serotype 6B [17]. Notably, however, the WHO putative threshold for protection against IPD may underestimate the protection against some serotypes and overestimate for others. Andrews et al. recently reported that much lower antibody concentrations are required to protected against some IPD causing serotypes, including serotypes 6B and 23F (protective threshold ≥0.16 ug/ml and ≥0.20 ug/ml, respectively), than proposed by the WHO threshold [18]. The clinical effectiveness of even a single dose of PCV is evident from case-control studies in which protection against IPD following receipt of a single dose before 7 or 12 months of age in the USA and UK, were 73% and 56%, respectively [19, 20].

4. A further factor to consider is that timing of the 1st dose of PCV may differentially impact on the immunogenicity of the vaccine. This could result from lower concentrations of maternal-derived transplacentally- acquired serotype specific capsular antibody with increasing age, which too could dampen the immune response to the first dose of PCV, the closer it is given to birth. Furthermore, maturation of the immune system in the first few weeks of life could theoretically result in heightened immunogenicity to the single dose of PCV when administered at 14 instead of 6 weeks of age. Also, theoretically priming of the immune system with diphtheria toxoid vaccine at 6 weeks of age could heighten the immune response of CRM_{197} constituted PCV when the first dose is iven at 14 weeks, rather than at 6 week of age. Considering the inherently reduced risk of pneumococcal disease during the first few months of life because of the current low risk of colonization by vaccine-serotypes, delaying PCV vaccination from 6 to 14 weeks of age is unlikely to significantly increase susceptibility to pneumococcal disease.
5. Also, the immune response following a booster dose after a single primary dose of PCV could be higher compared to after a two-dose primary series. That lower vaccine antigen exposure during the primary series can accentuate the immune responses to a booster dose of conjugate vaccine, is supported by studies on *Haemophilus influenzae* type b conjugate vaccine (HibCV). In a study looking at alternate HibCV dosing concentrations in South Africa, children who received a 1/10th of the approved dose of HibCV had greater antibody responses following the booster dose of vaccine compared to those who received the full-strength dose [21].

In view of the above, a study is underway in the United Kingdom, to evaluate whether the country can transition from a 2+1 to 1+1 PCV dosing schedule. This is undertaken with supporting evidence of reduced circulation of the commonly colonizing PCV serotypes 3-4 years after routine

immunisation with the 2+1 dosing schedule, as manifest by lower prevalence of colonization in different age-groups as well as notably reduction in incidence of vaccine-serotype IPD in all age-groups independent of whether targeted for PCV vaccination. Similarly, in South Africa where PCV was introduced into the public expanded program on immunisation (EPI) since 2009, there have been reductions in vaccine-serotype colonization and IPD across PCV-vaccinated and -unvaccinated age-groups. Alternate PCV dosing studies, including only a single dose in the 2nd year of life (0+1) and 1+1 dosing schedules are also underway in Fiji and Vietnam, despite PCV never having been previously used in the public immunisation programs of those countries.

This study will evaluate the immunogenicity of a reduced dosing schedule of PCV10 and PCV13, in which children will receive a primary dose at either 6 or 14 weeks of age, followed by a booster dose at 9 months of age (1+1 schedule), and compare this immune response to those who receive a two dose primary series (at 6 and 14 weeks of age) and booster dose at 9-months (2+1 schedule).

Although the public immunization program in South Africa currently vaccinates children with PCV13, the rational for including the PCV10 study arms relates to the possibility that PCV10 could replace PCV13 in the future. Such a decision could be premised on ongoing cost-effectiveness analysis, as well as that PCV10 has been shown to offer cross-protection against serotypes 6A and 19A (after booster dose)[22, 23], effectively protecting against 12 serotypes. Furthermore, the effectiveness of PCV13 against serotype 3 remains uncertain[24, 25], effectively potentially also only protecting against 12 serotypes. Consequently, there is likely minimal difference in protection against overall IPD between PCV10 and PCV13, and more so in the context of a setting such as South Africa where there has been a reduction in circulation of these vaccine serotypes because of the established PCV childhood vaccine program.

Aims and objectives

Primary study objective:

1. To evaluate the vaccine-serotype specific geometric mean antibody concentrations (GMC) one month following the booster dose (administered at 9 months of age) of differing 1+1 dosing schedule (1st dose at either 6 or 14 weeks of age) compared to the immune response following a 2+1 dosing schedule (6+14 weeks and booster at 9 months) of the same vaccine formulation (i.e. PCV10 or PCV13).

   Endpoint: The serotype-specific GMC measured 1 month after the 9-month booster dose for each 1+1 vaccine group and comparing it to the 2+1 group of the same vaccine.

Non-inferiority criteria: The lower limit of the 95% confidence interval of the ratio of GMC of the 1+1 and 2+1 dosing schedules being >0.5 for at least 10 of the PCV13 serotypes and eight of the PCV10 serotypes.

Secondary study objectives:

Immunogenicity outcomes (all study endpoints will be stratified by vaccine formulation; i.e. PCV13 and PCV10):

1. To evaluate the percentage of children with vaccine-serotype specific serum IgG antibody concentration above the WHO-defined putative threshold for protection (≥0.35 µg/mL) at 9 months of age, prior to the booster dose of differing 1+1 dosing schedules (i.e. primary dose given at either 6 or 14 weeks of age) compared to that of children who received a 2 dose primary series (i.e. 2+1 dosing schedule group)..

   Endpoint: The percentage of participants with serotype-specific IgG antibody concentration ≥0.35 µg/mL for each of the pneumococcal serotypes measured prior to the booster dose of PCV at 9-months of age for each 1+1 vaccine group and comparing it to the 2+1 group of the same vaccine formulation.

2. To evaluate the percentage of children with vaccine-serotype specific serum IgG antibody concentration above the WHO-defined putative threshold for protection (≥0.35 µg/mL) one month following the booster dose of differing 1+1 dosing schedules (i.e. primary dose given at either 6 or 14 weeks of age and booster at 9 months of age) compared to that of a 2+1 dosing schedule.

   Endpoint: The percentage of participants achieving a serotype-specific IgG antibody concentration ≥0.35 µg/mL for each of the pneumococcal serotypes measured 1 month after the 9-month booster dose for each 1+1 vaccine group and comparing it to the 2+1 group of the same vaccine formulation.

   Non-inferiority criteria: The lower bound of the 95% CI for the difference between the two groups (1+1 less 2+1) in the proportion of participants achieving an IgG concentration of ≥0.35 µg/mL would be >-0.10 for at least 10 of the PCV13 serotypes and eight of the PCV10 serotypes.

3. To evaluate the proportion of children with vaccine-serotype specific serum IgG antibody response above the modified serotype-specific correlate of protection against IPD as proposed by Andrews et al. [18] one month following the booster dose of differing 1+1 dosing compared to the immune response following a 2+1 dosing schedule.

   Endpoint: The percentage of participants achieving a serotype-specific IgG antibody concentration above the serotype-specific threshold proposed by Andrews et al., as correlate for protection against IPD 1-month after the booster dose of PCV for each 1+1 vaccine group and comparing it to the 2+1 PCV group of the same formulation.

   Non-inferiority criteria: The lower bound of the 95% CI for the difference between the two groups (1+1 less 2+1) in the proportion of participants achieving an IgG concentration of a specified threshold proposed by Andrews et al. for at least 10 of the PCV13 serotypes and eight of the PCV10 serotypes.

4. To evaluate the number of children with vaccine-serotype specific serum IgG antibody concentration above the WHO-defined putative threshold for protection (≥0.35 µg/mL, the modified serotype-specific correlate of protection against IPD as proposed by Andrews et al. [18] and GMCs one month following the first dose of PCV in children receiving the 1+1 dosing schedule (i.e. primary dose given at either 6 or 14 weeks of age) compared to one-month following the second dose of PCV in the 2+1 PCV group, stratified for the individual vaccine formulation (PCV10 and PCV13).

   Endpoint 4.1 Percentage of participants achieving a serotype-specific IgG antibody concentration ≥0.35 µg/mL for each of the PCV serotypes 1 month after the 1st dose in the 1+1 PCV groups, and one-month following the 2nd primary series PCV group in the 2+1 dosing arm, stratified by vaccine formulation.

   Endpoint 4.2 Percentage of participants achieving a serotype-specific IgG antibody concentration modified serotype-specific correlate of protection against IPD as proposed by Andrews et al. [18] for each of the PCV serotypes 1 month after the 1st dose in the 1+1 PCV groups, and one-month following the 2nd primary series PCV group in the 2+1 dosing arm, stratified by vaccine formulation.

Endpoint 4.3 Compare the GMCs for each of the PCV serotypes 1 month after the 1st dose in the 1+1 PCV groups, and one-month following the 2nd primary series PCV group in the 2+1 dosing arm, stratified by vaccine formulation.

5. To evaluate the number of children with vaccine-serotype specific serum IgG antibody concentration above the WHO-defined putative threshold for protection (≥0.35 µg/mL), the modified serotype-specific correlate of protection against IPD as proposed by Andrews et al. [18] and GMCs one month following the first dose of PCV in children receiving the 1+1 dosing schedule at either 6 weeks of age compared to those who received it at 14 weeks of age one-month following the first dose, stratified for the individual vaccine formulation (PCV10 and PCV13).

   Endpoint 5.1: Percentage of participants in the 1+1 groups achieving a serotype-specific IgG antibody concentration ≥0.35 µg/mL for each of the PCV serotypes 1 month after the 1st dose of PCV between those receiving the 1st dose at 6 weeks compared to those receiving it at 14 weeks of age, stratified by vaccine formulation.

   Endpoint 5.2: Percentage of participants in the 1+1 groups achieving a serotype-specific IgG antibody concentration modified serotype-specific correlate of protection against IPD as proposed by Andrews et al. [19] for each of the PCV serotypes 1 month between those receiving the 1st dose at 6 weeks compared to those receiving it at 14 weeks of age stratified by vaccine formulation.

   Endpoint 5.3: Compare the GMCs for each of the PCV serotypes 1 month after the 1st dose between those receiving the 1st dose at 6 weeks compared to those receiving it at 14 weeks of age, stratified by vaccine formulation.

6. To evaluate the proportion of children with vaccine-serotype specific opsonophagocytic activity (OPA) ≥1:8 (a more robust correlate of protection against IPD), as well as those proposed by Andrews et al. as a correlate of protection, one month following the booster dose of differing 1+1 dosing schedules compared to the immune response following a 2+1 dosing schedule on a random subset (20%) of participants from each group.

   Endpoint 6.1: The percentage of participants in a subset achieving a serotype-specific OPA titer ≥1:8 (i.e. lower limit of quantification of the assay) for each of the pneumococcal

serotypes measured 1-month after the 9-month booster dose for each 1+1 vaccine groups and compared to the 2+1 group of the same vaccine formulation.

Endpoint 6.2: The percentage of participants in a subset achieving a serotype-specific OPA titer greater or equal to serotype-specific threshold proposed by Andrews et al. as a OPA correlate of protection against IPD for each of the pneumococcal serotypes measured 1 month after the 9-month booster dose for each 1+1 vaccine group compared to the 2+1 group of the same vaccine formulation.

7. Compare the vaccine-serotype specific serum OPA geometric mean titers (GMT) one month following the booster dose of differing 1+1 dosing schedules compared to the immune response following a 2+1 dosing schedule on the subset in whom OPA are undertaken.

   Endpoint: The lower limit of the 95% confidence interval of the ratio of OPA GMT one-month following the booster dose of PCV of the 1+1 and 2+1 dosing schedules being >0.5 for at least 10 of the PCV13 serotypes and eight of the PCV10 serotypes.

8.

To evaluate GMCs of serotype specific antibody concentrations at 18 months of age in the different 1+1 dosing arms compared to the 2+1 dosing group.

Endpoint: The lower limit of the 95% confidence interval of the ratio of GMC of the 1+1 and 2+1 dosing schedules being >0.5 for individual serotypes.

Colonization outcome:

9. To compare the prevalence of vaccine-serotype (stratified by PCV10 and PCV13 serotypes) and non-vaccine serotype nasopharyngeal colonization at 9, 15, and 18 months of age for the 1+1 dosing schedule groups compared to the 2+1 groups.

   Endpoint: Prevalence of overall vaccine-serotype colonization and stratified by PCV10 and PCV13 serotypes at 9, 15 and 18 months of age in the 1+1 compared to 2+1 dosing arms, stratified for the individual PCV formulation.

Safety outcome:

10. The number of participants reporting adverse events in the 1+1 dosing groups and the 2+1 dosing groups throughout the study including specifically physician diagnosed all-cause

clinical pneumonia (and vaccine-serotype IPD), including out-patient or hospitalized episodes.

Exploratory study objectives:

11. Evaluate the percentage of children with vaccine-serotype specific serum IgG antibody concentration above ≥0.5 µg/mL and ≥1.0 µg/mL, at one month following the booster dose of differing 1+1 dosing schedules (i.e. primary dose given at either 6 or 14 weeks of age and booster at 9 months of age) compared to that of a 2+1 dosing schedule of the same vaccine formulation (i.e. PCV10 or PCV13).

    Endpoint: The percentage of participants achieving a serotype-specific IgG antibody concentration ≥0.5 µg/mL and ≥1.0 µg/mL for each of the pneumococcal serotypes measured 1 month after the 9-month booster dose for each 1+1 vaccine group and comparing it to the 2+1 group of the same vaccine formulation.

12. To evaluate the vaccine-serotype specific GMC at 18 months of age of differing 1+1 dosing schedule compared to the immune response following a 2+1 dosing schedule (6+14 weeks and booster at 9 months) of the same vaccine formulation (i.e. PCV10 or PCV13).

    Endpoint: The serotype-specific GMC measured at 18-month booster of age between each of the 1+1 vaccine group and also comparing each of the 1+1 to the 2+1 group, stratified for the same vaccine formulation.

Study design & methods:

Study Design

This will be a randomized, open-label study (laboratory personnel will however be blinded) in which participants are randomized to one of two (primary dose at either 6 or 14 weeks of age) 1+1 dosing schedules of PCV10 or PCV13, or to a 2+1 schedule of these vaccines. The study is designed to show non-inferiority in immune responses following the booster dose of PCV (either PCV10 or PCV13), in children randomized to a 1+1 dosing schedule compared to those who receive a 2+1 dosing schedule. A total of 600 participants will be randomized in a 1:1:1:1:1:1 ratio to one of the six groups. The study will be undertaken at an experienced research site in Johannesburg, South Africa, where the 600 children born to HIV-uninfected women are expected to be enrolled over a 12 month period.

This trial will evaluate the safety and immunogenicity of a 1+1 dosing schedule of PCV10 or PCV13 in HIV-unexposed South African infants. The selection of HIV-unexposed infants is based on mitigating against the increased risk of HIV-exposed-uninfected children to developing IPD during the first year of life as shown prior to the PCV-immunisation era [26]. The immunogenicity of the CRM-197 PCV formulation and PCV10 has, however, been shown to be similar between HIV-exposed and HIV-unexposed infants [27, 28]. In addition, we propose to evaluate whether the timing of the first dose of PCV (either at 6 or 14 weeks of age), would influence the immune response to the vaccine. The immunogenicity will be measured one month post the primary series of either one or two doses of vaccine, at the time of the booster dose of PCV and one-month following the booster dose. Additionally, we propose to evaluate the medium-term antibody levels at 18 months of age.

This study will be undertaken in a setting where there are robust prevailing data on the incidence of IPD and prevalence of serotype specific colonization, including data from studies dating back to mid-1990s. Although PCV10 has three fewer serotypes included in it, there is supporting evidence to show cross-protection against serotype 6A and possibly serotype 19A (especially after a booster dose of vaccine), hence, effectively the vaccine coverage for PCV10 would be similar to that for PCV13, as serotype 3 (not included in PCV10) is relatively uncommon (<2% of invasive isolates prior to PCV immunisation) in South African children [29, 30]. Both PCV13 and PCV10 are licensed for use in South Africa.

Although the immunogenicity of the 2+1 dosing schedule of PCV10 and PCV13 per South African public immunisation program recommendation has been previously evaluated [10, 31], we propose including such a dosing arm as a comparator group in the current study, since changes in circulation of the vaccine serotypes could theoretically influence the immune responses to even

this dosing schedule. Furthermore, considering the low incidence of PCV13-serotype IPD among HIV-unexposed children in South Africa in 2012 (1 per 10 000, which has likely declined even further since), it is unlikely that we would have any vaccine-serotype IPD cases among the study cohort of 600 participants against which to measure the relative efficacy of the different PCV formulations. In lieu of determining whether there is any likely clinical offset of a reduced dosing schedule, we propose to evaluate serotype specific pneumococcal colonization during the study. Although colonization is a pre-requisite to developing pneumococcal disease, the majority of pneumococcal colonizing events are asymptomatic, with the prevalence of PCV13 serotype colonization having decreased from 29% in 2010 to 14% in 2012, with further declines anticipated to have occurred since then (data from 2014/5 are currently being analyzed).

Sample size calculation:

The study is powered (80%) to demonstrate non-inferiority following the booster dose of PCV in the 1+1 compared to the 2+1 groups. To control the type I error for the demonstration of non-inferiority for 10 of the 13 PCV13 serotypes or 8 of the 10 PCV10 serotypes, adjustment of the 1-sided alpha was applied ($\alpha=10/13*0.025\approx 8/10*0.025=0.02$). Thus the non-inferiority will be declared if the lower limit of the 95% confidence interval for the ratio of the geometric means is greater than 0.5 for at least 10 of the PCV13 serotypes and eight of the PCV10 serotypes. Each 1+1 arm will be compared separately to the 2+1 arm for the specific vaccine formulation.

Sample size calculations assumed: (a) power of at least 80%; (b) two-sided type I error of 0.05; (c) standard deviation of the log antibody concentrations of 0.4; (d) the true ratio of the geometric means to be 1.05; and (e) non-inferiority criteria described above.

A sample size of 91 per group would provide at least 80% power to declare non-inferiority in 10 out of the thirteen PCV13 serotypes or 8 out of the ten PCV10 serotypes (Table 1). The sample size will be adjusted upward by 10% to allow for loss to follow-up and early study withdrawals. The total number to be enrolled will thus be 600, including 300 in the PCV13 cohorts and 300 in the PCV10 cohorts.

Table 1: Sample size calculations based on study definition of non-inferiority, overall power of 80% and a type I error ≤0.05.

Computed Sample Size per Group			
Ratio of GMC[1]	Std Dev[2]	Actual Power	Sample Size per Group
1.25	0.3	0.984	80
1.25	0.4	0.984	141
1.25	0.5	0.983	219
1.25	0.6	0.983	314
1.12	0.3	0.984	52
1.12	0.4	0.984	91
1.12	0.5	0.983	141
1.12	0.6	0.983	202
1.00	0.3	0.983	36
1.00	0.4	0.984	64
1.00	0.5	0.983	98
1.00	0.6	0.983	141

[1]Ratio of GMC = The assumed true ratio of GMC between 1+1 and 2+1 groups. A ratio of 0, 1.12 and 1.25 is equivalent to a 0, 0.05 and 0.10 difference in the log(base 10) antibody concentrations, respectively. [2]Std Dev = standard deviation of the log antibody concentration. It is assumed the standard deviation is the same in both the 1+1 and 2+1 groups.

Study population:

Any child who has not as yet received any of the vaccines scheduled at 6 week of age will be screened for participation in this study. All participants need to fulfill all inclusion and exclusion criteria, as detailed below, prior to randomization into the study.

Study inclusion criteria:

1. Signed informed consent by the parent/guardian of the child;
2. Born to an HIV-uninfected women, based on testing undertaken as part of standard of care during the last trimester of pregnancy;
3. Had not received any vaccine other than BCG and OPV (routinely given at birth) prior to enrolment;
4. Birth weight >2499g AND weight of child >3.5 kg at time of proposed randomization;
5. Aged 42-56 days of age at time of enrolment;
6. Available for the duration of the study;
7. Child is healthy based on medical history and physical examination of the study-staff.

Exclusion criteria:

1. Any clinically significant major congenital abnormalities;
2. Previous hospitalization for a respiratory illness following discharge from hospital after birth;
3. Receipt of any other investigational drug/vaccine. Co-enrollment into non-investigational studies, including epidemiology studies, is allowed;
4. Any previous PCV vaccination;
5. Known allergy to any of the vaccine components;
6. Febrile illness (axillary temperature ≥37.8°C) at time of enrolment. These participants are eligible if the temperature resolves for at least 48 hours and they remain within the study defined window periods;
7. Planned relocation to outside of the study area during up until age of 2 years;
8. Receipt of blood transfusion or any other blood products (including immunoglobulins) since birth. Receipt of such products during the course of the study, will require withdrawal of the child from the study;
9. History of confirmed pneumococcal disease since birth;
10. Any known or suspected immunodeficiency condition which could affect immune response to vaccination.

Study procedures:

Study population

The study will be undertaken in Soweto, South Africa. Parents of prospective study participant will be identified at the time of birth at Chris Hani-Baragwanath Academic Hospital (CHBAH) or at one of the neighboring primary health care clinics, including midwife operated units and the immunisation clinics at these facilities prior to receiving their vaccine at 6 weeks of age.

Study randomization

Participants consented for study-participation will be randomized into one of 6 study arms in a 1:1:1:1:1:1 ratio. The six groups will include children randomized to either one of the two 1+1 dosing schedules (1st dose given at either 6 or 14 weeks of age) and the 2+1 dosing group (per current South African public immunisation schedule at 6 weeks, 14 weeks and 9 months of age), using either PCV10 or PCV13. The booster dose in all study groups will be given at 9 months of age. Participants eligible for enrolment will be allocated a sequential study number and randomization undertaken manually with allocation to study group for specific study-numbers having been generated by the study statistician using blocks of 30.

Study vaccine

Both study-vaccines, i.e. PCV10 (Synflorix®) and PCV13 (Prevnar-13®), are currently licensed in South Africa.

PCV13 (Prevnar-13®) contains saccharides from pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F individually conjugated to nontoxic diphtheria toxin cross-reactive material 197 (CRM197). The vaccine is formulated to contain 2.2 µg of each saccharide, except for 4.4µg of 6B, per 0.5-mL dose. The vaccine is formulated with 5 mM succinate buffer, 0.02% polysorbate 80, and 0.125 mg of aluminum as aluminum phosphate, per 0.5-mL dose.

PCV10 (Synflorix®) consists of 1 µg capsular polysaccharide for serotypes 1, 5, 6B, 7F, 9V, 14, and 23F, and 3 µg for serotype 4, each individually conjugated to protein D of non-typeable H. influenzae, and 3 µg of capsular polysaccharide of serotypes 18C and 19F conjugated to tetanus and diphtheria toxoids, respectively. The PCV vaccines will be procured commercially. Study vaccines will be administered by qualified medical staff, with PCV being administered intramuscularly by injecting 0.5 mL into the anterolateral thigh muscle of the left leg at the vaccination visits, with the details (i.e., date of administration, randomization number, site of administration) recorded on the case report forms (CRF).

In addition, children will receive the other vaccines included in the public immunisation program (including Hexaxim®, measles and rotavirus vaccine) per recommended public immunisation schedule. All routinely administered injectable vaccines given concurrently with PCV will be administered in a limb other than where the PCV vaccine was given. All the routinely administered vaccines included in the pubic immunisation program will be sourced through the immunisation program by RMPRU, which is an accredited immunisation facility of the Department of Health. The dosing schedules for the PCV Groups and the other routinely administered vaccines are shown in Table 2. As a benefit to the children, all participants will be assigned to receive either a single dose of Hepatitis A vaccine or varicella vaccine at 15 months of age, both of which are registered in South Africa, but neither of which are included in the public immunisation program. Allocation to varicella or Hepatitis A vaccine will be based on whether the child's study randomization number ends in an even (Hepatitis A vaccine) or odd number (Varicella vaccine). In the event of unavailability of either of these vaccines during the study, the alternate vaccine will be provided to the child.

The PCV and other vaccines will be stored at 2-8°C in a temperature- monitored refrigerator at the RMPRU pharmacy, with control of vaccine stock undertaken by the site-pharmacist. The refrigerator is secure with limited access and temperature monitoring is undertaken on a 24/7

automated system, with alerts being sent to the pharmacist and other study staff in the event of excursions outside of the defined range.

Trial visit details

Visit 1 (6 weeks of age, day 42-56 of life):
For all participants, informed consent will be signed, inclusion and exclusion criteria reviewed, medical history will be collected and physical examination (including weight and length measurements) and randomization will be performed.
Blood draw for testing of baseline antibody concentrations against vaccine preventable diseases
All participants will receive Hexaxim® and Rotavirus vaccine as per EPI.
Group 1 & 3 participants will also receive PCV10/ PCV13 (as per randomisation).
All participants will receive a visit appointment card, and the next visit will be scheduled.

Visit 2 (10 weeks of age; 28-35 days post visit 1):
All participants will have a physical examination (including weight and length measurements) and will receive Hexaxim® vaccine as per EPI.
Group 1 participants will have a blood draw for ELISA for PCV vaccine serotypes (28-35 days post vaccine administration).
All participants: next visit will be scheduled.

Visit 3 (14 weeks of age; 28-35 days post visit 2):
All participants will have a physical examination (including weight and length measurements) and will receive Hexaxim® and Rotavirus vaccine as per EPI.
Group 2 & 3 participants will also receive PCV10/ PCV13 (as per randomisation).
All participants: next visit will be scheduled.

Visit 4 (18 weeks of age; 28-35 days post visit 3):
All participants will have a physical examination (including weight and length measurements).
Group 2 & 3 participants will have a blood draw for ELISA for vaccine serotypes (28-35 days post vaccine administration).
All participants: next visit will be scheduled.

Visit 5 (6 months of age; day 180 ±14 days):
All participants will have a physical examination (including weight and length measurements) and will receive measles vaccine as per EPI.

All participants: next visit will be scheduled.

Visit 6 (9 months of age; day 270 ±14):
All participants will have a physical examination (including weight and length measurements) and will receive PCV10/ PCV13 (as per randomisation).
A nasopharyngeal swab will be taken from all participants prior to vaccine administration for culture/ PCR detection of PCR serotypes.
All participants will have a blood draw for draw for ELISA for vaccine serotypes
All participants: next visit will be scheduled.

Visit 7 (10 months of age; 28-35 days post visit 6):
All participants will have a physical examination (including weight and length measurements).
All participants will have a blood draw for ELISA for PCV vaccine serotypes AND PCV OPA and vaccine preventable diseases (28-35 days post vaccine administration).
All participants: next visit will be scheduled.

Visit 8 (12 months of age; day 365 ± 14 days):
All participants will have a physical examination (including weight and length measurements) and will receive measles vaccine as per EPI.
All participants: next visit will be scheduled.

Visit 9 (15 months of age; day 450 ± 14 days):
All participants will have a physical examination (including weight and length measurements) and will receive Hexaxim® vaccine as per EPI.
All participants will receive benefit vaccine (Hepatitis A or Varicella).
A nasopharyngeal swab will be taken from all participants prior to vaccine administration for culture/ PCR detection of PCR serotypes.
All participants: next visit will be scheduled.

Visit 10 (18 months of age; day 540 ± 14 days):
All participants will have a physical examination (including weight and length measurements).
All participants will have a blood draw for ELISA for vaccine serotypes (end of study).
A nasopharyngeal swab will be taken from all participants for culture/ PCR detection of PCR serotypes.
Study termination visit.

Table 2a: Study Schedule of Events including the time points at which blood will be obtained by vecupuncture for analysis of the immunogenicity endpoints, and nasopharyngeal swabs collected for evaluation of colonization: **Group 1 (1+1, 6 weeks)**

Visit number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Age	**6 weeks**	**10 weeks**	**14 weeks**	**18 weeks**	**6 months**	**9 months**	**10 months**	**12 months**	**15 months**	**18 months**
Age (days)	Day 42-56	V1+28-35	V2 +28-35	V3 +28-35	180 ±14	270 ±14	V6 +28-35	365 ±14	450 ±14	540 ±14
ICF signed	X									
Inclusion/ exclusion criteria	X									
Medical history	X									
Physical examination	X	X	X	X	X	X	X	X	X	X
Randomisation	X									
Grp 1a (PCV10)[1]	PCV10[2]					PCV10[2]				
Grp 1b (PCV13)	PCV13[2]					PCV13[2]				
Blood draw: Serum for ELISA of PCV vaccine serotypes[5] (3ml)	X	X				X	X			X
Blood draw: Serum for PCV OPA in subset[6] (2ml)	X						X			
Blood draw: EPI[#]	X					X				
Hexaxim®[3]	X	X	X						X	
Rotavirus vaccine[3]	X		X							
Measles vaccine[3]					X			X		
Varicella or Hepatitis A vaccine[4]									X	
NP swab[9]						X			X	X

[1]: Refers to vaccination with respective PCV13 or PCV10 vaccine to which randomized. [2]All children will receive the same PCV formulation as the primary series as a booster dose. [3]Provided as standard of care in the public immunisation program, per indicated time points. [4]Proposed vaccine to all children as a potential benefit for study participation. Participants whose randomization number ends in an even number will receive Hepatitis A vaccine and those with an odd number the Varicella vaccine. [5] Serum samples taken for ELISA of the vaccine serotypes. [6] serum sample taken for opsonophagocytic activity (OPA) which will be tested for in a subset of patients. [9]Naspharyngeal (NP) swab taken using a flocked swab and transported in STGG medium, for culture/PCR detection and serotyping. [#] Blood draw for antibody testing of vaccine preventable diseases included in EPI

Table 2b: Study Schedule of Events including the time points at which blood will be obtained by vecupuncture for analysis of the immunogenicity endpoints, and nasopharyngeal swabs collected for evaluation of colonization: **Group 2 (1+1, 14 weeks)**

Visit number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Age	**6 weeks**	**10 weeks**	**14 weeks**	**18 weeks**	**6 months**	**9 months**	**10 months**	**12 months**	**15 months**	**18 months**
Age (days)	Day 42-56	V1+28-35	V2 +28-35	V3 +28-35	180 ±14	270 ±14	V6 +28-35	365 ±14	450 ±14	540 ±14
ICF signed	X									
Inclusion/ exclusion criteria	X									
Medical history	X									
Physical examination	X	X	X	X	X	X	X	X	X	X
Randomisation	X									
Grp 2a (PCV10)			PCV10[2]			PCV10[2]				
Grp2b (PCV13)			PCV13[2]			PCV13[2]				
Blood draw: Serum for ELISA of PCV vaccine serotypes[5] (3ml)	X			X		X	X			X
Blood draw: Serum for PCV OPA in subset[6] (2ml)	X						X			
Blood draw: EPI[#]	X					X				
Hexaxim®[3]	X	X	X						X	
Rotavirus vaccine[3]	X		X							
Measles vaccine[3]					X			X		
Varicella or Hepatitis A vaccine[4]									X	
NP swab[9]						X			X	X

Table 2c: Study Schedule of Events including the time points at which blood will be obtained by vecupuncture for analysis of the immunogenicity endpoints, and nasopharyngeal swabs collected for evaluation of colonization: **Group 3 (2+1)**

Visit number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Age	**6 weeks**	**10 weeks**	**14 weeks**	**18 weeks**	**6 months**	**9 months**	**10 months**	**12 months**	**15 months**	**18 months**
Age (days)	Day 42-56	V1+28-35	V2 +28-35	V3 +28-35	180 ±14	270 ±14	V6 +28-35	365 ±14	450 ±14	540 ±14
ICF signed	X									
Inclusion/ exclusion criteria	X									
Medical history	X									
Physical examination	X	X	X	X	X	X	X	X	X	X
Randomisation	X									
Grp 3a (PCV10)	PCV10[2]		PCV10[2]			PCV10[2]				
Grp3b (PCV13 EPI)	PCV13[2]		PCV13[2]			PCV13[2]				
Blood draw: Serum for ELISA of PCV vaccine serotypes (3ml)	X			X		X	X			X
Blood draw: Serum for PCV OPA in subset (2ml)	X						X			
Blood draw: EPI[#]	X					X				
Hexaxim®[3]	X	X	X						X	
Rotavirus vaccine[3]	X		X							
Measles vaccine[3]					X			X		
Varicella or Hepatitis A vaccine[4]									X	
NP swab[9]						X			X	X

Laboratory methods

The laboratory tests will be undertaken at RMPRU and its collaborator.

Immunogenicity assays:

The volume of blood collected from each participant at enrolment, at one month following the 1st dose in the 1+1 arms (Groups 1a, 1b, 2a, 2b) and following the 2nd dose in the 2+1 arms (Groups 3a and 3c) will be approximately 3ml. In addition, at one month following the booster dose and at 18 months of age, an additional 5ml of blood will be collected. Each sample will be labeled with a unique laboratory coded number, so that laboratory personnel will be blinded to the participant identify and randomization. Clotted blood samples will be delivered to the RMPRU laboratory within 4 hours of collection, centrifuged and the serum aliquoted and stored at -70°C until ready for testing. Residual serum samples will be stored for future testing, including assessing immune response to other administered vaccines. The participant's parent/legal guardian may decline to have the sample tested for any other reason than requested for this study, and also to have the sample destroyed at any time.

ELISA assay for serotype-specific IgG concentrations

Serotype specific antibody concentrations will be undertaken in all participants for all pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) included in PCV13, to avoid the laboratory staff being unblinded as to which arm the child might have been allocated to. Samples will be tested at the RMPRU laboratory, using standardized methods and reference sera as recommended by WHO. The ELISA assays will be undertaken in samples obtained one-month following the 1st dose in Groups 1 and 2, and one month following the 2nd dose in Groups 3a and 3b. In addition, ELISA will be performed on samples obtained one-month after the booster dose of PCV (10 months of age) and at 18 months of age.

OPA assays to the 13 pneumococcal serotypes

The OPA assays will be undertaken at the WHO pneumococcal assay Reference Laboratory of Dr David Goldblatt (London, United Kingdom).

A random sample of 20% of enrolled cases (n=20) will be tested from each of the study arm using blood samples obtained from one-month following the booster dose of PCV.

Antibody titres against vaccine preventable diseases

Antibody titres to diphtheria-toxoid, tetanus toxoid, anti-FHA, anti-PT and anti-Hepatitis B surface antigen will be evaluated using a multiplex assay on the Luminex platform at the RMPRU. Measles antibody will be measured by standard ELISA assay using commercially available

assays. Assays testing for rotavirus IgA and IgG will be set-up at the RMPRU, in collaboration with the diarrheal laboratory at Centre for Disease Control and Prevention; USA.

Nasopharyngeal swabs for pneumococcus identification/ culture and serotyping

Nasopharyngeal swabs will be collected using a flocked swab at 9, 12 and 18 months of age. The samples will be stored in STGG medium until tested. The first line testing will use standard culture methods and serotyping per established methods recommended by WHO.

Safety monitoring

The study will undertake passive surveillance for hospitalizations of study participants, including for pneumonia and invasive pneumococcal disease, throughout the study. A Data and Safety Monitoring Board (DSMB) will be established to review whether any of the hospitalizations are possibly due to pneumococcal disease (with a specific focus on IPD and radiologically confirmed pneumonia) and determine whether there is any trend to an excess of cases in any of the 1+1 dosing arms compared to the 2+1 dosing arm. Summary reports of all hospitalizations, radiologically confirmed pneumonia and IPD (including serotype data) will be provided to the DSMB on a quarterly basis, and more frequently if requested. The incidence (per 100,000) of PCV13 vaccine-serotype IPD among HIV-unexposed children in 2012 in Soweto was 20.6, 0, 5.6 and 0 in age groups <10 weeks, 10wks to 6 months, 6.1-12 months and 9-12 months of age, respectively. As such no cases would be expected in a sample size of 600 participants, a hold on further enrolment into the study will, however, be implemented if there are more than three cases of PCV13 serotype IPD across any of the 1+1 dosing schedules if occurring at least 4 weeks after receipt of the first dose of PCV in that group. The DSMB will determine whether the study should then be discontinued. If the study is discontinued all the children in the 1+1 dosing arms will default to a 2+1 dosing schedule if age appropriate or receive another dose of PCV as required. The DSMB will also be tasked with evaluating the post-booster data from the study, to determine whether the children in any of the 1+1 dosing arm should be offered an additional dose of PCV in the presence of significant inferior immune responses to the PCV schedule

Toxicity Management

It is anticipated that vaccine-associated adverse events (AEs) will occur, but that these will be minor local reactions and side effects. All AEs will be managed appropriately according to the situation.

Toxicities will be classified by the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, dated December 2004, (Clarification dated August 2009), which is available on the web (http://rsc.tech-res.com/safetyandpharmacovigilance).

A clinic visit will be required within 72 hours for vaccine-related (in the opinion of the investigator) adverse reactions Grade ≥3. Other Grade <3 reactions will be managed as per standard of care.

Examples of Adverse Events of Grade ≥ 3 that may be related to the vaccines in the absence of an alternative explanation:
i. Abnormal laboratory values, signs and symptoms or diagnoses.
ii. Local AEs, including pain, tenderness, redness, and swelling post vaccination.
iii. Systemic AEs, including feverishness, irritability, post vaccination.

ALL Grade ≥2 adverse events regardless of association to vaccine will be recorded on the appropriate CRF. Management of the infant will be as per local standard of care.

Participant Management

Screening and study entry may occur on the same day; however, entry may be delayed up to 28 days after screening, if required.

History at screening/ entry should include details of pregnancy, labour and delivery (including gestational age, birth weight, mode of delivery, APGAR scores, pregnancy or L&D complications), history of all immunizations received by infant, and where possible, maternal immunization details, details of infant admissions and illnesses.

Physical exam at screening/entry should include vital signs (temperature, blood pressure, heart rate, and respiratory rate), weight and complete physical exam, including developmental milestones as per local standards. After entry, physical exam should include vital signs, weight, and targeted exam based on current signs and symptoms.

Participant and mother will remain in the clinic for at least 30 minutes after vaccination so that clinic personnel can observe for any potential adverse reactions to the vaccine. Equipment, supplies, and properly skilled medical personnel must be immediately available for emergency use in the event of an unexpected adverse reaction.

Antipyretics should not be routinely given in anticipation of adverse events after vaccination, but should not be withheld if symptoms occur. Antipyretics should be recorded on the memory aid and reported to the site staff. Breastfeeding is permitted and encouraged in this study and the information recorded.

Criteria for Study Discontinuation

The participant's parent/ legal guardian refuses further treatment and/or follow-up evaluations.
The investigator determines that further participation would be detrimental to the participant's health or well-being.
The participant's parent/ guardian fails to comply with the study requirements so as to cause harm to him/ her or seriously interfere with the validity of the study results.

SAE Reporting Requirements for this Study

The SAEs for which expedited reporting (within 24 hours of site awareness thereof) are required for this study will include: infant death and invasive pneumococcal disease.

Grading Severity of Events

The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, dated December 2004, (Clarification dated August 2009), is used and is available on the RSC web site (http://rsc.teches.com/safetyandpharmacovigilance).

Monitoring

Safety and tolerability of the study vaccine will be monitored by means of AEs and toxicity reports presenting laboratory and clinical data.
The data to be reviewed by the protocol team will be pooled across randomisation arms. It is required that these data be entered into the database within 48 hours of the time at which the results of the laboratory tests or clinical examinations become available. These reports will be discussed by the Core Team (comprised of the Study Chair and other co-investigators as well as the data manager and study-statistician) at monthly meetings. Interpretation of vaccine-relationship to AEs will be based on the type of event, the relationship of the event to the time of vaccine administration, the known biology of the vaccine and the investigators' medical judgment. A vaccine-related AE refers to an AE for which there is a possibly, probably or definite relationship to the administration of the vaccine. The investigators determine the relationship to the vaccine using

the following definitions: a) Unable to judge; b) Not related; c) Probably not related; d) Possibly related; e) Probably related; f) Definitely related.

In addition to monthly toxicity reviews by the Core Team, the study will be monitored by the DSMB. DSMB members will be independent of the study and have no perceived conflict of interest. The committee will meet annually either via conference call or face-to-face to review relevant data. The Chair of the DSMB will be mandated to report the DSMB's comments to the Gates foundation and Protocol Team.

Ethics considerations and Participant Information and Consent

The study will be conducted in accordance with the general principles of the International Ethical Guidelines for Biomedical Research Involving Human Participants (Council for International Organizations of Medical Sciences 2002), Guidelines for GCP (ICH 1996), and the Declaration of Helsinki (World Medical Association 2008). The study will be submitted for approval to the University of the Witwatersrand Human Research Ethics Committee and the Medical Control Council (MCC) prior to enrolment of any participant.

The personal identifier data of study participants will not be disclosed in any reports, publication, or other disclosures. Participant randomization numbers will be de- identified from any personal identifiers. The Informed Consent Form will be compliant with ICH GCP and the requirements of the University of the Witwatersrand Human Research Ethics Committee. The parent/ legal guardian of the potential participant will be informed of the study objectives, procedures and possible risks, and be required to consent in writing before any study procedure is undertaken on the child. All consenting will be undertaken by adequately qualified and trained study medical or nursing staff. The informed consent will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the participant's parent/ guardian. Should the consenting parent demise during the course of follow-up of the infant, the other parent of the child if available will be requested to consent for the continued participation of the child in the study. Should the mother have been the consenting parent and the father of the child is unavailable for consenting and not involved in the care of the child, the person taking on the role of primary care-giver to the child will be informed of the study. The continued participation of the infant will depend upon agreement thereof by the new primary care-giver, as indicated by signed consent, and in conjunction with notification thereof on a case-by-case basis to the HREC.

The parent/guardian will be able to withdraw consent at any stage during the course of the study, following which she/he will be referred to the nearest immunisation facility for completion of the child's vaccination.

Participant's Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified only by a coded number to maintain subject confidentiality. All records will be kept in a secured area. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by regulatory authorities or their designates.

Biohazard Containment

As the transmission of blood borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention.

All infectious specimens will be sent using the ISS-1 SAF-T-PAK mandated by the International Air Transport Association Dangerous Goods Regulations-Packing Instruction 602. Refer to individual carrier guidelines (e.g., Federal Express or Airborne) for specific instructions.

Publication of study results

The results of the study will be published in peer-review journals and in accordance with the Bill and Melinda Gates Foundation open-access policy on publications.

Data entry, management and analysis

Data will be collected on study-specific data collection forms, and entered into specially designed databases. Database will be developed in MS Access or SQL, and double data entry will be performed. All data collected from participants will be stripped of any identifiers that reveal the identity of that individual (beyond the use of participant ID).

Data will be managed and analyzed with the assistance of Cleo Hwinya (data manager) and Alane Izu (statistician), based at University of the Witwatersrand/ Respiratory and Meningeal Pathogens Research Unit. Data will be analysed using SAS™, STATA® and R statistical packages.

The following data variables will be collected/ recorded for study participants when available:

- Maternal age: Date of birth
- Infant date of birth, gender, gestational age at delivery, birth weight, mode of delivery, APGAR scores, pregnancy and delivery complications
- Informed consent procedure: Date, language, how many people signed and who, questions asked, Witness
- Randomization procedure details, randomization group
- Vaccine details for all vaccines administered as part of trial and EPI: make, name, manufacturer, lot number, expiry date, route and site of administration.
- Local and systemic adverse reactions, admissions and other illnesses requiring medical care during course of trial.

Protection of human research participants

The major risk to the study participants relate to those included in the 1+1 dosing schedule, whose risk for disease might be slightly increased due to sub-optimal immune responses to the dosing schedule compared to those who receive the 2+1 dosing schedule. The incidence (per 100,000) of PCV13 vaccine-serotype IPD among HIV-unexposed children in 2012 in Soweto, South Africa was 20.6, 0 and 5.6 in age groups <10 weeks, 10weeks to 6 months and 6.1-12 months of age, respectively. As such, no cases would be expected in a sample size of 600 participants[4]. Considering that the circulation of the vaccine-serotype is likely to have decreased even further since 2012, we expect this risk of vaccine-serotype IPD also to have declined since 2012. Recent South African data comparing post-vaccine (2015) and pre-vaccine years (2005-2008), indicates that the overall IPD incidence (per 100,000 population) declined from 9.9 cases prior to PCV immunisation to 4.4 in 2015, including a PCV7-serotype decline of 87%; and PCV13-serotype (additional 6 serotypes) IPD declined of 75%. In tandem, there was a significant increase in non-vaccine-serotype IPD of 18% (95%CI:11% - 24%). Among children <2 years IPD rates declined by 97%, including a 93% reduction in PCV13-serotype IPD. Non-vaccine serotypes increased significantly only in the 45—64 year old (39%; 95%CI:28%, 49%) and >64 years age groups (56%; 95%CI:38%,69%). The predominant non-vaccine serotypes in 2015 were serotypes 12F (82/447, 18%) and 8 (16/99, 16%), neither of which are included in PCV10 or PCV13.

Furthermore, excluded from this study are children born to HIV-infected women, who are at increased risk of pneumococcal disease, even though the incidence of vaccines-serotype IPD in HIV-infected children has declined by >80% since the early 2000s. Also, it is unlikely that vaccinating 600 (2.0%) of the annual birth cohort of 28,000 in Soweto would have any impact on the community wide transmission of the vaccine-serotypes.
To offset the above marginal increased risk of severe vaccine-serotype IPD (incidence <10/100,000 in children <2 years age), the study will offer all study participants a single dose of either varicella or hepatitis A vaccine, neither of which are included in the current public immunisation program. The exact choice of vaccine will be finalized based on its availability in South Africa.
Other risks for participation in the study are minor and relate to the discomfort caused by study procedures such as vecupuncture for obtaining blood samples and nasopharyngeal swabs.

An additional risk to the project, is the Department of Health perception of the project. To mitigate against this, the PI has already started discussion with the Department of Health Deputy-Director General responsible for immunisation (Dr Yogan Pillay), as well as discussed the proposed study

with the National Advisory Group for Immunisation (NAGI), both of whom are supportive of the study.

Possibly community perception of an "inferior" schedule of PCV being provided to some study participants, will be mitigated by engaging with the Community Advisory Board prior to initiation of this study, as well as offsetting any risk by offering the study participants with either Hepatitis A or varicella vaccine, which are not provided in the public immunisation program. Also, the immunogenicity data will be reviewed following completion of the 1-month post PCV booster dose, and if the immune responses in the 1+1 group are shown to be significantly inferior to that of the 2+1 schedule group, the DSMB will be requested to deliberate whether the affected children in the 1+1 dosing arm should receive a further dose of PCV.

Costs to participants

Participants will incur no extra costs based on participation in the study. No additional study-specific visits are required. The parent/caregiver-child pair will be reimbursed with R220 for attending scheduled study visits to cover incidental expenses..

Review board approval:

The protocol and consent forms will be approved by the Human Research Ethics committee (HREC), University of the Witwatersrand, and the Medicines Control Council (MCC) prior to initiation of the study.

Notification of participants of their individual results:

Staff involved in the management of study participants will be notified of relevant results.

Intellectual property:

The data accumulated from this study will be stored at RMPRU. The data will be made publically available, within one year of completion of the study to any investigators or BMGF nominated partners, who wish to use the data to address any specific questions not directly addressed under the study objectives and which the data would lend itself to. The primary manuscript of the study will be submitted for peer-review within 6 months of the last subject, last visit in the study. Any transfer of data, will be governed, by the terms of the local Ethics Committee (HREC). Data sharing will be done on a collaborative basis, with the site PI (or his nominee) being included in any further interrogation of the data. The data will be provided in the format in which it has been entered at RMPRU with the necessary data dictionary.

Disseminating results to the public:

Summary of results will be reported to the HREC on completion of the study. Results may be presented at professional clinical meetings and national or international scientific meetings. Results will be submitted for publication in a peer-reviewed journal, where they will also be available to the public.

Time frames for study conduct

HREC submission date:	7th July 2016
MCC submission date:	1st July 2016
Planned Initiation of enrolment:	1st October 2016

Funding for the study

This study will be funded by a grant from the Bill and Melinda Gates Foundation (Grant number: OPP1152352).

References

1. Loo JD, Conklin L, Fleming-Dutra KE, Deloria Knoll M, Park DE, Kirk J, et al. Systematic review of the effect of pneumococcal conjugate vaccine dosing schedules on prevention of pneumonia. *Pediatr Infect Dis J*. 2014;33 Suppl 2:S140-151.
2. Madhi SA, Groome MJ, Zar HJ, Kapongo CN, Mulligan C, Nzenze S, et al. Effectiveness of pneumococcal conjugate vaccine against presumed bacterial pneumonia hospitalisation in HIV-uninfected South African children: a case-control study. *Thorax*. 2015.
3. Pilishvili T, Lexau C, Farley MM, Hadler J, Harrison LH, Bennett NM, et al. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *J Infect Dis*. 2010;201(1):32-41.
4. von Gottberg A, de Gouveia L, Tempia S, Quan V, Meiring S, von Mollendorf C, et al. Effects of vaccination on invasive pneumococcal disease in South Africa. *N Engl J Med*. 2014;371(20):1889-1899.
5. Loo JD, Conklin L, Fleming-Dutra KE, Knoll MD, Park DE, Kirk J, et al. Systematic review of the indirect effect of pneumococcal conjugate vaccine dosing schedules on pneumococcal disease and colonization. *Pediatr Infect Dis J*. 2014;33 Suppl 2:S161-171.
6. Tregnaghi MW, Saez-Llorens X, Lopez P, Abate H, Smith E, Posleman A, et al. Efficacy of pneumococcal nontypable Haemophilus influenzae protein D conjugate vaccine (PHiD-CV) in young Latin American children: A double-blind randomized controlled trial. *PLoS Med*. 2014;11(6):e1001657.
7. Madhi SA, Cohen C, von Gottberg A. Introduction of pneumococcal conjugate vaccine into the public immunization program in South Africa: translating research into policy. *Vaccine*. 2012;30 Suppl 3:C21-27.
8. Fleming-Dutra KE, Conklin L, Loo JD, Knoll MD, Park DE, Kirk J, et al. Systematic review of the effect of pneumococcal conjugate vaccine dosing schedules on vaccine-type nasopharyngeal carriage. *Pediatr Infect Dis J*. 2014;33 Suppl 2:S152-160.
9. Conklin L, Loo JD, Kirk J, Fleming-Dutra KE, Deloria Knoll M, Park DE, et al. Systematic Review of the Effect of Pneumococcal Conjugate Vaccine Dosing Schedules on Vaccine-type Invasive Pneumococcal Disease Among Young Children. *Pediatr Infect Dis J*. 2014;33 Suppl 2:S109-118.
10. Jones SA, Groome M, Koen A, Van Niekerk N, Sewraj P, Kuwanda L, et al. Immunogenicity of seven-valent pneumococcal conjugate vaccine administered at 6, 14 and 40 weeks of age in South African infants. *PLoS One*. 2013;8(8):e72794.
11. GAVI. http://www.gavi.org/support/nvs/pneumococcal/.
12. Nzenze SA, Shiri T, Nunes MC, Klugman KP, Kahn K, Twine R, et al. Temporal changes in pneumococcal colonization in a rural African community with high HIV prevalence following routine infant pneumococcal immunization. *Pediatr Infect Dis J*. 2013;32(11):1270-1278.
13. Nzenze SA, von Gottberg A, Shiri T, van Niekerk N, de Gouveia L, Violari A, et al. Temporal Changes in Pneumococcal Colonization in HIV-infected and HIV-uninfected Mother-Child Pairs Following Transitioning From 7-valent to 13-valent Pneumococcal Conjugate Vaccine, Soweto, South Africa. *J Infect Dis*. 2015;212(7):1082-1092.
14. Dagan R, Givon-Lavi N, Greenberg D, Fritzell B, Siegrist CA. Nasopharyngeal carriage of Streptococcus pneumoniae shortly before vaccination with a pneumococcal conjugate vaccine causes serotype-specific hyporesponsiveness in early infancy. *J Infect Dis*. 2010;201(10):1570-1579.
15. Madhi SA, Violari A, Klugman KP, Lin G, McIntyre JA, von Gottberg A, et al. Inferior quantitative and qualitative immune responses to pneumococcal conjugate vaccine in infants

with nasopharyngeal colonization by Streptococcus pneumoniae during the primary series of immunization. *Vaccine*. 2011;29(40):6994-7001.
16. Vakevainen M, Soininen A, Lucero M, Nohynek H, Auranen K, Makela PH, et al. Serotype-specific hyporesponsiveness to pneumococcal conjugate vaccine in infants carrying pneumococcus at the time of vaccination. *J Pediatr*. 2010;157(5):778-783 e771.
17. Madhi SA, Izu A, Violari A, Cotton MF, Panchia R, Dobbels E, et al. Immunogenicity following the first and second doses of 7-valent pneumococcal conjugate vaccine in HIV-infected and -uninfected infants. *Vaccine*. 2013;31(5):777-783.
18. Andrews NJ, Waight PA, Burbidge P, Pearce E, Roalfe L, Zancolli M, et al. Serotype-specific effectiveness and correlates of protection for the 13-valent pneumococcal conjugate vaccine: a postlicensure indirect cohort study. *Lancet Infect Dis*. 2014;14(9):839-846.
19. Andrews N, Waight PA, Borrow R, Ladhani S, George RC, Slack MP, et al. Using the indirect cohort design to estimate the effectiveness of the seven valent pneumococcal conjugate vaccine in England and Wales. *PLoS One*. 2011;6(12):e28435.
20. Whitney CG, Pilishvili T, Farley MM, Schaffner W, Craig AS, Lynfield R, et al. Effectiveness of seven-valent pneumococcal conjugate vaccine against invasive pneumococcal disease: a matched case-control study. *Lancet*. 2006;368(9546):1495-1502.
21. Nicol M, Huebner R, Mothupi R, Kayhty H, Mbelle N, Khomo E, et al. Haemophilus influenzae type b conjugate vaccine diluted tenfold in diphtheria-tetanus-whole cell pertussis vaccine: a randomized trial. *Pediatr Infect Dis J*. 2002;21(2):138-141.
22. Hausdorff WP, Van Dyke MK, Van Effelterre T. Serotype replacement after pneumococcal vaccination. *Lancet*. 2012;379(9824):1387-1388; author reply 1388-1389.
23. Hausdorff WP, Hoet B, Schuerman L. Do pneumococcal conjugate vaccines provide any cross-protection against serotype 19A? *BMC Pediatr*. 2010;10:4.
24. Miller E, Andrews NJ, Waight PA, Slack MP, George RC. Effectiveness of the new serotypes in the 13-valent pneumococcal conjugate vaccine. *Vaccine*. 2011;29(49):9127-9131.
25. Moore MR, Link-Gelles R, Schaffner W, Lynfield R, Holtzman C, Harrison LH, et al. Effectiveness of 13-valent pneumococcal conjugate vaccine for prevention of invasive pneumococcal disease in children in the USA: a matched case-control study. *The lancet Respiratory medicine*. 2016;4(5):399-406.
26. Nunes MC, von Gottberg A, de Gouveia L, Cohen C, Moore DP, Klugman KP, et al. The impact of antiretroviral treatment on the burden of invasive pneumococcal disease in South African children: a time series analysis. *AIDS*. 2011;25(4):453-462.
27. Madhi SA, Adrian P, Cotton MF, McIntyre JA, Jean-Philippe P, Meadows S, et al. Effect of HIV infection status and anti-retroviral treatment on quantitative and qualitative antibody responses to pneumococcal conjugate vaccine in infants. *J Infect Dis*. 2010;202(3):355-361.
28. Madhi S, Koen A, Jose L, Cutland C, Adrian P, Violari A, et al. PRIMARY VACCINATION WITH 10-VALENT PNEUMOCOCCAL NON-TYPEABLE HAEMOPHILUS INFLUENZAE PROTEIN D CONJUGATE VACCINE (PHiD-CV) IN HIV-EXPOSED-INFECTED, EXPOSED-UNINFECTED AND UNEXPOSED-UNINFECTED SOUTH AFRICAN INFANTS. *ESPID, 30th Annual Meeting of the European Society for Paediatric Infectious Diseases, Thessaloniki, Greece, May 8–12, 2012*. 2012.
29. Plosker GL. 10-Valent pneumococcal non-typeable haemophilus influenzae protein D-conjugate vaccine: a review in infants and children. *Paediatr Drugs*. 2014;16(5):425-444.
30. von Gottberg A, Cohen C, de Gouveia L, Meiring S, Quan V, Whitelaw A, et al. Epidemiology of invasive pneumococcal disease in the pre-conjugate vaccine era: South Africa, 2003-2008. *Vaccine*. 2013;31(38):4200-4208.

31. Madhi S, Koen A, Jose L, Cutland C, Adrian P, Violari A, et al. Immunisation with 10-valent pneumococcal NTHi protein D conjugate vaccine (PHiD-CV) according to different schedules in HIV unexposed/uninfected South African infants. *ESPID, 30th Annual Meeting of the European Society for Paediatric Infectious Diseases, Thessaloniki, Greece, May 8–12, 2012*. 2012.