Full Title: A Phase IV, Experimental Human Pneumococcal Challenge (EHPC) model to investigate *Streptococcus pneumoniae* Serotype 3 (SPN3) colonisation following PCV15, a Double Blind Randomised Controlled Trial (DBRCT) in healthy participants aged 18 – 50 years in the UK.

Short title: **RATIONALE-15:** ca**R**riage to **A**ssess pro**T**ect**I**on **O**f **N**ew pneumococc**AL** vaccin**E**s- PCV**15**

Internal Reference Number: OVG 2024/02

Ethics Ref: 24/SC/0388

IRAS Project ID: 1010658

Protocol Version and Date: V1.4 18 March 2025

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Sponsor:	University of Oxford
Funder:	Merck Sharp & Dohme (MSD)

Chief Investigator Signature:

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Conflict of Interest

DMF is a member of of UK Dept. Health and Social Care's (DHSC) Joint Committee on Vaccination & Immunisation (JCVI).

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee and Regulatory Authorities unless authorised to do so.

1. Contents

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3. LAY SUMMARY

Streptococcus pneumoniae (pneumococcus) is a bacterium that causes just under four million serious infections every year. It is normal for pneumococcus bacteria to live in the noses of healthy adults and children as part of the nasal microflora without causing harm. This is called “carriage”. But the bacteria can still be passed on to other people. If they are at-risk, for example elderly, or very young, or have pre-existing health conditions, the pneumococcus bacteria can cause pneumonia, which can cause serious life-threatening illness.

Pneumococcus bacteria are surrounded by a sugar capsule. But the capsule does not always have the same components. As a result, the bacteria are classified into more than 100 different types. To make them effective, the vaccines that are currently available contain the sugar capsules of the most common pneumococcal types that cause disease.

One such vaccine - PCV13 - has been effective globally in protecting against pneumococcus disease. It works because it controls the “carriage” (how a person carries the bacteria in their nose) of 13 types of the bacteria. Vaccines giving protection against other types of the bacteria are also becoming available worldwide. PCV15 is similar to PCV13 and protects against two additional types of the bacteria so may offer more protection.

In this study, which lasts 2 months and is funded by Merck Sharp & Dohme (MSD), we want to find out if using PCV15 can protect against “carriage”. To do this, we will use a well-established method that we have already used with more than 2,000 people safely in other research. This involves “challenging” volunteers by putting a small amount of the pneumococcus bacteria into their noses. In this study, before they are challenged, volunteers will either be vaccinated with the real PCV15 vaccine or a dummy (“placebo”). Researchers will then be able to compare the two groups to find out who the vaccine protected and who it did not.

After the study everyone who takes part and fit into certain criteria (see section 11.9) will be given antibiotics to clear the pneumococcus colonisation. They will also be regularly monitored during the study to ensure their safety.

We will ask a very small number of volunteers to have a biopsy to collect tissue samples from inside their nose before and after being vaccinated with PCV15. This will help researchers to understand more about how the immune system responds to the vaccine.

The information we gain in this project will help us understand how exactly this vaccine protects people against pneumococcus. This means we will be able to improve both this vaccine and future pneumococcal vaccines to protect many lives in future around the world.

4. SYNOPSIS

Trial Title	A Phase IV, Experimental Human Pneumococcal Challenge (EHPC) model to investigate Streptococcus pneumonie Serotype 3 (SPN3) colonisation following PCV15, a Double Blind Randomised Controlled Trial (DBRCT) in healthy participants aged 18 – 50 years in the UK
Internal ref. no.	OVG 2024/02
Trial registration	NCT06731374 – ISRCTN91656864
Sponsor	University of Oxford
Funder	Merck Sharp & Dohme
Clinical Phase	Phase IV
Trial Design	Double blind (participant and observer) randomised controlled trial (DBRCT) to investigate the effect of PCV15 vaccination versus placebo on pneumococcal colonisation using the EHPC SPN3 model in healthy adults
Trial Participants	Healthy adults aged 18 – 50 years (inclusive)
Planned Sample Size	Recruitment target up to 106 participants enrolled (84 participants to complete primary endpoint and up to 5 participants for exploratory nasal biopsy cohort)
Follow-up Duration	For main study cohort: 56 days from enrolment. Nasal Biopsy cohort: 70 days from enrolment.
Primary Objective	To compare the rate of acquisition of experimental SPN3 colonisation for 28 days following experimental human pneumococcal challenge (EHPC) at 1 month post PCV15 vaccination compared to placebo defined by classical culture from nasal wash (NW)
Secondary Objective	To determine the density and duration of experimental SPN3 colonisation for 28 days following EHPC at 1-month post PCV15 vaccination by classical culture and molecular methods from NW
	To compare vaccine-induced immune responses to those who receive PCV15 versus control before and after experimental SPN3 challenge
Exploratory Objectives	To compare selected immune parameters both at cellular and humoral level between the nasal mucosa and systemic circulation, including secondary lymphoid tissue
	To characterise the transcriptional changes in immune cells in response to vaccination and experimental challenge
	To describe symptoms following EHPC with SPN3
	To characterise nasal cells gene expression alterations and their visualisation (spatial location) within the nasal tissue induced by PCV15 vaccination.
Inoculum strain and dose	*Streptococcus pneumoniae* SPN3 (Clade Ia, strain LIV014-S3) – Single inoculation at 80,000 colony-forming unit (CFU)/naris

Investigational Products	Pneumococcal Conjugate Vaccine 15-valent, PCV15 (VAXNEUVANCE; Merck, Sharp & Dohme LLC, a subsidiary of Merck & Co, Inc., Rahway, NJ, USA [MSD])
Dose Regimen	1 dose, 0.5mL, given at day 0
Route	Intramuscularly (IM) into the deltoid region of the arm

5. ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
CI	Chief Investigator
CRA	Clinical Research Associate (Monitor)
CRF	Case Report Form
CRO	Contract Research Organisation
CT	Clinical Trials
CTA	Clinical Trials Authorisation
CTRG	Clinical Trials and Research Governance
DBRCT	Double Blind Randomised Controlled Trial
DMC/DMSC	Data Monitoring Committee / Data Monitoring and Safety Committee
DSUR	Development Safety Update Report
EHPC	Experimental Human Pneumococcal Challenge
ENT	Ear Nose and Throat
GCP	Good Clinical Practice
GP	General Practitioner
GTAC	Gene Therapy Advisory Committee
HRA	Health Research Authority
IB	Investigators Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IM	Intramuscular
IMP	Investigational Medicinal Product
IPD	Invasive Pneumococcal Disease

IRB	Independent Review Board
LMICs	Low- and middle-income countries
MHRA	Medicines and Healthcare products Regulatory Agency
MSD	Merck Sharp and Dohme
NHS	National Health Service
OXTREC	Oxford Tropical Research Ethics Committee
PBMC	Peripheral Blood Mononuclear cells
PCV	Polysaccharide conjugated vaccine
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
PPV	Pneumococcal Polysaccharide Vaccine
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RES	Research Ethics Service
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SDV	Source Data Verification
SMPC	Summary of Medicinal Product Characteristics
SOP	Standard Operating Procedure
SPN	*Streptococcus pneumoniae*
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
TMG	Trial Management Group
U&Es	Urea and electrolytes
ULN	Upper Limit of Normal
WHO	World Health Organisation
WOCBP	Woman of Child Bearing Potential

6. BACKGROUND & RATIONALE

6.1. Streptococcus pneumoniae

Streptococcus pneumoniae (SPN, pneumococcus) is a major global cause of morbidity and mortality due to community acquired pneumonia, bacterial meningitis and bacteraemia. The burden of pneumococcal infection is high in low- and middle-income countries with over 1 million fatalities per year[1].

Pneumococcus is classified by serotypes based on capsular components. All pneumococcal serotypes can cause serious disease, but only a few serotypes are responsible for the majority of pneumococcal infections. The clinical spectrum ranges from invasive disease in normally sterile sites (e.g. septicaemia, pneumonia with bacteraemia, septic arthritis, osteomyelitis, mastoiditis, meningitis) to non-invasive infections such as pneumonia without bacteraemia, otitis media and sinusitis[2]. Otitis media is the most common reason for antibiotic prescriptions in young children worldwide, with pneumococcus as one of the main bacterial pathogen causing these infections[3,4].

Colonisation of the human upper respiratory tract by pneumococcus is frequent, with a prevalence of 40%–95% among infants[5], 5%–25% among adults ≥18 years old[6–9] and 0%–10% among adults ≥65 years old[8,9]. Pneumococci adhere to the epithelial cells of the nasopharynx, but in most cases, this does not lead to pneumococcal disease[10,11]. However, pneumococcal nasopharyngeal colonisation is considered a prerequisite for pneumococcal disease and a source of transmission.

6.2. Pneumococcal Vaccines

In the UK, there are currently two types of vaccine formulation licensed and recommended for the prevention of pneumococcal infections: the pneumococcal polysaccharide vaccine (PPV-23) and the pneumococcal conjugate vaccine (PCV13, PCV15). PPV-23 consists of partially purified pneumococcal capsular polysaccharide and is given as a part of the national immunisation schedule to adults ≥ 65 years old and clinical risk groups ≥2 years old[12]. PPV-23 contains polysaccharides from 23 different serotypes (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F). PCV13 (Prevnar-13) and PCV15 (VAXNEUVANCE) are recommended for infants under one year of age as part of the UK's national immunisation programme and for children and adults in certain in risk groups. PCV13 contains purified polysaccharides of the capsular antigens for 13 pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9B, 14, 18C, 19A, 19F, 23F), that are individually conjugated with a non-toxic diphtheria toxin (CRM197, CRM is cross-reactive material).PCV15 contains the same 13 serotypes from PCV13 as well as two additional serotypes, 22F and 33F[13].

High-income and middle-income countries use PPV23 and PCV13 for the elderly and at-risk and immunosuppressed adults[14] with countries varying on using PPV23 alone, PCV13 alone or both in combination[15,16]. Some countries are starting to recommend the newer PCVs for use in adults[17].

Regional UK data showed an invasive pneumococcal disease (IPD) incidence increase between 2014 and 2018, primarily due to non-PCV13 vaccine type (VT) serotypes in all age groups, especially in adults, particularly older adults. SPN 8, 12F and 9N were responsible for 37.5% of IPD cases. However, PCV13 VTs were responsible for 20.1%, mainly due to SPN serotype 3 (SPN3) (45.9% of PCV13-type disease)[17]. For individuals, evidence suggests PCV13 protects against SPN3. A post hoc analysis of RCT data showed a 61.5% (95% CI: 17.6% to 83.4%) reduction in clinical community-acquired pneumonia due to SPN3 in older adults[18], while a meta-analysis showed protection against SPN3 IPD in children[19]. The sole RCT of PCV13 and colonisation in children found no reduction in SPN3 colonisation[20].

In the whole UK population, SPN3 increased modestly following PCV7 introduction in the childhood NIP, declined following the introduction of PCV13 and then increased to above pre-PCV7 levels[21]. Lower population-level protection may result from several hypothetical mechanisms. Limited vaccine impact on SPN3 colonisation is possible. This limitation could result from the specific characteristics of the SPN3 capsule: SPN3 grows as a mucoid colony and sheds its non-covalently bound capsule, seemingly as a defence against antibody-mediated phagocytosis[22,23], which, in theory, can overwhelm the protective capacity of vaccine-induced antibodies. Post-vaccination antibody levels are lower for SPN3 than other PCV13 serotypes in both the serum and lungs[24], and may not substantially reduce colonisation[20]. New PCV vaccines are being formulated to try to increase the efficacy against SPN3 and therefore reduce the burden of disease caused by this serotype.

6.2.1. PCV15 (VAXNEUVANCE) Vaccine and Protection Against SPN3

VAXNEUVANCE (Merck, Sharp & Dohme LLC, a subsidiary of Merck & Co, Inc., Rahway, NJ, USA [MSD]) is a 15-valent PCV containing capsular polysaccharides from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 22F, and 33F adjuvanted with aluminium phosphate. In the UK, PCV15 is licenced for infants under one year of age as part of the UK's national immunisation programme and for children and adults in certain in risk groups[13].

PCV15 has been studied in infants, adults and at-risk groups in several clinical trials showing its safety and efficacy against the serotypes it contains[17,25–36]. Results from a Phase 3 trial of vaccinated adults ≥50 years of age have shown PCV15 met non-inferiority criteria compared with PCV13 for the 13 shared serotypes (based on a using a 2-fold non-inferiority margin for the ratio of OPA geometric mean titers [GMTs] [PCV15/PCV13] post vaccination). In addition, PCV15 met superiority criteria compared to PCV13 for SPN3 (based on a super-superiority margin of 1.2 for the ratio of the OPA GMTs [PCV15/PCV13] and a superiority margin of 0 for the difference in proportions of participants with ≥4-fold rise)[17].

Studies in infants have been conducted to test the safety and immunogenicity of different vaccine schedules. In the 2+1 studies, PCV15 met noninferiority criteria for each of the 13 shared serotypes and superiority criteria for serotypes 22F and 33F, based on IgG response rates and IgG geometric mean concentration (GMC) ratios at 30 days post-booster as compared with PCV13[26,29]. In the 3 + 1 study, noninferiority (for all 15 serotypes) and superiority (for SPN3, 22F, and 33F) assessments were made at post dose 3 and post dose 4[25].

These data shows that PCV15 broadens PCV coverage without significant loss of immunogenicity and has improved immunogenicity against SPN3 in both adults and infants.

6.3. Experimental Human Pneumococcal Challenge (EHPC) model

Controlled human infection models (CHIMs) have been vital in vaccine development and exploring host-pathogen interactions, a notable example being the licensure of the first malaria vaccine[37]. CHIMs typically recruit young, healthy participants with a low risk of severe outcomes. Our experimental human pneumococcal challenge (EHPC) model allows us to test vaccine efficacy (VE) against experimental colonisation in a timely and cost-effective manner, requiring fewer participants than phase III clinical trials[38–40]. It enables the assessment of immunological responses to nasopharyngeal colonisation and vaccine-induced immune responses which would be protective against SPN carriage. Participants are given predefined SPN doses by nasal administration, with some becoming colonised at a duration and density typical of natural colonisation episodes. Samples

including nasal washes (NW), nasal cells and blood are taken to assess colonisation and anti-pneumococcal immune responses.

A great advantage of the EHPC model is that the onset and duration of a colonisation episode are known, allowing for precise measurement of colonisation density over time. Over 2000 participants have been challenged with several serotypes across different cohorts (age, at-risk groups) and at various doses (10,000–320,000 colony forming units (CFU)/100 µL), showing that serotypes have different colonisation rates. Participants are carefully selected to reduce the risk of them developing pneumococcal disease.

In order to better understand natural and vaccine immunity to SPN3 we developed a SPN3 challenge model in healthy adults 18-50 years of age. Different strains of SPN3 at different doses were tested. This model was shown to be safe and we obtained colonisation rates comparable to the established SPN6B challenge model[41]. These strains were used in a phase IV double-blind randomised controlled trial to investigate the effect PCV13 and PPV23 on pneumococcal colonisation using SPN3 EHPC compared with placebo[42]. We have recently developed an additional SPN3 EHPC model in healthy adults using 2 different SPN3 strains isolated from the UK (LIV014-S3) and Malawi (10V). This study aimed to assess whether sequential inoculation increases colonisation rates and how this double exposure influences immunity pre-disposing acquisition and impacts on transmission[43]. Results obtained after 43 participants inoculation with strain LIV014-S3 at 80,000 CFU/naris, showed that a single inoculation provides 72% attack rate which increases to an overall experimental carriage of 86% after booster inoculation. In addition, strain 10V at the same dose, showed a 54% after first inoculation to and overall of 72% after second inoculation. The model for both strains is safe and suitable for vaccine evaluation studies[44].

The EHPC model has been used to study vaccine efficacy against carriage. A previous study assessing the impact of PCV13 on colonisation in a double blind-randomised controlled trial (DBRCT) showed that PCV13 provided 78% protection against experimental colonisation with SPN6B pneumococcus versus placebo when detected by classical microbiology[45]. Protection correlated with the ability of the participants' nasal washes to agglutinate the bacteria[46]. Reanalysis of our EHPC PCV-13 trial samples using molecular methods (qPCR targeting lytA and 6ABcps genes) showed that PCV13 conferred 29% protection against acquisition of 6B pneumococcus. qPCR identified subjects vaccinated with PCV13 who had low density colonisation which was undetectable by classical microbiological methods. Very importantly, PCV13 resulted in significant reduction (by qPCR 3-4 logs of CFU) in colonisation density in participants who became colonised, suggesting that the major effect of PCVs on colonisation is to reduce density rather than to completely prevent acquisition[47].

In this clinical study we seek to assess the direct effect of PCV15 on experimental colonisation with SPN3 (LIV014-S3 strain) 1 month after vaccination, as well as the vaccine-induced serotype-dependent immune responses (antibody levels and function and B cell responses) that correlate with protection against colonisation

6.4. Potential Risks to Participants

Pneumococcus is responsible for infections including otitis media (OM), sinusitis, pneumonia, bacteraemia and meningitis. The milder forms of infection (OM, sinusitis) are many times more common than the serious invasive forms of the disease. Due to inoculating participants with pneumococcus, there is a very low risk of OM, sinusitis, pneumonia, bacteraemia and meningitis[48]. While the risk to individuals of developing any infection is very low (10% adults experience natural

colonisation at any time[7]) and the incidence of invasive disease is 20/100,000 patient years[49]), the study is designed to ensure that any risk of invasive disease is minimal (strict inclusion/exclusion criteria, strict safety follow-up, pneumococcal strain that is sensitive to antibiotics and healthy young adult study cohort).

This study can be safely run based on the following experience and provisions:

- The research team has 14 years of experience in human challenge studies, following very similar protocols and facing similar risks as previous studies.
- The selected pneumococcal serotype (SPN3) is fully antibiotic sensitive and has been safely tested in 60 healthy participants without safety concerns.
- Participant selection and exclusion criteria reduce the excess risk of invasive pneumococcal disease associated with comorbid conditions.
- Participant education regarding the risks of study participation, provision of a safety information leaflet, and close interaction with the study staff.
- Rigorous and frequent monitoring of development of symptoms and body temperature.
- Provision of standby antibiotics to reduce time to treatment, if it is required.
- 24-hour emergency telephone contact with researchers (including individual daily monitoring for the first 7 days following inoculation), to facilitate access to hospital and/or prompt treatment if required.

Within the safety information sheet, participants will be warned to look out for specific symptoms relating to infections caused by Streptococcus pneumoniae. That includes: fever (>37.5 ℃), shivering, headache, new rash, drowsiness, cough, shortness of breath, sore throat, earache or symptoms of an eye infection.

We now have experience of inoculating and following over 2000 participants using several serotypes, in different adult cohorts and at a range of doses with participants being experimentally colonised, naturally colonised and not colonised during our studies. We have had two separate SAEs reported during these challenge studies, both classified as unrelated to the study protocols and study conduct.

In the event that symptoms occur during the inoculation follow up period, participants will contact the team on the day that the symptoms are noted. The clinical team will assess if the participant's symptoms are potentially consistent with pneumococcus disease (for example, ear pain, sore throat, cough or fever) in which case the participant will be seen in person by the clinical study team for medical assessment to determine if antibiotics should be commenced, irrespective of the colonisation status at that point. Participants may be reviewed in the study clinic or asked to attend an NHS healthcare treatment facility directly at investigator discretion.

6.5. Specific Risks Related to PCV15

The most commonly reported solicited adverse reactions in adults 18 through 49 years of age described in the PCV15 Summary of Product Characteristics (SmPC) were: injection-site pain (75.8%), fatigue (34.3%), myalgia (28.8%), headache (26.5%), injection-site swelling (21.7%), injection-site erythema (15.1%) and arthralgia (12.7%). The majority of solicited adverse reactions were mild (based on intensity or size) and of short duration (≤3 days); severe reactions (defined as an event that prevents normal daily activity or size of injection site reaction >10 cm) occurred in ≤1.5% of adults across the clinical program.

Across all clinical trials investigating PCV15, among participants 18 years of age and older who received PCV15 serious adverse events within 30 days postvaccination were reported by 0.4% of recipients. In a subset of these studies, among those who received PCV15, serious adverse events within 6 months postvaccination were reported by 2.5% of recipients. There are no risks of developing pneumococcal pneumonia attributable to a serotype(s) in PCV15 (i.e. given the modification of the infective organism and according susceptibility of that organism to antibiotics). However, these modifications on the vaccine does not preclude of incidental vaccine type pneumococcal disease post vaccination.

6.6. Other Trial-related Risks

The majority of sampling methods utilised are not invasive and have no associated risks. The following have potential for mild, self-limiting risks:

- Venepuncture may cause slight pain, bruising, light-headedness or fainting. The amount of blood collected during the study will be within the NHS Blood and Transplant guidelines for blood donation.
- Intramuscular injections carry a risk of bleeding in patients with very low platelet counts or coagulopathies. A baseline full blood count (with a platelet count) taken prior to vaccination will allow exclusion of participants with this risk. Intramuscular injections may cause pain, swelling and tenderness at the injection site but this is not long lasting.
- Collection of nasal cells taken during the trial may cause discomfort, eye watering or minor local bleeding.
- Collection of throat swabs may cause slight discomfort and may elicit a gag reflex.
- Collection of nasal Wash: participants may swallow saline which may taste salty.
- Collection of nasopharyngeal/nasal swab: this may cause some discomfort, eye watering or a minor local bleeding.
- Inferior turbinate/post-nasal biopsy (For a subset only): Participants may have mild nasal discomfort or rhinorrhea following the procedure, which typically settles within 48 hours. Rarely, subjects undergoing nasal biopsy may have an adverse reaction to the local anaesthetic used. The risk of local bleeding which may require further outpatient ENT intervention such as nasal packing or cautery is <1%. Rarely, subjects may need to be admitted for observation to an ENT acute ward for management of bleeding. Surgical intervention to manage epistaxis in the operating theatre following this procedure is extremely rare. All participants who undergo a nasal biopsy will receive a safety telephone review at 24 hours post-procedure and will have 24/7 access to a study doctor for up to 3 weeks post procedure.

The medical tests carried out during the trial screening and follow up have the potential to find incidental medical problems that may require referral of participants for further investigation. Participants will be informed of these, and, with their consent, their general practitioner will be contacted.

6.7. Potential Benefits to Participants

The participants will benefit from receiving PCV15 vaccine which protects against 15 pneumococcus serotypes. However, the risk of pneumococcal disease in the study cohort is minimal. At the point of unblinding, PCV15 will be offered for participants in the placebo arm that wish to take the vaccine. No

specific additional medical care will be provided through participation, and medical procedures are performed with the aim of determining eligibility and safety during the trial.

7. OBJECTIVES AND ENDPOINTS

7.1. Primary Objective

To compare the rate of acquisition of experimental SPN3 colonisation for 28 days following experimental human pneumococcal challenge (EHPC) at 1 month post PCV15 vaccination compared with placebo defined by classical culture from nasal wash (NW).

7.2. Primary Outcome Measures

The rate of experimental SPN3 colonisation determined by the presence of experimental SPN3 in NW by classical culture at any evaluated timepoint (D2, 7, 14, 28) following EHPC at 1 month after vaccination in PCV15 versus control.

7.3. Secondary Objectives

a) To determine the density of experimental SPN3 colonisation for 28 days following EHPC at 1-month post PCV15 vaccination by classical culture and molecular methods from NW.
b) To determine the duration of experimental SPN3 colonisation for 28 days following EHPC at 1-month post PCV15 vaccination by classical culture and molecular methods from NW.
c) To compare vaccine-induced immune responses to those who receive PCV15 versus control before and after experimental SPN3 challenge.

7.4. Secondary Outcome Measure

a) The density of experimental SPN3 colonisation in NW at each and any timepoint (D2, 7, 14, 28) by classical culture and molecular methods following EHPC at 1-month after vaccination in PCV15 vs control.
b) The duration of experimental SPN3 colonisation following EHPC at 1-month after vaccination between the first and the last NW in which SPN3 is detected by classical culture and/or molecular methods in PCV15 and control.
c) Assessment of immune responses including but not limited to: mucosal and systemic antibody levels, antibody functionality and cellular populations levels before and after vaccination and EHPC.

7.5. Exploratory Objectives

a) To compare selected immune parameters both at cellular and humoral level between the nasal mucosa, secondary lymphoid tissue and systemic circulation.
b) To characterise the transcriptional changes in immune cells in response to vaccination and experimental challenge.
c) To describe symptoms following EHPC with SPN3.
d) To characterise nasal cells gene expression alterations and their visualization (spatial location) within the nasal tissue induced by PCV15 vaccination.

7.6. Exploratory Outcome Measures

Exploratory analysis will be detailed in the Laboratory Analysis Plan. Sample analysis for the completion of exploratory endpoints, with appropriate consent may be performed under the ethically approved Oxford Vaccine Centre (OVC) Biobank protocol (REC 21/SC/0161).

a) Analysis of antibody level, function, inflammatory markers and cell populations (cellular and transcriptome level) in nasal mucosa, secondary lymphoid tisssue and systemic circulation and compare data with those generated at the nasal mucosa.
b) Determination of gene induction and regulation to identify patterns in individuals who become experimentally colonised versus those who remain protected.
c) The presence of mild or moderate symptoms as recorded on a Likert scale in participants with SPN3 within the first 7 days after EHPC.
d) Analysis of the spatial microenvironment and transcriptomics of the nasal tissue before and 28 days after PCV15 vaccination.

8. TRIAL DESIGN

This is a Phase IV Double Blind (participant and observer) Placebo Controlled Randomised Controlled Trial (DBRCT) that will assess the superiority of PCV15 against placebo in healthy adults 18-50 years old exposed to an Experimental Human Pneumococcal Challenge (EHPC). Participants will be randomised 1:1 to receive PCV15 or placebo. We estimate a colonisation rate of 60% for the placebo group (84 participants with available endpoints, or up to 106 participants enrolled after adjusting for 20% attrition).

One month following randomisation and vaccination with PCV15 or placebo, all participants will be intranasally inoculated with *Streptococcus pneumoniae* serotype 3 (SPN3). Participants will be inoculated with a pure culture of a well-characterised, fully sequenced amoxicillin-sensitive pneumococcal serotype 3 (Clade Ia, strain LIV014-S3). Follow-up for 28 days will occur in the clinic with assessment of laboratory measures of the acquisition of nasal pneumococcal colonisation and of immune response after which participants will be required to take a 5-day course of antibiotics. Participants will be considered enrolled into the trial at vaccination.

Exploratory Nasal Biopsy cohort: From the up to 106 participants enrolled, 5 participants (not included in the primary endpoint sample size) will be asked to consent for a nasal biopsy procedure during screening visit and a second nasal biopsy 28 days after PCV15 vaccination. This cohort will not be blinded as only PCV15 will be provided. These participants will not be inoculated and the study will terminate 21 days after second biopsy visit. Participants will be considered enrolled after first nasal biopsy procedure.

Study visit procedures and sampling can be found in section 10.

The study is sponsored by the University of Oxford with two sites: Oxford (Centre for Clinical Vaccinology and Tropical Medicine) and Liverpool (Liverpool School of Tropical Medicine). The Experimental Human Pneumococcal Challenge model is well established on both sites.

9. PARTICIPANT IDENTIFICATION

9.1. Trial Participants

Healthy participants 18-50 years of age.

9.2. Inclusion Criteria

Participants must satisfy all the following criteria to be eligible for the study:

1. Adults between 18 to 50 years old (inclusive) at the time of enrolment.
2. Medically healthy, such that according to investigator judgement, hospitalisation within the study period is not anticipated, and the participant appears likely to be able to remain a study participant through to the end of protocol-specified follow-up. Planned elective procedures for pre-existing conditions may be allowable.
3. Fluent spoken English – to ensure a comprehensive understanding of the research project and their proposed involvement.
4. Able to attend the scheduled visits and to comply with all study procedures, including internet access for the recording of electronic diary after inoculation.
5. Willing and able to give informed consent for participation in the study.
6. Willing to allow confirmation of past medical history either through provision of, or access to, a medical record summary or other medical documentation or allowing investigators to obtain a copy of their medical history from their GP practice or accessed via electronic patient records.
7. Willing to allow their GP and/or consultant, if appropriate, to be notified of participation in the study.
8. Willing to provide their national insurance number or passport number to be registered on The Over-Volunteering Prevention System (TOPS).
9. *For participants of childbearing potential only*: willing to use effective contraception for the duration of the study AND to have a pregnancy test on the day of screening and challenge.

9.3. Exclusion Criteria

Participants may not enter the study if any of the following apply:

1. **Research Participants**:
    a. Participation in another research study, in which procedures performed could compromise the integrity of this study (such as significant volumes of blood taken), or are planning to do so within the trial period
    b. Currently a participant in a previous EHPC trial within the last 2 years
2. **Vaccination** (self-reported or confirmed from GP questionnaire or medical records/summary if deemed necessary at clinician discretion):
    a. Have had any previous pneumococcal vaccination in the past 5 years (including in a research study)
    b. Planned vaccination during the study
3. **Allergy:**
    a. Have an allergy to penicillin or amoxicillin (for main study cohort only)

   b. History of a bleeding disorder (e.g., Factor deficiency, coagulopathy, or platelet disorder), or prior history of significant bleeding or bruising following IM injections or venepuncture
   c. Have previous anaphylaxis or severe adverse reaction to any component/excipient of the vaccine or **to any** vaccine
   d. Allergy to Lidocaine local anaesthetic (for nasal biopsy cohort only)
4. **Health History** (self-reported or confirmed from GP questionnaire or medical records/summary if deemed necessary): moderate ill health including but not limited to:
   a. Asplenia or dysfunction of the spleen
   b. Chronic respiratory disease (e.g. asthma [on medication], COPD, emphysema, bronchiectasis)
   c. Chronic heart disease (e.g. angina, ischaemic heart disease, chronic heart failure) [controlled stable hypertension +/- angina may be included].
   d. Chronic kidney disease (e.g. nephrotic syndrome, kidney transplant, on dialysis)
   e. Chronic liver disease (e.g. cirrhosis, biliary atresia, hepatitis)
   f. Chronic neurological conditions
   g. Connective tissue disease
   h. Dementia
   i. Diabetes mellitus (including diet controlled)
   j. Immunosuppression or history of receiving immunosuppressive therapy – at the discretion of the investigator
   k. Individuals with cochlear implants
   l. Individuals with major cerebrospinal fluid leaks (e.g. following trauma, major skull surgery, or requiring CSF shunt)
   m. Recurrent otitis media.
   n. History of significant unexplained bleeding after a surgical or dental procedure (for nasal biopsy participants only)
   o. Have any **uncontrolled** medical/surgical/mental health conditions at the discretion of the study doctor.
   p. Major **pneumococcal illness** requiring hospitalisation within the last 10 years.
   q. Significant mental health condition (e.g previous admissions in a psychiatric unit, at the discretion of the clinician) that would impair the participant's ability to participate in the study
5. Taking **Medications**:
   a. Any medication that may affect the immune system in the last 3 months (e.g. systemic steroids [IM/IV], Roaccutane, disease modifying anti-rheumatoid drugs)
   b. Long-term use of antibiotics (see also section of Temporary Exclusion Criteria)
   c. Use of any medication or other product (prescription or over-the-counter) for symptoms of rhinitis or nasal congestion within the last 1 month
   d. Use of any medication affecting blood clotting (any oral/injectable anticoagulants)
6. **Female participants** who are pregnant, lactating or intending on becoming pregnant during the study
7. **Direct caring role or close contact with individuals at higher risk of infection** (for main study cohort only):
   a. Children under 5 years of age
   b. Chronic ill health or immunosuppressed adults

c. Older adults

8. **Smoker:**
    a. Current or ex-smoker (regular cigarettes/cigars/e-cigarette/vaping/smoking of recreational drugs) in the last 6 months
    b. Previous significant smoking history (more than 20 cigarettes per day for 20 years or the equivalent [>20 pack years])
9. **Suspected or known current alcohol or drug abuse, as per investigators discretion**
10. **Overseas travel** during the follow-up period (from the time point of inoculation to antibiotic treatment or completion of the 28 day follow up period post inoculation)
11. **Any other issue which**, in the opinion of the study staff, may:
    a. Put the participant or their contacts at risk because of participation in the study
    b. Adversely affect the interpretation of the study results, or
    c. Impair the participant's ability to participate in the study
12. Study site staff or a partner or dependent child of study site staff

9.4. Temporary Exclusion Criteria

Occurrence of any illness, incidental finding, adverse event or laboratory adverse event, which in the opinion of the Investigator, requires further time and/or investigation to resolve or stabilise prior to a dose of vaccine or challenge being administered

The following are temporary exclusion criteria to **inoculation**:

1. Current illness and/or acute illness within 14 days of inoculation
2. Positive COVID-19 swab whether symptomatic or asymptomatic within 14 days of inoculation
3. Antibiotic use within 28 days of inoculation
4. Vaccination with an approved COVID-19 vaccine in the 14 days preceding inoculation, if participant is recommended to take the vaccine by their GP or due to unforeseen circumstances.

The following are temporary exclusion criteria to **vaccination**.

1. Vaccination with an inactivated vaccine/mRNA in the 14 days or with a live vaccine in the 28 days preceding study vaccination

The following are temporary exclusion criteria to **nasal biopsy**:

1. Antibiotic use (during the study): delay nasal biopsy for at least 1 week from last date of therapy
5. Dental infections: delay nasal biopsy for at least 2 weeks after last day of illness

Potential participants who are temporarily excluded at screening or prior to vaccination may be re-screened at a later date to assess inclusion into the study. There is no limit to re-screen a potential participant, however, participants would be re-consented if the time since initial written informed consent was greater than 3 months. Participants who meet any of the temporary exclusion criteria to inoculation may have their inoculation delayed until resolution of the temporary exclusion criteria or, if this is not possible, will not analysed as per protocol. If a participant unexpectedly requires a vaccination during the study period, they will remain in the study and will be considered as a part of an intention-to-treat subgroup for the purposes of analysis.

10. TRIAL PROCEDURES

10.1. Schedule of Procedures Table: Screening Visit (all participants)

Table 1 Schedule of Screening Procedures

Visit Number	S
Visit type	Screening
Timeline[1]	-90/-1 or -90/-21 days before D0[7]
Visit Procedures	
Informed consent[2]	X
Consent quiz	X
Review inclusion and exclusion criteria	X
Record demographic data	X
Concomitant medication and medical history[3]	X
Vital signs (heart rate, temperature, blood pressure) and height and weight	X
Screening physical examination	X
TOPS initial check and registration *www.tops.org.uk* [4]	X
Record emergency contact information	X
Urine Samples	
Pregnancy test (POCBP only)[5] point of care test	X
Blood Samples[6]	
HBsAg, HCV Ab, HIV serology	X
Haematology and Biochemistry (FBC, LFTs, U+Es)	X
Nasal Biopsy Cohort only	
Coagulation screen	X

[1]Additional unscheduled screening visits may occur (for example: to repeat a blood test)
[2]Consent discussion and obtaining formal written consent and screening visit procedures may occur over two separate visits
[3]Medical history provided by online screening questionnaire will be reviewed with participant
[4] TOPS: The Over-volunteering Prevention System
[5]Alternatively hCG may be added to the blood sample for pregnancy testing
[6]Additional repeat blood draws may be required (for example: if there is a problem with the sample or abnormality in the results)
[7]Timeline of -90/-1 days refers to main study cohort and -90/-21 days to the nasal biopsy cohort
POCBP: participants of childbearing potential

10.2. Schedule of Procedures Table: Nasal Biopsy, Vaccination, Challenge and Follow up Visits

Table 2 Schedule of Procedures main study

Visit Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**PCV15 vaccination for placebo[6]**	**Unsched[1]**
Days relative to vaccination	**D0**	**D7**	**D23**	**D28[i]**	**D30**	**D35**	**D42**	**D56**	After unblinding	**Unsched[1]**
Time window (days)		±2	-7/+1	-2/+14	±1	±2	±5	±5		
Visit Procedures										
Review eligibility	X			X						
Vaccine randomisation	X									
Vaccination	X								X	
SPN3 Inoculation				X						
Vital signs	X	X	X	X	X	X	X	X	X[2]	X
Targeted medical history[2]	X	X	X	X	X	X	X	X		X
Targeted physical exam[2]	X	X	X	X	X	X	X	X	X	X
Adverse Event Collection										
SAEs/SARs collection and review	X	X	X	X	X	X	X	X		X
AEs/AESIs collection and review				X	X	X	X	X		X
Electronic diary (eDiary)										
eDiary started				X[7]						
Sample Collection[3]										
Pregnancy test[4]	X			X					X	
Biochemistry, Haematology [LFTs, U+Es, FBC]			X							X[2]
Nasal Wash[5]	X		X		X	X	X	X		X
Throat swab[5]	X		X		X	X	X	X		X
Nasal cells[5*]	X	X	X		X	X		X		
Research bloods[5]	X			X				X		
Respiratory bacterial +/- viral swabs[5]	X		X							X[2]

[1] As required for example to repeat a blood test or for clinical review. Samples and assessments if clinically indicated [2] Optional, if indicated [3] Additional samples may be taken, to repeat a planned sample, upon review of data or for safety reasons [4] WOCBP only, point of care test. This procedure may be performed at any time in the study at the discretion of the study team e.g. if clinically indicated. [5]Samples to be taken before vaccination *Minimum of 40 study participants (20 at each site). [6]For participants in the placebo arm that wish to take PCV15. It will occur after unblinding, either within 3 months or before the expiry date of vaccine stock, whichever is sooner [i]Inoculation [7]E-Diary will be completed by participants daily from D28 to D35 and will be reviewed daily by the clinical team [i]Inoculation. Blood volumes: Some tests tube types and tube volumes may vary slightly between sites for safety and for research bloods, according to local specifications. Total blood volume taken will not exceed that of two blood donations (the limit in a year is three donations for women and four for men, according to the Guidelines for the Blood Transfusion Services in the UK, 8th edition).

Table 3 Schedule of Procedures for nasal biopsy cohort

Visit Number	**Biopsy 1**	**Phone 1**	**1**	**2**	**3**	**Biopsy 2**	**Phone 2**	**Phone 3**	**Unsched[1]**
Days relative to vaccination	**D-21**	**1 day post biopsy**	**D0**	**D7**	**D23**	**D28**	**1 day post biopsy**	**21 days post biopsy**	**Unsched[1]**
Time window (days)	-90/-21	1-3 post biopsy		±2	-7/+1	-2/+14	1-3 post biopsy	14-28 post biopsy	
Visit Procedures									
Review eligibility	X		X			X			
Vaccination			X						
Vital signs	X		X	X	X	X			X
Targeted medical history[2]	X		X	X	X	X			X
Targeted physical exam[2]	X		X	X	X	X			X
Phone safety review		X					X	X	X
Adverse Event Collection									
SAEs/SARs collection and review	X	X	X	X	X	X	X	X	X
AEs collection and review	X	X	X			X	X	X	X
Sample Collection[3]									
Pregnancy test[4]	X		X			X			
Nasal Wash[5]			X		X				X
Throat swab[5]			X		X				X
Nasal cells[5]			X	X	X				
Research bloods[5]	X		X	X		X			
Safety bloods[6]									X[2]

Respiratory bacterial +/- viral swabs[5]			X						X
Nasal Biopsy	X					X			

[1] As required for example to repeat a blood test or for clinical review. Samples and assessments if clinically indicated [2] Optional, if indicated [3] Additional samples may be taken, to repeat a planned sample, upon review of data or for safety reasons [4] POCBP only, point of care test. This procedure may be performed at any time in the study at the discretion of the study team e.g. if clinically indicated. [5]Samples to be taken before vaccination Procedures (biopsy and other visit procedures) can occur over two different days within visit window [6]Safety bloods are not required for the exploratory nasal biopsy cohort as participants will not be inoculated. Blood volumes: Some tests tube types and tube volumes may vary slightly between sites for safety and for research bloods, according to local specifications. Total blood volume taken will not exceed that of two blood donations (the limit in a year is three donations for women and four for men, according to the Guidelines for the Blood Transfusion Services in the UK, 8th edition).

10.3. Recruitment

Advertisements for recruitment will be distributed through methods including but not limited to posters, leaflets, websites, newspapers, radio, public engagement events, and/or social media, using advertising material containing wording from approved study documents to invite participation in the study. Potential participants may be contacted by methods including but not limited to email, telephone, and/or mail, using an approved invitation letter.

The details of other recruitment methods which may be used are outlined below:

- **Email campaign**: We may contact representatives of local tertiary education establishments and local employers and ask them to circulate approved posters and a link to the study website by email or hard copy.
- **Volunteers' databases**: Direct email and link may be sent to members of the public who have registered their interest in potentially volunteering for clinical trials. These are secure databases where members of the public registered here have given consent to have their details recorded and be contacted expressly for the purpose of being notified when a trial opens for recruitment. They understand this is not a commitment to participating for any trial they are contacted about.
- **Media advertising**: Approved local media, newspaper and website advertisements may be placed in locations relevant for the target age group with brief details of the study and contact details for further information.
- **Website advertising**: Description of the study and copy of the information booklet may be placed on study websites and other appropriate platforms for vaccine trial advertising.
- **Social media**: Approved advertisements may be placed on trial social media accounts or targeted social media platform advertisements including, but not restricted to, Twitter, Facebook and Instagram.
- **Exhibitions**: Advertising material and/or persons providing information relating to the study may exhibit using stalls or stands at exhibitions and/or fairs, such as University Fresher's Fairs.
- **Royal Mail Leaflet**: Royal Mail door-to-door service with delivery of invitation letters enclosed in envelopes may be sent to every household within certain postcode areas.

10.4. Online Screening Questionnaire

Information about the study will direct participants to the study website, where a full participant information sheet will be available. Participants will also have access to the study team contact details to communicate with the team directly. Participants who are willing to proceed will be asked to complete an initial online 3-part questionnaire.

Part 1: No identifiable information (such as name/address) will be collected at this stage. Will include major inclusion and exclusion criteria. If a volunteer is deemed ineligible based on any of the replies, they be informed, the questionnaire will stop, and no demographic information will be recorded. Answers will be stored on a secure University of Oxford server and will be deleted at the end of recruitment period for all participants who do not proceed to participate in the study. Those eligible will be directed to e-consent in part 2.

Part 2: E-consent for access to and storage of medical history and vaccination records (via the volunteer GP or NHS databases) and recording and storage of personal information. Completion of the e-consent directs the volunteer to part 3.

Part 3: Records demographic information, NHS number, medical history, and medication use.

Participants that remain eligible will be invited for a full screening and consent visit, where their full eligibility will be assessed by a member of the clinical research team. Clarification of history provided can be discussed with the volunteer by telephone, prior to the face-to-face screening visit.

Where potential participants are not able or willing to complete the online screening and e-consent for storing and accessing medical records, they can be invited to attend a face-to-face screening.

10.5. Informed Consent

Written and verbal versions of the Participant Information and Informed Consent will be presented to the participants detailing no less than: the exact nature of the trial; what it will involve for the participant; 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 trial at any time for any reason without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal.

Participants will be asked to also consider the exploratory nasal biopsy cohort. Participants will be provided with a separate participant information sheet and will undertake a separate written research consent from the main study consent as procedures and study duration will be different. Before the nasal biopsy, the ENT surgeon performing the procedure will consent the participants for the biopsy procedure using a NHS consent form.

Videos, audio, and animations may be used to make the Participant Information and Informed Consent more accessible and the scripts for these will be submitted for approval.

A consent quiz may be used, to test the participants understanding of the study having read or viewed the Participant Information. Where answers are in-correct, the person obtaining the consent will focus the consent discussions on these areas until the participant demonstrates understanding.

The participant will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their GP, or other independent parties to decide whether they will participate in the trial. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent. Doctors and nurses who obtain the consent must be suitably qualified and experienced and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the participant. The original signed form will be retained at the trial site.

10.6. Screening and Eligibility Assessment

Once written informed consent has been received, the following baseline assessments as outlined in the schedule of procedures table (section 10.1) will be performed and recorded as part of the assessment of inclusion/exclusion criteria:

- Participant demographics: e.g., age, sex, and ethnicity
- Medical history
- Contraception: POCBP are asked if they are willing to use effective contraceptive measures for the duration of the study (section 12.11)

- Use of concomitant medication and vaccinations (including over the counter medications, vitamins, illicit drug use and herbal supplements)
- Recording of resting pulse, blood pressure, oral temperature, weight, and height
- Physical examination including (but not limited to) cardiovascular, respiratory, abdominal, and gross neurological examination
- Urine pregnancy test (POCBP only, section 10.21)
- Blood samples (see table 1)
- Next of kin contact details

Medical history, including vaccination and prescribed medication may be collected by participant recall, summaries and verified from the GP records or accessed via the electronic patient record. Participants' GPs will be notified of an individual's participation in the study. A participant identity check will be performed according to local policy.

The maximum duration allowed between screening and vaccination is 90 days. To avoid unnecessary additional procedures, if the appropriate screening information (including investigation results) are available for a volunteer from a screening visit of another study, these results may be used to assess eligibility, provided screening assessment occurred within 90 days.

Table 4 Baseline assessment and STOP criteria

Clinical history and examination	STOP if unexplained or concerning findings on history or examination
Engagement with research team	STOP if the research team have concerns about participant's ability to commit to frequent communication and safety checks
Illness during study	STOP if participant develops a medical condition or commences medication while on the study that would meet the exclusion criteria
Full blood count	STOP if Hb <10g/L STOP if total WCC <1.5x10^9/L STOP if total WCC >12x10^9/L STOP if platelets <75x10^9/L
Resting SpO_2	STOP if <94%

10.7. Definition of Enrolment

Enrolment will occur at vaccination. For the exploratory nasal biopsy procedure, enrolment will occur during initial biopsy visit. GPs will be notified at the time of enrolment that the subject is taking part in the study.

10.8. Randomisation

Randomisation and vaccination will occur at the Centre for Clinical Vaccinology and Tropical Medicine (Oxford) or the Liverpool Life Sciences Accelerator Building (Liverpool) by the un-blinded team. At the vaccination visit (see Table 2) participants will be randomised to two groups to receive:

- PCV15 vaccine IM and inoculation 28 days later with SPN3
- 0.9% saline IM and inoculation 28 days later with SPN3

The randomisation ratio is 1:1. A statistician at OVG will generate a randomisation list using stratified block randomisation stratified based on participant's sex (male/female) and study sites. The final randomisation list will be sent to an IT manager at OVG to upload into a web-based randomisation system. The web-based randomisation system will ensure the allocation concealment is valid. The final randomisation list is only accessible by the unblinded team, the IT manager and the trial statistician. Continued consent and eligibility will be confirmed with the participant (i.e. that no changes have occurred since their Screen visit and vital sign measurements taken) prior to randomisation and vaccination. Randomisation of participants will be carried out by unblinded study staff. Due to staff capacity, the unblinded staff may continue to perform follow-up visits, however, they will be prevented from performing the nasal wash sampling and the inoculation procedure to eliminate bias and maintain observer blinding. Details of back-up randomisation procedures for study sites will be captured in the statistical analysis plan.

Participants in the exploratory nasal biopsy cohort will not be randomsised as only PCV15 will be administered for this group.

10.9. Blinding

10.9.1 Participant Blinding

Participants will remain blinded until the completion of the last participant, last study visit (Day 56). Unblinded clinical team members will prepare the vaccine separately to the participant and administer the vaccines/placebo to the participants in a blinded manner, shielding the vaccine formula, syringe and box from the participant when it is administered.

Participants will be unblinded by the clinical team upon confirmation of the last participant, last visit. Participants will be notified of the vaccine they had received during the study. Participants that received placebo will be invited to attend the clinic for administration of a single dose of PCV15 vaccination. Participant's GP will be informed of the date of vaccination, vaccine name, batch number and formulation the participant had been given.

Participants in the exploratory nasal biopsy cohort will not be blinded as only PCV15 vaccine will be administered for this group.

10.9.2 Staff Blinding

The designated statistician for generating randomisation schedule and the study personnel involved in the preparation and administration of IMP will be unblinded to the treatment assignments. No unblinded stakeholders will be involved in the assessment of adverse events related to the vaccine or any clinical assessments following inoculation or in study endpoint sample analysis. Unblinded personnel will be able to complete follow up visits and take research samples that are not part of the primary endpoint (nasal wash). Core medical doctors will remain blinded to the vaccination allocation throughout the study to ensure thorough assessment of symptoms, AEs and SAEs.

The vaccine preparation and administration will be done by designated unblinded personnel (pharmacist or clinician or nurse). These vaccine related procedures will be performed out of the view of participant as well as blinded study staff. Both vaccine and placebo have distinct appearances when observed through the syringes. Hence, the syringes will be masked with a blinding tape before administration such that the contents of the syringe are not visible to the participant. An accountability label with the participant details will be affixed on the masked syringe prior to administration. The participant may be asked to wear a physical blindfold for the vaccine administration only to ensure blinding is maintained. The blindfold can be removed once the

vaccine is administered and the syringe is disposed of in the sharps bin. After administration, the unblinded study personnel will complete the vaccine record to be filed in the CRF and the eCRF. The unblinded part of the vaccination form will be sealed in an envelope with a sticker across the seal stating the study ID number and date (stored in the participant CRF). This can be used to unblind in an emergency if required.

Unblinded personnel:

- Statisticians generating the randomisation list
- Data management team
- Unblinded Monitors
- Pharmacists/nurses/health professional responsible for IMP administration across all sites

Blinded individuals:

- Chief Investigator
- Co-Investigators
- Clinical staff not involved in the IMP administration
- Laboratory staff
- Analytical laboratory staff
- Analysing statistician
- Participants
- Blinded Monitors

Staff working with the exploratory nasal biopsy cohort will not be blinded as only PCV15 vaccine will be administered for this group.

10.9.3 Unblinding/Code-breaking

Participants will be notified by the study team of whether they received the vaccine or placebo at the time of unblinding. Participants in the placebo arm will be offered PCV15.

Scheduled unblinding will occur after all participants have completed the D56 visit.

Unblinding may also occur at an earlier time point in the event of occurrence of SAEs, SARs or SUSARs or for any circumstances in consideration by the CI or Sponsor delegated Investigator.

Circumstances may arise in which unblinding is required for one specific participant. Examples of this include when a participant has a Serious Adverse Reaction (SAR) or requires medical intervention which would be influenced by whether they have received the vaccine or control. Unblinding will be undertaken according to trial specific working instruction and group allocation will be sent to the attending clinician.

In emergency situations the investigator may need to break the blind immediately, or as quickly as possible. The investigator will therefore have access to the emergency unblinding web-based system 24 hours a day (REDCap), and additionally unblinding information will be available in the participant's CRF (in a sealed envelope). They will have the final decision and unilateral right to unblind in this emergency situation. The Chief Investigator will not need to be involved in the decision to unblind or be able to delay unblinding in an emergency situation. The local site Principal investigator will have responsibility for documenting and informing the Chief Investigator promptly of any unblinding.

At the time of scheduled unblinding, the participants and their GP will be informed of their vaccine or placebo allocation.

10.10. Study Visits

The procedures to be included in each visit are documented in the schedule of procedures tables (section 10.1 and 10.2). Each visit is assigned a time-point and a window period, within which the visit should be conducted. Whether a visit can occur out of window will be decided on a case-by-case basis by the study investigators. All visits, except nasal biopsy procedure, will take place at the study site. Nasal Biopsy procedures will take place in a NHS acute hospital setting.

10.11. Nasal Biopsy Visit

For participants in the nasal biopsy group, there will be two nasal biopsies visits: one within screening window and the second one within visit 4 window. This visit will take place in an acute hospital outpatient setting and the biopsy will be performed by a trained Ear Nose and Throat (ENT) surgeon. During this visit, participants will undertake a separate additional written consent for the procedure which will be performed or re-confirmed on the day of the biopsy by the operator. Nasal biopsies will be collected following NHS procedures. After nasal biopsy procedure, participants will be observed as per local NHS hospital guidelines before leaving the facility. 24hrs after biopsy a phone safety review will be conducted by study team. Participants will be asked to contact the study team within 3 weeks after the procedure if there is any concern.

10.12. Vaccination Visit

The visit procedure for the vaccination visit will be as follows:

- Ensure that participant consent remains valid and verbally confirm continued consent
- Obtain and document interim medical history since the screening visit including medication use and other vaccinations, and check eligibility criteria, specifically temporary exclusion to vaccination, and perform a targeted physical examination (if required to reassess eligibility)
- Record oral temperature, pulse, and blood pressure
- Perform urine pregnancy test for POCBP (section 12.11)
- Collect respiratory samples (according to section 10.2)
- Perform blood draw
- Administer vaccine by IM injection into the deltoid muscle of the non-dominant arm (preferentially)
- Observe participant for a minimum of 15 minutes following vaccine administration
- Schedule/confirm the subsequent visits.

10.13. Inoculation Visit

10.13.1 Preparation of Challenge agent for administration

Streptococcus pneumoniae SPN3 strain LIV014-S3 was isolated from a healthy young natural carrier participant of the Clinical trial: Experimental Human Pneumococcal Challenge (EHPC) Model: *Streptococcus pneumoniae* Nasopharyngeal Experimental carriage study of Attenuated Strains – proof of concept in healthy adults: working towards vaccines for pneumonia Single-blind randomised controlled trial (REC 18/NW/048). The inoculum batch of strain LIV-014-S3 will be prepared at in positive pressured rooms following the Liverpool Vaccine Group SOP for the Preparation and Storage of *Streptococcus pneumoniae* inoculum stocks which follows GMP-like procedures. The same SOP has been followed for the growth of *S. pneumoniae* inoculum stocks for the following clinical trials: CTA 25753/0001/001-000, 2014-000944-13 and CTA 12155/0215/001-0001 and more recently has been used to prepare this same strain for *S. pneumoniae* strain LIV014-S3 for the following study: Serotype 3 Experimental Human Pneumococcal Challenge (EHPC) study protocol: dose ranging and reproducibility in a healthy volunteer population (REC 22/NW/0051, IRAS 306700)[43]. Briefly, a mid-log broth culture of *Streptococcus pneumoniae* SPN3 (Clade Ia, strain LIV014-S3) will be frozen at -80°C in aliquots of glycerol-enriched media. Frozen aliquots will be thawed and checked for bacterial number (colony forming units [CFU] per ml), and purity. The stability of the stock concentration is checked 3 days after preparation and then weekly before the study starts to check that the stock concentration remains stable. Stability tests are then conducted every 3 months. These checks will first be carried out in our laboratory and then identification and penicillin sensitivity will be confirmed in a reference laboratory (UKHSA). Reports on sensitivities, stability and sequence of the stock are stored in the study TMF and shared with the study Sponsor, if requested.The stock vials will be transferred in a secured and tempeture controlled package using an approved courier in line with the material transfer agreement for transfer of pneumococcus strains and stocks already in place between both institutions and Oxford SOP (OVGL003). Upon receipt of the stock, the PI or appropriate PI delegated research staff will maintain accurate records of receipt and condition of all challenge stock vials in accordance with the site SOPs.

On experimental inoculation days, aliquots will be thawed, washed twice, and re-suspended at the correct density. We will aim for a dose of 80,000 CFU/100µl per naris of the SPN3 inoculum (inoculum dose determined in the Serotype 3 EHPC study protocol, REC 22/NW/0051)[43]. This study was a SPN3 dose ranging and reproducibility study that showed the SPN3 Challenge Model to be safe and achieved a colonisation rate of 79% for SPN3 on a single incoulation and 86% on a double incoulation (unpublished study by the EHPC group).

10.13.2 Accountability for the challenge strain

The investigator at site will be responsable for adequate and accurate accountability of *Streptococcus pneumoniae* SPN3 vials prepared for administration to participants. The investigator or designee will administer the study *S. pneumoniae* vials only to individuals included in this study following the procedures described in this study protocol, associated documents and site SOPs. The vial number, date, dosage and time of administration will be recorded.

The study team will track all vials of *Streptococcus pneumoniae* SPN3 that have been used, administered to participants and wasted, within an accountability log.

10.13.3 Inoculation (28 days post vaccination)

The inoculum will be administered according to local SOPs. Briefly, the participant will be seated in a semi-recumbent position. Using a P200 micropipette, 0.1ml of pneumococcus-containing-fluid will be instilled into each nostril. After inoculation, the participant will remain in this position for up to 15 minutes. The participant is given a safety pack containing:

- Thermometer
- Safety information leaflet (including how to take temperature and symptoms of pneumococcal infection)

- Emergency Contact card containing participant study number and study team contact details
- Amoxicillin 500mg 5-day supply

After inoculation, study team will provide participant with access and training to use the electronic diary (ediary) (on REDCap with link sent via email)

Participants will be instructed to monitor the development of any symptoms at home and report them to the clinical team immediately. Home monitoring of symptoms will include a clear flow chart of the necessary intervention should any symptoms develop (see participant safety information sheet). Participants are asked to contact the team daily for the first 5 days following inoculation with their temperature recording and any symptoms before 1200hrs. This is as a safety precaution in the rare incidence of developing pneumococcal infection. Should they not make contact by the specified time, a member of the research team will contact the participant. If no contact is made, then a prior defined 'secondary contact' will be telephoned. Participants will have access to a 24/7 on-call telephone number until the end of the study. Patients reporting symptoms potentially consistent with pneumococcal disease (for example, ear pain, sore throat, cough or fever) will be seen in person by the clinical study team for medical assessment and begin a course of amoxicillin if the study clinician assesses that symptoms are possibly due to pneumococcal infection.

Inoculation will be performed by trained staff from the clinical or laboratory team that are blinded to the vaccine allocation. **Participants in the nasal biopsy cohort will not be inoculated.**

10.14. Follow-up Visits

Follow up visits require the following:

- Review of AESIs/SAEs related to inoculation as appropriate (refer to section 12), since the last visit
- Review of concomitant medications and vaccinations since the last visit
- Review and transfer SAE/SARs to vaccination via the Yellow Card Scheme: www.mhra.gov.uk/yellowcard
- Review laboratory blood tests
- Record oral temperature, pulse rate, and blood pressure
- Perform blood draw
- Collect respiratory samples (according to section 10.2)
- Schedule/confirm the subsequent visits and re-iterate participant requirements such as ediary entries

In exceptional circumstances, where a participant is unable to attend a follow-up visit in person, the visit may be conducted by telephone to ensure adequate collection of safety data. In this case, all above procedures will take place except recording of vital signs, nasal washes and blood samples.

Participants will be followed up post-vaccination at days 7 and 23, and post inoculation at days 2, 7, 14 and 28. Participants in the nasal biopsy cohort will only be followed up post vaccination at days 7 and 23 and post-biopsy at days 1 and 21. Participants will have investigations and samples collected as per Tables 2 and 3.

10.15. Unscheduled Visits

Additional visits or procedures may be performed at the discretion of investigators, for example for further medical history and physical examination, additional blood tests or other investigations if clinically relevant.

10.16. Electronic Diary (ediary)

Following inoculation, participants will have access to an electronic diary system (ediary) using their personal email address, to allow them to self-report solicited and unsolicited AEs. Participants will be asked to grade any symptoms from 0-3 on a Likert scale. This format has been previously utilised in the EHPC studies, therefore this will facilitate direct comparison of SPN3 with other previously utilised serotypes. Sore throat symptoms have been noted by the EHPC DSMC to be an 'adverse event of special interest' (AESI) in the Experimental Human Pneumococcal Challenge (EHPC) Model: Establishing SPN3 Challenge Model REC reference 19/NW/0238 study, that has occurred with an increased frequency when compared to previous EHPC studies (in both carriers and non-carriers). As a result, any participants reporting sore throat symptoms during a clinic visit will be assessed and graded using an additional pharyngitis assessment. Participants will be advised to ensure the clinical team are contacted with any moderate to severe symptoms as soon as possible.

Training for the diary will be given at the inoculation visit. Local site clinical teams will have access to the ediary, to review data inputted by participants and participants will be asked to complete their diaries before 1200h allowing for clinical review during office hours. The results will be reviewed daily from inoculation (D28) to the following 7 days (D35). The system automatically sends an email to investigators if a grade 3 AE has been inputted by a participant. The electronic system automatically sends a reminder to the participant and to the clinical teams if the ediary hasn't been completed for the previous 24-hour period. Daily checks of the alerts are required to facilitate prompt action where necessary. The study team will remind participants via phone, text or email to complete the ediary if not being completed. Participants experiencing AEs by Day 7 of the diary will have their e-diary period extended until the resolution of symptoms.

10.17. Participant Samples

Samples will be taken from each participant as outlined in the study procedures tables (section 10). The screening samples are for assessment of eligibility. Immunology and microbiology samples are research samples for analysis as outlined in the Laboratory Analysis Plan.

10.18. Clinical Laboratory Samples

The analysis of safety blood samples will be carried out at an accredited local clinical laboratory; samples will be destroyed following local (NHS) analysis.

- Haematology:
  - Full Blood Count (Including: Haemoglobin, platelet count, total white cell count, neutrophil count, lymphocyte count, eosinophil count)
  - Clotting for nasal biopsy cohort
- Biochemistry:
  - Urea and Electrolytes (Including: Sodium, Potassium, Urea and Creatinine)
  - Liver Function Tests (Including: ALT, ALP, Bilirubin, Albumin)
- Diagnostic serology (screening only):
  - Screening tests for Hepatitis B, Hepatitis C and HIV infection (Including: HBsAg, HCV antibodies, HIV antibodies)

Additional safety blood tests may be performed if clinically relevant at the discretion of the medically qualified investigator(s). Tests for HIV, Hepatitis B and C are carried out as part of the screening investigations for this trial. Hepatitis B and C are notifiable organisms listed under the UK Health Protection (Notification) Regulations 2010 and if suspected will be reported to the volunteers GP for further investigation and reporting. This will be included in the Participant Information Sheet (PIS) and Informed Consent Form (ICF).

10.19. Microbiology Samples

The following samples will be obtained during the study and we will be processed and analysed at the University of Oxford and Liverpool School od Tropical Medicine:

Nasal wash (NW): will be performed using our established SOP to collect nasal flora/ pathogen specimens and soluble biomarkers[41,50] . Briefly, 5ml of saline is instilled and held for a few seconds in the nares before being allowed to drip into a sterile pot; this is usually repeated up to 20ml in total. In the event of nasal wash loss (for example, if the participant coughs, sneezes, or swallows) the procedure may be repeated to obtain an adequate specimen (≥10ml return).

Throat swabs: will be obtained for detection of viral and bacterial pathogens by microbiological and molecular techniques. Swabs may also be taken at scheduled and unscheduled visits, if participants develop any respiratory symptoms outside the usual study schedule. The participant's tongue will be depressed using a tongue depressor exposing the palatopharyngeal arch. The sample is taken by making five small circular motions of the palatopharyngeal arch in contact with the mucosa whilst avoiding the participant's tongue. Performing throat swabs prior to nasal washes will ensure that the oropharynx is not inadvertently contaminated with nasal pathogens prior to throat swab sampling. Normally, up to 2 swabs may be taken at each time point, with additional swabs as required if the participant is symptomatic.

Nasopharyngeal and nasal swabs will be obtained for detection of viral and bacterial pathogens. Regular nasopharyngeal or nasal samples will be taken by study staff to monitor SPN3 carriage dynamics). If the participant is symptomatic, additional swabs may be taken at study and unscheduled visits.

10.19.1 Determination of Colonisation

Colonisation will be defined by the result of NW taken at 2, 7, 14- and 28-days post inoculation. NW will be plated on to culture media and incubated overnight at 37°C in 5% carbon dioxide (CO_2). Colonies will be confirmed *as S. pneumoniae* using classical microbiological techniques may include but not limited to (i) typical draughtsman-like colony morphology, (ii) the presence of α-haemolysis, (iii) optochin sensitivity, (iv) solubility in bile salts and (v) Gram-positive diplococci. Typing by latex agglutination will be performed using a commercial kit to confirm pneumococcal serogroup. Results from the cultured nasal wash will also be confirmed using Polymerase Chain Reaction (PCR) based methods of bacterial detection. SPN3 isolates will be frozen at -80ºC for storage to allow for confirmation of experimental colonisation by sequencing when required. Colonisation will be assess at both study sites.

10.19.2 Molecular Methods of Detection

DNA will be extracted from NW samples using our well-defined protocols[41] . SPN3 detection will be done by multiplex qPCR for *lytA* and SPN3 *cpsA* genes. This technique will enable us to detect individuals who are potential carriers with very low bacterial density. This multiplex qPCR is well established and validated in our laboratory at the Liverpool School of Tropical Medicine.

Respiratory pathogens multiplex qPCR for detection and quantification will be performed on DNA and RNA of stored swab and/or nasal wash to detect all common respiratory pathogens.

As per our previous published data we will measure several parameters of humoral and cellular responses and the associations of these with both acquisition and clearance of colonisation.

10.20. Immunology Samples

Immunology samples will be used to measure immunological parameters before and after vaccination and EHPC. Details of immunological assessments will be described in the Laboratory Analysis Plan. The following samples will be obtained during the study and will be analysed at the University of Oxford laboratories:

Nasal cells: will be collected after nasal wash using flocked swabs and/or a nanosampling method in which cells are obtained through minimally-invasive superficial nasal scrape (rhinoprobe). Scrapes can be taken multiple times with no significant side effects[51,52]. Up to 4 samples will be obtained at each nasal sampling visit. If no cells are visible on the rhinoprobe/swab following sampling, the sample will be repeated. These samples will be used to assess vaccine and challenged- induced mucosal immune responses.

Blood sampling: Venepuncture will be performed by trained, experienced staff. Blood will be collected to measure safety parameters and assess eligibility, and for laboratory measures including, but not limited to serum immunoglobulins, PBMC populations, and host RNA expression. Up to 60ml of blood will be taken at each of the clinic visits.

Nasal biopsy tissue: will be collected on two occasions before and after vaccination (Day 28) for up to 5 participants in the nasal biopsy cohort. This tissue will be collected from the inferior turbinate and/or the postnasal space/adenoid following NHS procedures. Participants will have up to 4 biopsies collected between the nasal sites, performed by a trained ENT surgeon in an acute hospital outpatient setting using local anaesthesia.

10.21. Urine Samples

For participants of childbearing potential only, urine will be tested for human chorionic gonadotrophin (hCG) at screening and immediately prior to vaccination, biopsy procedure and inoculation. Urine samples will be destroyed immediately following screening result. Alternatively, hCG blood sampling may be used instead.

10.22. Sample Handling for trial purposes

The study outcome measures will be evaluated as per the outcome measures and timepoints specified in section 7. Samples will be handled based on local agreed SOPs and Laboratory Analysis Plan. Samples will be analysed as described in the Laboratory Analysis Plan, some may occur on fresh samples, other samples may be frozen to allow analysis to be batched. Laboratory sample handling and analysis will be compliant with GCP.

Some study samples including but not limited to serum and PBMCs may be sent to the study funder for analysis of exploratory immunological endpoints such as vaccine induced antibody levels, antibody functionality and cellular responses. Consent from participants to share samples with the funder will be sought.

10.23. Early Discontinuation/Withdrawal of Participants

Each participant can exercise their right to withdraw from the study at any time without giving a reason. The participant information sheet (PIS) and presentation will inform participants that they may withdraw from the study at any time and that this will not affect any care they receive within the NHS. In addition to consent being withdrawn by a participant, the investigator may discontinue a participant from the study at any time for the following, although not exhaustive, reasons:

- The investigator considers it necessary for participant safety
- Significant non-compliance with study requirements
- The participant is lost to follow up

In circumstances pertaining to the safety of the participant, the DSMC chair, DSMC committee or Investigator may choose to discontinue further vaccination and/or specific study procedures for an individual participant. However, participants should otherwise continue to attend the follow up visit schedule and follow up procedures unless they withdraw consent for this. Such circumstances may include the following non-exhaustive reasons:

- Pregnancy (further details on management of participants who become pregnant are provided in section 12.11)
- If a medication is started that would affect the study results, for example, intercurrent use of antibiotics
- An AESI which results in an inability to continue to comply with study procedures
- Ineligibility (either arising during the study or in the form of new information not declared or detected at screening)

Withdrawal from the study will not result in exclusion of existing data generated by the participant from analysis. Participants can request that their samples are destroyed at any point during or after the study (although data that has already been generated from samples that have been analysed up to that point will be retained). The reason for withdrawal, if given, will be recorded in the eCRF.

10.24. Definition of End of Trial

The clinical phase of the study ends when the last participant completes their last visit. Recruitment and follow up of participants from initial recruitment activities until the last participant finishes their last study visit are planned to be completed within 2 years. The end of the trial will be complete when all assays providing data for primary and secondary endpoints have been completed.

11. INVESTIGATIONAL PRODUCT

The term ‘investigational product’ applies to PCV15 (VAXNEUVANCE) in this study.

11.1. PCV15 (VAXNEUVANCE)

VAXNEUVANCE (PCV15) is a 15-valent PCV, adsorbed pneumococcal polysaccharide conjugate vaccine produced by Merck, Sharp & Dohme LLC, a subsidiary of Merck & Co, Inc., Rahway, NJ, USA (MSD). It has

marketing authorisation and is licensed for active immunisation for the prevention of invasive disease, pneumonia and acute otitis media caused by *S. pneumoniae* in infants, children and adolescents from 6 weeks to less than 18 years of age. Vaxnuevance is also indicated for active immunisation for the prevention of invasive disease and pneumonia caused by *S. pneumoniae* in individuals 18 years of age and older. It contains 2 µg of purified capsular polysaccharides from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F and 4.4 µg of serotype 6B individually conjugated to CRM_{197} carrier protein (30 µg), adsorbed on aluminium phosphate (125 µg) per 0.5ml (1 dose) suspension for injection. It comes in a pre-filled syringe and is a homogeneous white suspension for injection. See product SmPC for full detailed description. MSD (the funder of the study) holds a market authorisation in the UK, under the number PLGB 53095/0090.

Any deficiency or medical event with the pre-filled syringe or incident with PCV15 will be reported to the manufacturer.

11.2. Saline Placebo (0.9% Sodim Chloride)

The placebo consists of 0.9% sodium chloride for injection, this will be ordered via NHS pharmacy and will be stored in the fridge (2°C - 8°C) so that the placebo is cold on injection to mimic the vaccine. Once the placebo has been removed from the fridge and accountability records are completed, the injection will be prepared and the syringe will be concealed with a label detailing the participant ID number, initials, date and time of preparation and expiry time as required.

11.3. Storage, Supply and Accountability of IMPs

PCV15 vaccine will be provided free of charge by MSD and the 0.9% saline for injection will be sourced and purchased from the NHS (or other reputable supplier). Vaccine/placebo will be stored, administered and managed according to relevant SOPs and manufacturer instructions.

The study vaccine/placebo will be stored at Centre for Clinical Vaccinology and Tropical Medicine and the Liverpool Life Sciences Accelerator Building at the appropriate temperature in a temperature monitored fridge in a restricted access room.

During the study, the vaccine/placebo will be removed from storage and administered immediately according to relevant SOPs and manufacturer's instructions. Vaccine/placebo accountability records will be maintained from the time of receipt to the time of destruction of IMP as per relevant SOPs. Vaccine/placebo accountability records will be kept blinded until the time of participant un-blinding and will only be accessible to the un-blinded clinical team.

11.4. Compliance with Trial Treatment

The study investigational product will be administered by trained study personnel and will be documented according to GCP guidelines and relevant SOPs. Issues related to compliance are therefore the responsibility of study personnel who have received appropriate training.

11.5. Packaging and Labelling of Trial Treatment

The packaging and labelling will be those provided by the manufacturer, as is standard practice.

11.6. Concomitant Medication

The use of all concomitant medication prescribed or over the counter, will be recorded in the eCRF. Concomitant medication that will result in temporary exclusion or withdrawal of participants from further vaccination or inoculation are detailed in section 9.3. There is otherwise no restriction on the use of concomitant medication.

11.7. Emergency Medication and Procedures

All clinical staff are trained in the acute management of anaphylactic reactions including the use of IM adrenaline. This is detailed in relevant site SOPs and adrenaline is available at all times of vaccine administration and subsequent observation.

11.8. Post-trial Treatment

The study IMP will not be continued beyond the trial period.

11.9. Other treatments (non-IMPs) and interventions

Participants with allergy to penicillin or amoxicillin will be excluded from the main study cohort (section 9.3). Study sites will have a pharmacy agreements for local pharmacies to provide the study Amoxicillin and do the overlabelling. The antibiotic will be dispensed to all participants at the first inoculation (V1 visit) and prescribed by a study doctor/dispensed using a Patient Group Directive (PGD). Participants will be instructed to take 500 mg of amoxicillin 3 times per day for 5 days under specific instances including:

- After the Day 28 visit if a carrier at >1 timepoint during the study without 2 consecutives negative nasal washes
- If unwell and/ or symptomatic and instructed to take by the study doctor, or
- If unwell and unable to contact the research team
- At an investigator discretion at any point

Participants will be given an antibiotic record card , which will also have instructions for taking antibiotics and possible side effects. They are also provided with an antibiotic record card if they are unable to access their e-diary. An MHRA leaflet with information about amoxicillin will be provided to participants.

Amoxicillin is usually generally well tolerated, however the most commonly reported side affects are diarrhoea, nausea and skin rash. All Reference Safety Information and further particulars of Amoxicillin can be found in the Summary of Product Characteristics (SmPC).

Antibiotics dispensed and returned will be tracked using accountability logs and local SOPs will be followed for destruction or retuned of any unused antibiotic.

In the unlikely scenario that a different antibiotic needs to be prescribed, the antibiotic to use will be informed by the challenge agent report on antibiotic sensitivity provided by UKHSA and antibiotics will be provided through NHS pharmacy.

12. SAFETY REPORTING

12.1. Adverse Event definitions

Table 5 Adverse Event definitions

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.
Adverse Event of Special Interest (AESI)	An adverse event of special interest (serious or non-serious) is one of scientific and medical concern specific to the vaccination or inoculum, for which ongoing monitoring and rapid communication by the investigator to the Sponsor can be appropriate. Such an event might warrant further investigation in order to characterise and understand it. Depending on the nature of the event, rapid communication by the trial Sponsor to other parties (e.g., regulators, DSMC) might also be warranted.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect Other 'important medical events' may also be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

	NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant 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.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the Reference Safety Information for the medicinal product in question set out: • in the case of a product with a marketing authorisation, in the approved summary of product characteristics (SmPC) for that product • in the case of any other investigational medicinal product, in the approved investigator's brochure (IB) relating to the trial in question.

12.2. Assessment of Severity

Severity grading will be graded according to scales based on the United States Food and Drug Administration (FDA) toxicity grading scales for healthy participants enrolled in preventative vaccine clinical trials.

The severity of AEs/AESIs will be assessed on the following scale:

GRADE 0	None
GRADE 1	Mild: Transient or mild discomfort (< 48 hours); No interference with activity; No medical intervention/therapy required
GRADE 2	Moderate: Mild to moderate limitation in activity – some assistance may be needed; no or minimal medical intervention/therapy required
GRADE 3	Severe: Marked limitation in activity, some assistance usually required; medical intervention/therapy required.

Specific grading scales for solicited AEs, vital signs and laboratory AEs will be according to scales listed in Appendix B.

AEs reported in the ediary will be severity graded by the participant and transcribed to the AE eCRF by study staff. AEs will be provided to the funder on quarterly basis via the study update report funder template using participant's ID only.

12.3. Assessment of Causality

The relationship of the an AE or SAE with the study procedures will be categorized as not related, possibly related, probably related or definitely related. The PI delegated blinded clinician will use clinical judgment to determine the relationship using the following definitions:

No Relationship		No temporal relationship to vaccine administration or *S. pneumoniae* inoculation or other study procedure; ***or*** Alternate aetiology (clinical state, environmental or other interventions); ***and*** Does not follow known pattern of response to vaccine administration and/or *S. pneumonia*e inoculation and/or other study procedure.
Related	Possible	Reasonable temporal relationship to vaccine administration or *S. pneumonia*e inoculation or other study procedure; ***or*** Event not readily produced by clinical state, environmental or other interventions; ***or*** Similar pattern of response to that seen to vaccine administration and/or *S. pneumonia*e inoculation and/or other study procedure.
	Probable	Reasonable temporal relationship to vaccine administration or *S. pneumonia*e inoculation or other study procedure; ***and*** Event not readily produced by clinical state, environment, or other interventions; ***or*** Known pattern of response seen to vaccine administration and/or *S. pneumonia*e inoculation and/or other study procedure.
	Definite	Reasonable temporal relationship to vaccine administration or *S. pneumonia*e inoculation or other study procedure; ***and*** Event not readily produced by clinical state, environment, or other interventions; ***and*** Known pattern of response seen to vaccine administration and/or *S. pneumonia*e inoculation and/or other study procedure.

12.4. Procedures for Collecting and Recording Adverse Events

Adverse Events will be recorded in either:

- the e-diary
- the eCRF

All AEs occurring from inoculation until 7 days after challenge that are observed by the Investigator or reported by the participant will be recorded in the eCRF/e-diary. Participants experiencing AEs by the 7th day will have their e-diaries extended until resolution of symptoms. Most common AEs will include sore throat, headache, rash, earache and cough.

All grade 3 solicited adverse events recorded in the e-diary will be followed up with the participant by the clinical team.

Unsolicited adverse events will be reviewed at clinic visits. If clarification of any event is required, then the study nurse or doctor will seek this from the participant during a clinical visit or by telephone call. Unsolicited adverse events recorded in the e-diary will be severity graded by the participant using the same Likert scale (0-3).

AEs will be recorded using the following guidance:

- Each AE should be recorded to represent a single diagnosis with any accompanying signs or symptoms (including abnormal laboratory values) anoted together.

The following information will be recorded in the CRF:

- Description of the AE/SAE
- The date of onset and end date
- Severity and assessment of relatedness to study procedure(s) (as judged by a medically qualified investigator)
- Action taken.

All SAEs/SARs and AEs will be followed until resolution, until the event is considered stable/resolved or until participants' last visit.

It will be left to the investigator's clinical judgment whether an AE is of sufficient severity to require the participant's removal from study. A participant may also voluntarily withdraw from the study due to what they perceive as an intolerable AE/SAE.

If either of these occurs, the participant should undergo an end of study assessment (where possible) and be given appropriate medical care (e.g. referral to their GP). If required, the investigator can refer the participant directly to hospital if the AE/SAE warrants it.

During the 3 week follow-up period post-nasal biopsy procedure, unsolicited adverse events will be recorded and casualty assigned.

12.5. Adverse Events of Special Interest (AESI)

Following inoculation until Day 56 (or until the time of completion of antibiotic treatment) AEs will be recorded in the CRF. If considered at least possibly related to the inoculum by a study physician, they will be reported as adverse events of special interest (AESIs) to the TMG and DSMC in the weekly safety report that is circulated. If patterns emerge with AEs, this may trigger a pause to the study by the DSMC where appropriate, this will be described in further detail in the DSMC terms of reference. AEs will be monitored by the blinded clinical team until the participants' last visit or stabilisation/resolution. If the AE meets the definition of an SAE, then they will also be reported as an SAE.

The following events will be considered AESIs:

- Invasive pneumococcal disease
- Otitis media (OM), periorbital cellulitis, sore throat (pharyngitis/tonsillitis assessment)
- AEs requiring a physician visit or Emergency Department visit which, in the opinion of the study staff, are related to the challenge

12.6. Procedures for Recording and Reporting Serious Adverse Events (SAEs) and Serious Adverse Reactions (SARs)

Safety of the participant including symptoms experienced and participant attendance to hospital/GP will be elicited at each visit. Serious adverse reactions/events (SARs/SAEs) will be reported from the signing of informed consent through the end of study participation (or until the completion of antibiotic treatment). All

SAEs must be recorded on a SAE reporting form on the electronic data capture system (REDCap) with causality assessed by the blinded investigator at site, as soon as possible, within 24 hours of discovery or reporting as serious. In the eventuality that REDCap is unavailable, a paper back-up reporting system can be used. All SAEs must be notified by email to CI-delegated investigators at the Oxford Vaccine Group within 24 hours of discovery or reporting as SAE

All SAEs will be reported to the Sponsor delegate as soon as the form is saved on REDCap, via automated email. The SAE reports will be sent to the DSMC Chair (or nominated designee) within 24 hours of notification of the event to CI-delegated Investigator, via an automated email. When paper back-up reporting system is used, the reporting investigator will be reminded in the SAE form to send the report to the Sponsor delegate as soon as possible, including instructions on how to send it. In this situation, the electronic SAE form on REDCap will also be completed once it is available. DSMC will be notified 24 hours after Sponsor delegate via email. Additional information received for an SAE (follow up or corrections to the original report) will be detailed on a new SAE form, indicating 'update'. All SAEs will be followed up until resolution, the event is considered stable or until a non-study causality is assigned. The DSMC will perform an independent review of SAEs as outline in section 15.5.

SAE and SUSARs should be reported by the site Principal Investigator to MSD's Global Pharmacovigilance ('MSD GVP') group (MSD GPV facsimile number at 0032 2404 5990), including all the follow-up information involving the study subject. Notifications shall be completed on an agreed format and contain the reporter's name and study subject ID. SUSAR information will be reported unblinded and randomisation codes for all other SAEs will be provided to MSD GPV at the end of the study. A full description of the reporting requirements is included in the funding agreement.

As the antibiotic is licensed and have extensive safety profile, only SARs/SAEs following antibiotic used will be recorded and reported using the Yellow Card reporting site. Any SAE or SAR related to the vaccine will be recorded in the Development Safety Update Report (DSUR), as per section 12.12.

12.7. Events Exempt from Immediate Reporting as SAEs

Hospitalisation (including inpatient or outpatient hospitalisation) for an elective procedure for a pre-existing condition that has not worsened unexpectedly does not constitute an SAE. Emergency department attendances should not routinely be reported as SAEs unless they meet the SAE definition described in section 12.1.

12.8. Expectedness

All serious adverse events and serious adverse reactions will be assessed for expectedness by the clinical Investigator. For SAEs that require reporting, expectedness of SARs will be determined according to the relevant RSI section (that is, section 4.8 of the Summary of Product Characteristics) of the SmPC for VAXNEUVANCE by the CI-delegated investigators at the Oxford Vaccine Group responsible for Sponsor assessment. The RSI within the SmPC will be the current Sponsor and MHRA approved versions at the time of the event occurrence. VAXNEUVANCE is being administered according to the marketing authorisation and therapeutic indications. Updates to the SmPC will be monitored annually at the time the Development Safety Update Report (DSUR) is due as per CTU process and any updates affecting the conduct, safety or results of the trial will be incorporated via substantial amendment. For assessment of expectedness in the DSUR, see section 12.12.

12.9. Suspected Unexpected Serious Adverse Reactions (SUSAR) Reporting

All SUSARs will be reported to the CI-delegated Investigators, Sponsor, relevant Research Ethics Committee, MHRA and to the funder. The CI will determine whether an SAE is considered expected. For fatal and life-threatening SUSARs, this will be done no later than 7 calendar days after the CI or delegate is first aware of the event. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days.

12.10. Procedure in the Event of Abnormal Findings

Laboratory parameters for inclusion/exclusion in the trial will be considered on an individual basis, with investigator discretion for interpretation of results and the need for repeated tests. Abnormal clinical (Appendix B) findings from medical history, examination or blood tests will be assessed as to their clinical significance throughout the trial. If a test result is deemed clinically significant, it may be repeated to ensure it is not a single occurrence or spurious result. If a test remains clinically significant, the participant will be informed and medical care arranged as appropriate and with the permission of the participant. Decisions to exclude the participant from enrolling in the trial or to withdraw a participant from the trial will be at the discretion of the Investigator.

12.11. Pregnancy and Contraception

Pregnant and breastfeeding/lactating women will be excluded from the study.

Participants of childbearing potential will be required to use an effective form of contraception. A woman is considered of childbearing potential (i.e. fertile) from the point following menarche until becoming post-menopausal, unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A post-menopausal state is defined as having no menses for 12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) level in the post-menopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhoea, a single FSH measurement is insufficient, and effective contraception would need to be used.

Contraception should have been initiated at least for one month prior to receiving the vaccine and for the duration of the study. Acceptable forms of effective contraception for participants of childbearing potential include:

1. Established use of oral, injected or implanted hormonal methods of contraception.
2. Intrauterine device (IUD).
3. Intrauterine hormone-releasing system (IUS).
4. Barrier methods of contraception (condom or occlusive cap with spermicide).
5. Bilateral tubal occlusion.
6. Vasectomised male partner if the vasectomised partner is the sole partner for the participant.
7. Sexual abstinence when this is in line with the preferred and usual lifestyle of the participant. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of exposure to IMP, and withdrawal methods are NOT acceptable methods of contraception.

8. Exclusive sex with a female partner(s).

Should a volunteer become pregnant during the trial this will be recorded and the Sponsor and the DSMC will be notified if appropriate. They will be followed up for clinical safety assessment with their ongoing consent and in addition will be followed until pregnancy outcome is determined. No further non-essential trial procedures will be performed (i.e. vaccination or inoculation), however, procedures such as appropriate antibiotic treatment may be required if pregnancy detected following inoculation. Follow up of any pregnancy may affect the study end date if pregnancy continues after the expected study end and outcome is recorded (or reported in event of congenital abnormality in accordance with section 12.6)

Male participants with female partners are not required to use barrier methods for the purposes of contraception, as the risks of vaccine excretion at mucosal surfaces and in semen are negligible.

12.12. Development Safety Update Report

The CI will submit a Development Safety Update Report (DSUR) once a year on the anniversary of clinical trials authorisation for this study throughout the clinical trial, or on request, to the MHRA, REC, Sponsor and funder. The CTU (on behalf of the Sponsor) will submit (in addition to the expedited reporting above) Development Update Safety Reports (DSURs) once a year throughout the clinical trial, or on request to the Competent Authority (MHRA in UK), Ethics Committee, HRA (where required), Host NHS Trust and Sponsor.

For assessment of SARs in the DSUR, the RSI that was approved at **the start of the safety reporting period** will be used. When there has been approved changes to the RSI by substantial amendment during the reporting period, the RSI used for the DSUR will differ to the RSI used to assess expectedness at the time of SAR occurrence for SARs which require expedited reporting

13. STATISTICS

13.1. Statistical Analysis Plan (SAP)

The plan for the statistical analysis of the trial is outlined below. A statistical analysis plan will be produced for this study and signed off before any formal analysis of the data.

13.2. Sample size determination

We will aim to recruit 84 effective participants with primary endpoint available in order to detect a 50% relative risk reduction in the experimental SPN3 colonisation acquisition rate (detected by classical microbiology) from 60% in the control group to 30% in the intervention (PCV15) group. The sample size was calculated to achieve 80% power and a type I error (alpha) of two-sided 0.05. Up to 106 participants will be recruited to ensure 84 complete the study, allowing for a up to 20% attrition rate. Natural carriers at the time of bacterial inoculation will be replaced, they will be allowed to continue in the study but data will not be in included in the population analysis.

In calculating the sample size, we have assumed:

- The colonisation rate of SPN3 in unvaccinated adults 18-50 years of age is 60% as determined by classical microbiology from a recent EHPC study[41]. Results of another SPN3 Challenge (Serotype 3 EHPC study protocol: dose ranging and reproducibility in healthy volunteer population -Challenge 3, REC

reference 22/NW/0051) study also reported a >60% attack rate in the control group for the selected 80,000 CFU dose[44].

- PCVs confers 50% relative risk reduction (Vaccine efficacy) in colonisation against SPN3 as determined by microbiology (78% risk reduction in colonisation against SPN6B seen in young healthy people in EHPC study[28], and 50% relative risk reduction in colonisation when comparing PCV-13 to control 1 month after vaccination.
- Approximately 10% screen failure rate and 10% dropout rate leading to non-completion from previous EHPC studies.

13.3. Analysis Populations

As the participants to be recruited are healthy adult volunteers and the primary objective is to establish the reduction in colonisation rate afforded by the study vaccine (PCV15) compared with the placebo, the Per Protocol (PP) population will be used for evaluation of the primary endpoint.

For the current study, the PP population is defined as all participants who:

- Have received the allocated study vaccine (or the actual vaccine received in case of randomisation error),
- Have been inoculated with SPN3, and
- Were not naturally colonised with pneumococcus on the day of the inoculation
- Have received no bias or interference that may interfere with potential vaccine effect or colonisation rate, either according to the protocol or in the view of the study investigators.

Description of the final population to be analysed for the primary endpoint will be reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) Statement.

If a participant later withdraws from the study, safety data up until that point will be included in the analysis. If participants are withdrawn before the specified 28-day period after inoculation to determine colonisation, they will be excluded in the primary analysis and a sensitivity analysis will be undertaken to explore different assumptions for the missing data and include those participants, if possible.

All the analysis populations are defined:

Populations	**Description**
Screening Set	The screening set consists of all participants screened for this study.
Full Analysis Set (FAS)	The FAS consists of all randomised participants who received the study IMP or control. Participants will be analysed according to their randomised arms.
Modified Intent-to-Treat (mITT) set	The mITT set consists of all participants in the FAS whose endpoints are available. Participants will be analysed according to their randomised arms.
Per-protocol set for efficacy	The PP set is defined as above. Participants will be analysed according to their vaccine received.

Solicited Safety Set	The solicited safety set consists of all participants in the FAS who filled in the symptom diary for at least one day.
Safety set	The safety set consists of all participants in the FAS and will be analysed according to their vaccine received. The safety set will be used for safety analysis expect the solicited AEs.

13.4. Analysis of Demographics and Baseline Characteristics

Descriptive statistics relating to participant characteristics at baseline will be calculated overall and by study arms. No formal statistical comparisons of baseline characteristics between randomised groups will be conducted.

13.5. Primary Outcome Analysis

The primary objective of this study is to determine the relative protective effect of PCV15 compared with the placebo group on the experimental SPN3 colonisation acquisition rates (detected by classical microbiology). The null and alternate hypotheses are:

H_0 : Colonisation $Rate_{PCV15}$ = Colonisation $Rate_{placebo}$

H_1 : Colonisation $Rate_{PCV15}$ ≠Colonisation $Rate_{placebo}$

Where colonisation rate is the proportion of participants with colonisation (presence/absence of SPN3 experimental Streptococcus pneumoniae at any time point post-inoculation) determined by the result of nasal washes taken at 2, 7, 14- and 28-days post-inoculation (see section Monitoring of Colonisation) in the population of “Per-protocol set for efficacy”.

The colonisation rates between the two arms will be compared using a generalised linear model adjusting for sites and gender as randomisation stratification variables, which will generate relative risk (RR) together with its 95% confidence intervals (CIs). The vaccine efficacy will be calculated as (1-RR)*100.

A secondary analysis of this primary endpoint will be conducted using the Kaplan-Meier method in the FAS population. Participants who withdrew or had potential interference with vaccine effect or colonisation will be censored in the analysis at the time of withdrawal or interference. Non-colonised participants will also be censored in the analysis at the end of the inoculation phase (Day 28 following inoculation).

13.6. Secondary Outcome Analysis

Immunogenicity data are expected to be highly skewed and will be log-transformed prior to analysis. Results will be presented as geometric means with 95% confidence intervals. Values below the limit of detection will be replaced by half the value of the lower limit.

Density of experimental pneumococcal colonisation at different time points post-inoculation will be available for those who have a recorded density (positive for colonisation) and will be analysed in two ways:

- The density will be summarised using number, mean, geometric mean, standard deviation, median, minimum and maximum at each time point. Log transformed density will be analysed using a linear mixed model, in which vaccine arm, time, interaction between vaccine arm and time as independent factors after taking the correlation of participants into account. Exchangeable covariance structure will be used. The geometric mean ratio between arms together with their 95% CIs at each time point will be derived.
- The area under the curve (AUC) of density of experimental pneumococcal colonisation will be derived and summarised using the above descriptive statistics and log AUC will be analysed using a generalised linear model with a single factor of vaccine arm. The geometric mean ratio between groups together with their 95% CIs will be derived.

The duration of SPN3 pneumococcal colonisation in the nasal wash collected from study participants following experimental pneumococcal inoculation is defined by the difference in days between inoculation and the last visit with confirmed SPN3 pneumococcal colonisation in the nasal wash. The duration of pneumococcal colonisation will be summarised using the above descriptive statistics. The comparison between the two arms will be done using t-test if the data is normally distributed, otherwise, non-parametric test will be used.

13.7. Exploratory Outcome Analysis

The analysis of the exploratory outcomes will be conducted following the main principles set in the final statistical analysis plan. Further exploratory analysis may be conducted if findings of scientific interest become apparent during the study or processing of the data.

13.8. Level of Statistical Significance

The significance level is two-sided 0.05. All confidence intervals for descriptive analyses will be reported as 95% confidence intervals.

13.9. Procedure for Accounting for Missing, Unused, and Spurious data

All available data will be used in the analyses and there will be no imputation for missing data.

14. DATA MANAGEMENT

The data management aspects of the study are summarised here, with details fully described in the Data Management Plan.

Each study participant will have a unique participant ID which will be allocated at the time of the screening visit. Names or identifying details are not included in any electronic file, containing study data. The exception to this is the electronic diaries, for which consent will be obtained to store the participant's email address, which is necessary for the system to function. Only site research staff and sponsor data managers have access to view the email address. Participant's personal information will be stored on a separate database not linked to the clinical database. Apart from clinical safety blood samples or samples which are sent to local clinical

Macedo BR, *et al*. *BMJ Open* 2025; 15:e106028. doi: 10.1136/bmjopen-2025-106028

laboratories and follow local sample labelling requirements, samples sent to laboratories for processing will be identified by trial number and participant number only.

14.1. Source data

Source documents are original documents, data, and records from clinical findings, observations and other study-related activities. Most source data will be electronic data, where the data is first recorded electronically into the eCRF into the Electronic Data Capture system (REDCap).

For a list of source data to be originally kept as paper documents, see Data Management Plan.

All documents will be stored safely under strict confidentiality and with restricted access. The participant will be referred to by the study participant number/code on study-specific documents, other than the signed consent forms, participant contact sheet and information for GPs and their national public health agency. Participants' details populated from the electronic database are kept in the form of an electronic participant and screening log located on a password protected network drive.

14.2. Access to data

Direct access to source data/documents will be granted by investigator/institution to authorised representatives from the Sponsor (or appointed by the Sponsor), Ethics Committees and regulatory authorities to permit trial-related monitoring, audits, and inspections.

14.3. Data Recording and Record Keeping

PI-delegated staff will populate the content of participants' CRFs and all the clinical data will be recorded directly into REDCap, or onto a paper source document for later entry into EDC if direct entry is not available. Any additional information that needs recording but is not relevant for the CRF (such as signed consent forms etc.) will be recorded on a separate paper source document. For details on those, see Data Management Plan. Laboratory data for secondary and exploratory endpoints will be stored on secure servers on the University of Oxford MSDIT network. External servers will be used by the relevant trusts when laboratory data has been shared, these external trusts have security in place. All documents will be stored safely and securely in confidential conditions.

The EDC system (CRF data) uses a relational database (MySQL/ PostgreSQL) via a secure web interface with data checks applied during data entry to ensure data quality. The database includes a complete suite of features which are compliant with GCP, EU and UK regulations and Sponsor security policies, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL and PostgreSQL database and the webserver will both be housed on secure servers maintained by Oxford Vaccine Group IT personal and local site IT personal. The servers are in a physically secure location in EU and data are backed up on secure servers operated by the University of Oxford IT Services physically located in EU zone. Backups will be stored in accordance with the IT department schedule of daily, weekly, and monthly retained for one month, three months, and six months, respectively. The IT servers provide a stable, secure, well-maintained, and high-capacity data storage environment. REDCap is a widely used, powerful, reliable, well-supported system. Access to the study's database will be restricted to the members of the study team by username and password.

Participant's personally identifiable information will be stored at the study site in compliance with GCP and regulatory and institutional requirements for the protection of confidentiality of volunteers. Identifiable information may be transferred between sites using an encrypted messaging service, such as between nhs.net or university email accounts.

Personal identifiable data will be recorded to plan and schedule visits, set reminders, track payments, and generate reports on participant management to enable the study teams to track recruitment and visit compliance. Where this information is recorded electronically, it will only be accessible through restricted specific networks, with only delegated study members able to gain access.

Each study participant will have a unique participant number which will be allocated at the time of screening. Names and/or identifiable details are not included in the clinical electronic database capture system. Storage of participant email addresses for REDCap electronic diaries and electronic medical records access informed consent forms will be required for the system to function, for which consent will be obtained. Only site research staff and data managers have access to view the email address. Participants will be identified by the unique study-specific participant number and/or code, allocated at the screening visit.

With the exception of clinical safety blood samples and any respiratory samples, which are sent to local clinical laboratories and follow local sample labelling requirements (typically including the participant's medical record number (MRN), NHS number, name, sex and date of birth), samples sent to other laboratories for processing will be identified by study number and participant number only.

The team statistician will retain the lists linking the participant numbers (participant number, laboratory number).

The study teams will use names and contact details to contact participants about the research study, and make sure that relevant information about the study is recorded for their care, in relation to their health during the study and to oversee the quality of the study. At the completion of the study, unless participants consent otherwise (e.g., requesting to be informed of other studies), participant's personal details will not be used to contact them other than exceptional circumstances concerning their safety. If consent is provided by participants to take part in another study carried out by the study site, personal information and medical information including blood test results may be accessed to avoid unnecessary repetition. If participants provide specific consent, we will use personal identifiable data to invite participants for future research.

Direct access will be granted to authorised representatives from (or appointed by) the Sponsor, host institution and the regulatory authorities to permit study-related monitoring, audits and inspections.

Bank details will be stored for 7 years unless otherwise stated in information sheet to align with local site financial policy.

14.4. Data Integrity

Data collection and storage will be inspected throughout the study by the Oxford Vaccine Group and monitoring will be carried out by an internal OVG monitor.

15. QUALITY ASSURANCE PROCEDURES

15.1. Investigator Procedures

Approved standard operating procedures (SOPs) will be used at all clinical and laboratory sites.

15.2. Risk Assessment

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and Standard Operating Procedures. A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the trial to reflect significant changes to the protocol or outcomes of monitoring activities. Approved and relevant SOPs will be used at all clinical and laboratory sites.

15.3. Monitoring

Monitoring will be performed according to GCP by monitors from the Oxford Vaccine Group internal quality assurance team. Following written SOPs, 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. Trial site(s) will provide direct access to all trial-related source data/documents and reports for the purpose of monitoring and auditing by the sponsor and inspection by local and regulatory authorities.

15.4. Data Safety Monitoring Committee (DSMC)

An independent DSMC will be appointed. There will be a minimum of three appropriately qualified committee members of whom one will be the designated Chair. The DSMC will operate in accordance with the DSMC charter, which will be established before recruitment starts. The Chair of the DSMC may also be contacted for advice where the Chief Investigator thinks independent advice or review is required.

15.5. DSMC reviews

A DSMC will review safety and colonisation rate data throughout the study. All roles and responsibilities of the DSMC will be outlined in detail in the DSMC charter. DSMC data reviews will be performed as follows:

1. Formal review of the safety profile and colonisation rate of the inoculum after the first 10 participants in the SPN3 challenge (without stopping the study) and at the end of the study.
2. Independent review following any SAE and severe AESIs regardless of relatedness to any of the study procedures throughout the study.
3. Unscheduled reviews on request of the study management committee at a frequency determined by the severity of reported adverse events.

From these reviews the DSMC will make recommendations to the study investigators on whether there are any ethical or safety reasons why the trial should not continue. A summary of all blinded and unblinded AEs and SAEs to date will be provided to the DSMC on request.

The DSMC will be supplied with a safety report at the end of the study, in the event of an SAE, or if requested at any time by the CI or DSMC members.

AEs such as (but not limited to) headache, cough, sore throat, rash and earache will be documented and reported together with the carriage rates to the TMG and DSMC in weekly safety reports.

The outcome of each DSMC review will be communicated directly to the research study team and TMG and documentation of all reviews will be kept in the site file.

The Chair of the DSMC will also be contacted for advice where the CI feels independent advice or review is required.

15.6. Communication Plan

For the interim safety reviews described above (section 15.5) the following process will apply:

1. The study statistician or data manager will provide a full safety report for review.
2. The lead fellow* will review the safety data with the CI/PI*.
3. An Interim Safety Review Report will be generated, signed and dated by the CI/PI* and/or the lead fellow* as applicable.
4. The report is shared with the DSMC by email ahead of the scheduled review and as outlined in the DSMC charter.
5. The outcome of the DSMC review will be shared with the study team and the TMG and filed in the TMF.

*Or designated individual

15.7. Trial Management Group

The CI, PI and study site investigators will form the trial management group (TMG) and will provide on-going management of the trial.

15.8. Protocol Deviation

Any deviations from the protocol will be documented in a protocol deviation form and filed in the trial master file. Each deviation will be assessed as to its impact on volunteer safety and study conduct. Significant deviations will be listed in the end of study report.

15.9. Audit and Inspection

The Quality Assurance team operates an internal audit program to ensure that the systems used to conduct clinical research are present, functional, and enable research to be conducted in accordance with study protocols and regulatory requirements. Audits include laboratory activities covering sample receipt, processing and storage and assay validation. The internal audits will supplement the external monitoring process and will review processes not covered by the external monitor.

The Sponsor, trial sites, and ethical committee(s) may carry out audits to ensure compliance with the protocol, GCP and appropriate regulations.

GCP inspections may also be undertaken by the MHRA to ensure compliance with protocol and the Medicines for Human Use (Clinical Trials) Regulations 2004, as amended. The Sponsor will assist in any inspections and will support the response to the MHRA as part of the inspection procedure.

15.10. Serious Breaches

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the Sponsor becoming aware of the breach.

A serious breach is defined as “A breach of GCP or the trial protocol which is likely to affect to a significant degree -

(a) the safety or physical or mental integrity of the participants of the trial; or

(b) the scientific value of the trial”.

If a serious breach is suspected, the Sponsor will be informed within one working day.

16. ETHICAL AND REGULATORY CONSIDERATIONS

16.1. Declaration of Helsinki

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki.

16.2. Guideline for Good Clinical Practice

The Investigator will ensure that this trial is conducted in accordance with relevant regulations and with Good Clinical Practice.

16.3. Approvals

Following Sponsor approval, the protocol, informed consent form, participant information sheet, and required material will be submitted to an appropriate Research Ethics Committee (REC), MHRA, regulatory authorities, and host institutions for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

16.4. Reporting

Once a year or on request throughout the clinical trial, the CI or their delegate will submit an Annual Progress Report to the REC committee, host organisations, funder (where required) and Sponsor. In addition, an End of Trial notification and summary report will be submitted to the MHRA, the REC, host organisations and Sponsor.

16.5. Transparency in Research

Prior to the recruitment of the first participant, the trial will have been registered on a publicly accessible database. Results will be uploaded to ISRCTN Database/CT.gov within 12 months of the end of trial (as declared by the CI or their delegate). Where the trial has been registered on multiple public platforms, the trial information will be kept up to date during the trial, and the CI or their delegate will upload results to all those public registries within 12 months of the end of the trial declaration.

16.6. Participant Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. All documents will be stored securely and only accessible by trial staff and authorised personnel. The trial will comply with UK General Data Protection Regulation (GDPR) and Data Protection Act 2018, which requires data to be anonymised as soon as it is practical to do so.

16.7. Participant Financial Compensation

It is not intended that financial factors influence an individual's decision to participate in this study. The fees will reflect remuneration and not financial coercion. We compensate participants for time, travel, inconvenience and discomfort. The sums offered are consistent with remuneration in other similar local and national studies and are detailed below:

Visit time per hour	£40
Travel expenses per visit	£30
Sample collection per visit	£20
Full Diary completion	£30
Nasal biopsy visit	£150
Per Safety Phone calls post-biopsy	£5

Based on the above table, participants will be compensated £110 for attending the screening visit even if not eligible and safety samples have been obtained. Participants will receive £110 for attending vaccination and inoculation visit and £90 for each of the trial follow-up visits.

Additional reimbursement for unscheduled visits at £90 per visit will be provided. This will not be given unless an unscheduled visit occurs. The total amount of compensation for an individual participant will depend on the actual number of visits attended and whether any repeat or additional visits were necessary. If a participant withdraws consent for continued participation in the trial or is withdrawn for any other reason, they will still be compensated for any trial visits they attended. Based on the study visits on the main study cohort, each participant will receive a maximum of £920 whereas for the nasal biopsy group participants will receive a maximum of £715. Participants will receive an additional amount based on whether unscheduled visits were required and how many occurred. If participants refer a friend and they take part in the study, they will receive a £10 payment or a shopping voucher of equivalent value.

The reimbursement provided is considered to be reasonable amounts to cover the costs of participating in this study, therefore there should not be any consequences for tax and benefit purposes.

17. FINANCE AND INSURANCE

17.1. Financing

Funding for this study has been provided by Merck, Sharp & Dohme LLC, a subsidiary of Merck & Co, Inc., Rahway, NJ, USA (MSD).

17.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London).

17.3. Contractual Arrangements

Appropriate contractual arrangements will be put in place with all third parties.

18. PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Data from the study may also be used as part of a thesis for a PhD or MD. A lay summary of the study results may be provided to participants at the end of the study.

19. ARCHIVING

Study data may be stored electronically on a secure server by the University IT team, and paper notes will be kept in a secure location at the study site(s) or as outlined in local sites SOPs. We will store the research data and any research documents with personal information, such as consent forms, securely for up to 25 years after the end of the study, or as per national regulatory requirements. Anonymised research data may be stored indefinitely due to regulatory requirements or for scientific benefit, but with 5 yearly reviews.

Participants' bank details will be stored for a minimum of 7 years or in line with the University of Oxford financial policy. Volunteers who only complete online screening (before informed consent) will not have data kept beyond the end of the trial. General archiving procedures will be conducted in compliance to SOP OVC020 Archiving.

20. APPENDIX A: Investigator Signature and Declarations

Statement of Compliance

The trial will be conducted in compliance with the protocol, the principles of Good Clinical Practice Guideline, Medicines for Human Use (Clinical Trials) Regulations 2004 (as amended) and all other applicable regulatory requirements.

Chief Investigator Approval, Agreement and Conflict of Interest statement

I have read the trial protocol and agree to conduct the trial in compliance with the protocol, the principles of Good Clinical Practice and all applicable regulatory requirements.

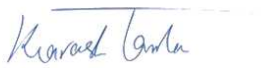
Conflict of interest statement:

Chief Investigator Dr Simon Drysdale	Signature 	Date: 31 Mar 2025

Lead Statistician Approval, Agreement and Conflict of Interest statement

I have read the trial protocol and agree to conduct the trial in compliance with the protocol, the principles of Good Clinical Practice and all applicable regulatory requirements.

Conflict of interest statement:

Lead Statistician Kiarash Tanha	Signature 	Date: 31 Mar 2025

Principal Investigator Approval, Agreement and Conflict of Interest statement

I have read the trial protocol and agree to conduct the trial in compliance with the protocol, the principles of Good Clinical Practice and all applicable regulatory requirements.

Conflict of interest statement:

Principal Investigator	Signature	Date:

21. APPENDIX B: Severity Grading Scales

Table 6 Laboratory Adverse Event Grading Scale

Adverse Event *	**Grade 1 (mild)**	**Grade 2 (moderate)**	**Grade 3 (severe)**
Sodium: hyponatraemia (mmol/L)	132 – 134	130 – 131	< 130
Sodium: hypernatraemia (mmol/L)	146	147	> 147
Potassium: hypokalaemia (mmol/L)	3.3 – 3.4	3.1 – 3.2	< 3.1
Potassium: hyperkalaemia (mmol/L)	5.4 – 5.5	5.6 – 5.7	> 5.7

Urea (mmol/L)	8.2 – 9.3	9.4 – 11.0	> 11.0
Creatinine (µmol/L)	132 – 150	151 - 177	> 177
ALT (IU/L)	50 – 112	113 – 229	> 229
AST (IU/L)	46 – 105	106 – 213	> 213
Bilirubin, with increase in LFTs (µmol/L)	23.1 – 25	26 – 31	> 31
Bilirubin, with normal LFTs (µmol/L)	23.1 – 33	34 – 41	> 41
ALP (IU/L)	143 – 272	273 – 402	> 402
Albumin (g/L)	28 – 31	25 – 27	<25
Haemoglobin: decrease from baseline value (g/L)	10 – 15	16 – 20	> 20
White cell count: Elevated (x 10^9/L)	11.10 – 15.00	15.01 – 20.00	> 20.00
White cell count: Depressed (x 10^9/L)	2.50 – 3.50	1.50 – 2.49	< 1.50
Neutrophil count (x 10^9/L)	1.50 – 1.69	1.00 – 1.49	< 1.00
Lymphocyte count (x 10^9/L)	0.75 – 0.89	0.50 – 0.74	< 0.50
Eosinophil count (x 10^9/L)	0.65 – 1.50	1.51 – 5.00	> 5.00
Platelet count (x 10^9/L)	125 – 149	100 – 124	< 100

Table 7 Vital Sign Adverse Event Grading Scale

Adverse Event *	**Grade 1 (mild)**	**Grade 2 (moderate)**	**Grade 3 (severe)**
Temperature (°C) **	38.0 – 38.4	38.5 – 38.9	39.0 – 40
Tachycardia (beats per minute)	101 – 115	116 – 130	>130
Bradycardia (beats per minute) ***	50 – 54	45 – 49	<45
Hypertension (systolic, mmHg)	141 – 150	151 – 155	>155
Hypertension (diastolic, mmHg)	91 – 95	96 – 100	>100
Hypotension (systolic, mmHg)	85 – 89	80 – 84	< 80

*Participant should be at rest for all vital sign measurements

**Oral temperature

***When resting heart rate is between 60 – 100 beats per minute. Clinical judgement will be used when characterising bradycardia amongst some healthy participants, for example conditioned athletes.

22. APPENDIX C: Amendment History

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
REC/MHRA Response	1.1	17 December 2024	Simon Drysdale, Carla Solorzano-Gonzalez	Removal of wording related to Docmail and CAG in section 10.3 Section 10.13. Additional information included regarding the challenge agent as requested by MHRA Also updated as part of the REC response: • Participant Information Sheet • Invitation Letter • Informed Consent Form Informed Consent Form Nasal biopsy Cohort
NSA01	1.2	07 January 2025	Simon Drysdale, Carla Solorzano-Gonzalez, Bruno Rocha de Macedo, Olga Mazur	Correction of typos throughout the protocol Exclusion criteria, section 9.3: participants that participated in an EHPC study in the previous 2 years will be excluded. Removal of "at the discretion of the study clinician" wording Table 1: Update on screening timelines to account for the biopsy cohort timelines Table 3: Removal of randomisation procedure from the table as the biopsy cohort won't be randomised Tables 2 and 3: Clarification that all samples in unscheduled visit should be

				optional and only obtained if clinically indicated Section 12.6: as requested by MHRA, we have clarified that AEs will be recorded from the signing of informed consent. Also as part of this amendment. Updates on: Online Screening Questionnaire to version 1.1: redistribution of the questions and correction of the automated messages Diary card to version 1.1: Removal of references to home sampling and correction of the grading table to match the study protocol
NSA02	1.3	10 March 2025	Hannah Robinson, Simon Drysdale, Bruno Rocha de Macedo, Carla Solorzano, Daniela Ferreira, Britta Urban, Julia Lustosa Martinelli	Protocol: Correction of some typos Table 2: We have clarified that nasal cells will be collected for a minimum of 40 participants (20 each site). Section 10.22: We have removed the references to the Biobank from this section and the schedule of events. A separate Biobank consent will not be used for this study as the study consent already contains an optional clause for participants to consent for future use of the research samples. Biobank allows for consent to be sought using the study ICF rather than on Biobank consent.

				Participant Information Sheet to version 1.2 and Nasal Biopsy Participant Information Sheet Cohort to version 1.1: Clarification that we will not use a separate consent for Biobank. Diary card to version 1.2: Addition of an extra column in the clinical symptoms tables to account for the end date if the symptom is still onfoinf after Day 7 as per study protocol Vaccination Card to version 1.1: Clarification on the sections that are applicable for the Oxford site. Clarification on the Randomisation review that needs to be performed before vaccination. Addition of an extra column on the Pre-Vaccination Checklist table (Page 1) with 'N/A (not applicable)" option to the question on changes being permitted by the protocol, in case no changes to medication/health have happened.
NSA03	1.4	18 March 2025	Emma Plested, Rawan Mahmud, Carla Solorzano-Gonzalez	Section 14.3: Clarification that safety and respiratory samples may contain participant's identifiable information. We have also updated Paricipant Information Sheet to version 1.3 and Nasal Biopsy Cohort Participant Information Sheet to version 1.2.

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