BMJ Open

Protocol for 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 in healthy participants aged 18–50 years in the UK (RATIONALE-15)

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To cite: Macedo BR, Solórzano C, Hyder-Wright A, *et al*. Protocol for 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 in healthy participants aged 18–50 years in the UK (RATIONALE-15). *BMJ Open* 2025;**15**:e106028. doi:10.1136/bmjopen-2025-106028

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (https://doi.org/10.1136/bmjopen-2025-106028).

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Received 03 June 2025
Accepted 03 November 2025

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ABSTRACT

Introduction *Streptococcus pneumoniae* serotype 3 (SPN3) remains a significant contributor to invasive pneumococcal disease globally, despite its inclusion in widely administered vaccines. The next generation of pneumococcal vaccines may confer better protection against this serotype, reducing disease burden. We describe an ethically approved protocol for a double-blind randomised controlled trial assessing the impact of VAXNEUVANCE (15-valent pneumococcal conjugated vaccine (PCV15)) and 0.9% saline (placebo) on the acquisition, density and duration of SPN3 carriage using a controlled human infection model.

Methods and analysis Healthy adults aged 18–50 years will be randomised 1:1 to receive PCV15 or placebo. Participants will be considered enrolled on the trial at vaccination. One month following vaccination, all participants will be intranasally inoculated with SPN3. Following inoculation, participants will be followed up on days 2, 7, 14 and 28 to monitor safety, SPN3 colonisation status, density and duration, as well as immune responses. The primary endpoint of the study is to assess the rate of SPN3 acquisition between vaccinated and unvaccinated participants defined by classical microbiological methods. Secondary endpoints will determine the density and duration of SPN3 colonisation and compare the immune responses between study groups. An exploratory cohort of 5 participants will be asked to consent to a nasal biopsy procedure during a screening visit and a second nasal biopsy 28 days after PCV15 vaccination. This cohort will only receive PCV15 and will not be challenged. Through this exploratory cohort, we will explore gene expression changes induced by PCV15 vaccination and their visualisation (spatial location) within the nasal tissue.

Ethics and dissemination This protocol has been reviewed by the sponsor, funder and external peer reviewers. The study is approved by the NHS Research and Ethics Committee (Reference: 24/SC/0388) and by the Medicines and Healthcare Products Regulatory Agency (Reference: CTA 21584/0485/001-0001).

Trial registration number NCT06731374 – ISRCTN91656864.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study will employ a controlled human infection model to evaluate the protection conferred by 15-valent pneumococcal conjugated vaccine (PCV15) against *Streptococcus pneumoniae* serotype 3 (SPN3) carriage and to investigate vaccine-induced immunity.
⇒ SPN3 is a leading cause of disease and using carriage as an endpoint, we may translate reductions in acquisition and density into potential decreases in disease incidence and community transmission.
⇒ Nasal biopsies before and after PCV15 vaccination may provide deeper insights into vaccine-induced mucosal immune responses.
⇒ This is a controlled infection model with healthy young adults; results may not be fully generalisable to at-risk populations.
⇒ This study uses a single SPN3 strain and will not provide information on the long-term vaccine protection against carriage.

INTRODUCTION

Burden of pneumococcal disease

Lower respiratory tract infections are the leading cause of death globally, with an estimate of 2.18 million deaths in 2021 of which over 500 000 were in children under 5 years of age.[1] More deaths are attributable to *Streptococcus pneumoniae* (SPN, pneumococcus) than to all other lower respiratory tract aetiologies combined, across age groups and income levels.[2] Access to pneumococcal vaccination has substantially reduced mortality, notably among children under 5 years of age. Adults over 65 years of age are the other group at high risk of mortality in which targeted pneumococcal vaccination should be sought.[1]

Pneumococci adhere to the epithelial cells of the nasopharynx, leading to colonisation (or carriage) and transmission. Colonisation is as frequent as 40%–95% among infants[3] with reducing rates in later childhood[4 5] until it reaches 5%–25% among adults ≥18 years old[3 6] and 0%–10% among adults ≥65 years old.[7] Carriage is usually transient and can be impacted by risk factors such as age, smoking history and sharing the household with children.[6] Colonisation is a prerequisite for infection and plays a key role in transmission, although most colonisation episodes will not lead to disease.[8]

There are more than 100 serotypes of pneumococcus classified according to capsule antigens. Currently licensed vaccines include the pneumococcal polysaccharide vaccine (PPV-23) and the conjugate vaccines (pneumococcal conjugated vaccines (PCVs), eg, PCV13, PCV15 and PCV20), included within paediatric and adult vaccine schedules, and are targeted towards serotypes causing the most disease and antibiotic resistance.[9 10] However, pneumococcal serotypes causing disease have continued to evolve due to selective pressure and in response to vaccine use. The development of new PCVs against relevant serotypes is critical for expanded protection globally while maintaining suppression of serotypes included in prior PCVs.[11]

Licenced pneumococcal vaccine and serotype 3 protection

High-income and middle-income countries employ PPV-23, PCV13, or a combination thereof for immunisation of older adults and those at risk.[12–15] In the UK (2014–2018), PCV13 serotypes accounted for 20% of invasive pneumococcal disease (IPD) cases, with serotype 3 comprising approximately half.[16] Although PCV13 demonstrates efficacy against serotype 3 (SPN3) in both adults and children,[17–19] antibody responses to SPN3 are comparatively lower,[20] potentially due to immune evasion mechanisms such as capsule shedding and mucoid phenotype.[21 22] Persistent SPN3 circulation post-vaccination may explain its continued role in IPD.[20] Notably, the only randomised controlled trial (RCT) assessing PCV13's impact on colonisation of SPN3, conducted in children, found no reduction in carriage.[23]

VAXNEUVANCE (MSD) is a 15-valent PCV containing the serotypes included in PCV13, with the addition of serotypes 22F and 33F. In the UK, PCV15 is licenced for infants under 1 year of age as part of the UK's national immunisation programme and for children and adults in certain at-risk groups.[24] 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.[11 15 25–35] 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 using a twofold non-inferiority margin for the ratio of Opsonophagocytic activity (OPA) geometric mean titres (GMTs) (PCV15/PCV13) post-vaccination). In addition, PCV15 met superiority criteria compared with 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 ≥four-fold fold rise).[15]

Experimental human pneumococcal challenge model

Controlled human infection models (CHIMs) could play a critical role in advancing vaccine research and elucidating host–pathogen dynamics. These models generally involve healthy young adults with minimal risk for severe disease. In the experimental human pneumococcal challenge (EHPC) model, participants receive a controlled intranasal inoculation of a defined dose of pneumococcus, leading to transient colonisation in some individuals at levels comparable with natural exposure. Therefore, this model enables efficient and cost-effective evaluation of pneumococcal vaccine efficacy (VE) against colonisation.[36–38] In addition, the collection of longitudinal samples allows the monitoring of the colonisation status, density and host immune response to both natural and vaccine-induced pneumococcal antigens.

To advance our understanding of both natural and vaccine-induced immunity to SPN3, a human challenge model has been established in healthy adults aged 18–50 years. Multiple SPN3 strains were evaluated across a range of inoculum doses, demonstrating a favourable safety profile and colonisation rates comparable with those observed with the well-characterised SPN6B model.[39] Selected SPN3 strains were subsequently used in a Phase IV, double-blind, RCT assessing the impact of PCV13 and PPV23 vaccination compared with placebo on pneumococcal colonisation, using the SPN3 EHPC model.[40]

Study rationale

Given the expanded serotype coverage and preserved immunogenicity of PCV15, along with its enhanced immune response to SPN3 in both adult and infant populations, this study aims to evaluate the vaccine's direct impact on experimental SPN3 colonisation 1 month post-vaccination. In addition, we will explore potential immunological correlates of protection by analysing humoral and cellular responses, including antibody quantity and function, as well as B cell-mediated immunity.

Table 1 Objectives and outcome measures

	Objectives	Outcome measures
Primary	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)	The rate of experimental SPN3 colonisation determined by the presence of experimental SPN3 in NW by classical culture at any evaluated time point (D2, 7, 14, 28) following EHPC at 1 month after vaccination in PCV15 versus control
Secondary	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	The density of experimental SPN3 colonisation in NW at each and any time point (D2, 7, 14, 28) by classical culture and molecular methods following EHPC at 1 month after vaccination in PCV15 versus control
	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	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
	To compare vaccine-induced immune responses to those who receive PCV15 versus control before and up to 28 days after experimental SPN3 challenge	Assessment of immune responses, including but not limited to: mucosal and systemic antibody levels, antibody functionality and cellular population levels before and after vaccination and EHPC
Exploratory	To compare selected immune parameters, both at the cellular and humoral level, between the nasal mucosa, secondary lymphoid tissue and systemic circulation	Analysis of antibody level, function, inflammatory markers and cell populations (cellular and transcriptome level) in the nasal mucosa, secondary lymphoid tissue and systemic circulation, and compare data with those generated at the nasal mucosa
	To characterise the transcriptional changes in immune cells in response to vaccination and experimental challenge	Determination of gene induction and regulation to identify patterns in individuals who become experimentally colonised versus those who remain protected
	To describe symptoms following EHPC with SPN3	The presence of mild or moderate symptoms as recorded on a Likert scale in participants with SPN3 within the first 7 days after EHPC
	To characterise nasal cells' gene expression alterations and their visualisation (spatial location) within the nasal tissue induced by PCV15 vaccination	Analysis of the spatial microenvironment and transcriptomics of the nasal tissue before and 28 days after PCV15 vaccination

EHPC, Experimental Human Pneumococcal Carriage; NW, Nasal Wash; PCV15, 15-valent pneumococcal conjugated vaccine; SPN3, Streptococcus pneumoniae serotype 3.

STUDY AIMS AND OBJECTIVES

This study aims to assess the rate of SPN3 acquisition by classical microbiology between vaccinated (PCV15) and unvaccinated (placebo) participants for 28 days after SPN3 challenge. We hypothesise that the colonisation rate will be different between the PCV15 vaccinated and the placebo groups, with a smaller acquisition rate in the PCV15 group. Secondary objectives include determining the density and duration of the SPN3 colonisation episode following challenge. Exploratory objectives will include the analysis of the immune responses induced after vaccination and challenge and the characterisation of the symptoms following SPN3 challenge. Primary, secondary and exploratory objectives and outcomes are detailed in table 1.

METHODS AND ANALYSIS

Trial design

This protocol has been reported following the standard protocol items: recommendations for interventional trials reporting guidelines (online supplemental material 1). The study is expected to run from January 2025 to December 2025.

This is a Phase IV Double Blind (participant and observer) Placebo Controlled RCT that will assess the superiority of PCV15 against placebo in healthy adults aged 18–50 years exposed to an EHPC. The study protocol V.1.4 from 18 March 2025 can be accessed in the online supplemental material 2. Participants will be randomised 1:1 to receive PCV15 or placebo (0.9% saline). One month following randomisation and vaccination with

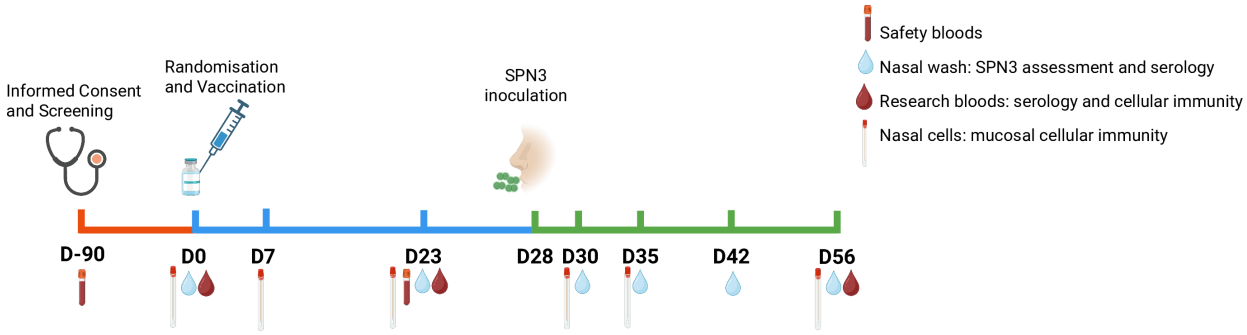

Figure 1 Trial timeline and samples collected for the main study cohort. SPN3, *Streptococcus pneumoniae* serotype 3. Created with BioRender.com.

PCV15 or placebo, all participants will be intranasally inoculated with 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). LIV014-S3 was isolated from a healthy young natural carrier participant of a previous EHPC study.[41] This strain was used in a recent dose-ranging and reproducibility study.[42] In that study, 43 participants were inoculated with 80 000 CF/ml and boosted with the same dose and inoculum 14 days after prime inoculation if colonisation was negative before then. Preliminary data show that a single inoculation provides a 72% attack rate, which increases to an overall experimental carriage of 86% after booster inoculation.

Follow-up for 28 days (days 2, 7, 14 and 28) will occur in the clinic with the assessment of laboratory measures of the acquisition of nasal pneumococcal colonisation and of immune response after which participants may require a 5-day course of antibiotics under certain circumstances (figure 1). Participants will be considered enrolled on the trial at vaccination.

The study will have an exploratory nasal biopsy cohort (figure 2) in which five participants (not included in the primary endpoint sample size) will consent to a nasal biopsy procedure to be performed 21 days prior to PCV15 vaccination and a second nasal biopsy 28 days afterwards. 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 the second biopsy visit. Participants will be considered enrolled after the first nasal biopsy procedure.

Study participants

Recruitment and study sites

Participants will be recruited using various 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.

Information about the study will direct volunteers to the study website where they will find the participant information sheet (online supplemental materials 3 and 4) and the study team's contact details. Volunteers willing to proceed will be asked to complete an initial online screening questionnaire that will be reviewed by the study team. Those eligible at this point 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 in Oxford (Centre of Clinical Vaccinology and Tropical Medicine, Oxford Vaccine Group (OVG)) and Liverpool (Accelerator Research Clinic, Liverpool School of Tropical Medicine, Liverpool Vaccine Group). If the participant meets the eligibility criteria, they will be invited to provide written informed consent with a delegated member of staff (online supplemental materials 5 and 6).

Participants will be compensated for their time, travel, inconvenience and discomfort.

Eligibility assessment

Healthy participants aged 18–50 years (inclusive) who are fluent in English and can give informed consent will be

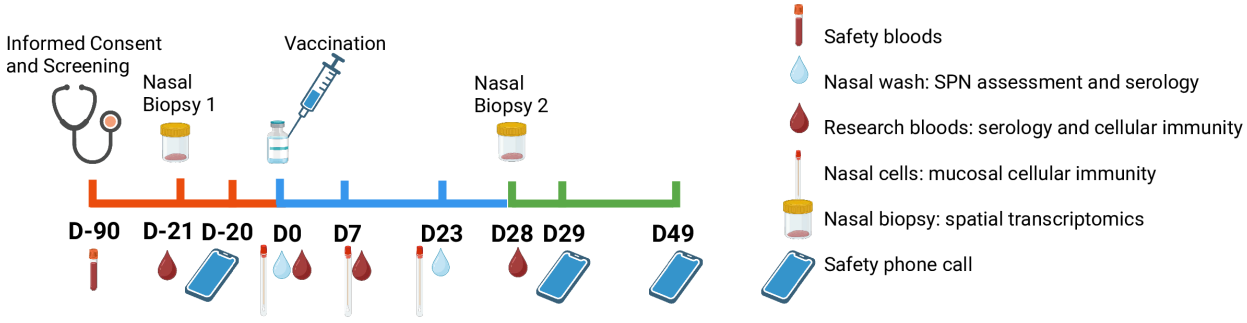

Figure 2 Trial timeline and samples collected for the exploratory nasal biopsy cohort. SPN, *Streptococcus pneumoniae*. Created with BioRender.com.

Box 1 Inclusion criteria

- ⇒ Adults between 18 and 50 years old.
- ⇒ Medically healthy and able to remain in the study.
- ⇒ Fluent spoken English.
- ⇒ Able to attend the scheduled visits and to comply with all study procedures.
- ⇒ Willing and able to give informed consent.
- ⇒ Willing to allow confirmation of past medical history.
- ⇒ Willing to allow General Practitioner (GP) and/or consultant, if appropriate, to be notified.
- ⇒ Willing to provide information for registration in the over volunteering prevention system.
- ⇒ For participants of childbearing potential only: willing to use effective contraception and to take a pregnancy test.

allowed to take part in the study. Other inclusion criteria are described in box 1.

Table 2 outlines the exclusion criteria for the main study and the nasal biopsy cohort.

Additionally, illness, an incidental finding, or an adverse event (AE) requiring investigation to resolve or stabilise prior to vaccine or inoculation will be temporary exclusion criteria. Table 3 describes the full list of temporary exclusion criteria.

Study procedures and interventions

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 unblinded team. At the vaccination visit, participants will be randomised into 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 based on participants' sex (male/female) and study sites.

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

Blinding

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 participants as well as blinded study staff. No unblinded stakeholders will be involved in the assessment of AEs related to the vaccine or any clinical assessments following inoculation or in study endpoint sample analysis. Core medical doctors will remain blinded to the vaccination allocation throughout the study to ensure thorough assessment of symptoms, AEs and severe adverse events (SAEs).

Participants will remain blinded until the completion of the last participant, last study visit (Day 56). Participants will be unblinded by the clinical team on confirmation of the last participant, last visit. Participants will be notified of the vaccine they had received during the study. Participants who received placebo will be invited to attend the clinic for administration of a single dose of PCV15 vaccination.

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

Vaccination

Participant's continued consent is verbally confirmed and interim medical history since the screening visit, including medication use and other vaccinations, is conducted. Observations are taken and recorded; targeted physical examination is performed as required. Eligibility criteria are reviewed and specifically, temporary exclusion to vaccination is completed. Participants of childbearing potential will have a urine pregnancy test performed as well as the other research samples. 1 dose (0.5 mL) of the study injection (PCV15 or placebo) is given IM into the deltoid muscle of the non-dominant arm (preferentially) and the participant is observed for a minimum of 15 min.

Pneumococcal inoculation

On day 28 post-vaccination, participants will be inoculated with SPN3. Participant consent and eligibility will be reviewed as described for vaccination visit. The inoculum will be prepared using local standard operational procedures. Briefly, a mid-log broth culture of amoxicillin-sensitive serotype 3 SPN (Clade Ia, strain LIV014-S3) will be frozen at −80°C in aliquots of glycerol-enriched media. On experimental inoculation days, aliquots will be thawed, washed twice and re-suspended at the correct density. Participants will be inoculated with a dose of 80 000 CFU/100 µl per naris of the inoculum.

All participants will be followed up for 28 days after inoculation and complete an electronic diary of solicited and unsolicited symptoms for 7 days following the inoculation visit (online supplemental material 7).

Participants will receive a study safety pack including safety information leaflet, study emergency contact card with a 24 hours phone number, an antibiotic pack and log and a paper back-up of the diary. Participants who report symptoms consistent with pneumococcal disease will be reviewed by a clinician (ideally in person) and may be instructed to take the antibiotic course.

Participants from the nasal biopsy cohort will not be inoculated.

Follow-up visits

Participants will be followed up for 28 days post-inoculation. During the visits, the study team will ensure that participant consent remains valid, obtain and document interim medical history, collect respiratory samples

Table 2 Exclusion criteria

Research participant	Participation in another trial that could compromise the integrity of this study or is planning to do so within the trial period
	Participant in a previous EHPC within the last 2 years
Vaccination	Any pneumococcal vaccination in the past 5 years
	Planned vaccination during the study
Allergies	Allergy to amoxicillin or penicillin (main study only)
	Previous anaphylaxis or severe adverse reaction to any component of the vaccine or to any vaccine
	Allergy to lidocaine anaesthetic (nasal biopsy cohort only)
Health history	Asplenia or dysfunction of the spleen
	Chronic respiratory disease (eg, asthma (on medication), COPD, emphysema, bronchiectasis)
	Chronic heart disease (controlled stable hypertension +/– angina may be included)
	Chronic kidney disease (eg, nephrotic syndrome, kidney transplant, on dialysis)
	Chronic liver disease (eg, cirrhosis, biliary atresia, hepatitis)
	Chronic neurological conditions
	Connective tissue disease
	Dementia
	Diabetes mellitus (including diet controlled)
	History of bleeding disorder or prior bleeding or bruising following IM injection or venepuncture
	Immunosuppression or history of receiving immunosuppressive therapy—at the discretion of the investigator
	Individuals with cochlear implants
	Individuals with major cerebrospinal fluid leaks
	Recurrent otitis media
	History of significant unexplained bleeding after a surgical or dental procedure (for nasal biopsy participants only)
	Have any uncontrolled medical/surgical/mental health conditions at the discretion of the study doctor
	Major pneumococcal illness requiring hospitalisation within the last 10 years
	Significant mental health condition
Taking medications	Any medication affecting the immune system in the last 3 months (systemic steroids, Roaccutane, anti-rheumatoid drugs)
	Long term use of antibiotics
	Use of medication or other product for symptoms of rhinitis or nasal congestion within the last 1 month
	Use of medication affecting blood clotting
Female participants	Pregnant, lactating or intending on becoming pregnant during the study
Direct caring role or close contact with individuals at higher risk of infection (main study only)	Children under 5 years of age
	Chronic ill health or immunosuppressed adults
	Older adults ≥65 years of age
Smoker	Current or ex-smoker in the last 6 months
	Previous significant smoking history (more than 20 pack years)
Alcohol or drug abuse	Known or suspected, as per investigator discretion
Overseas travel	During the challenge follow-up period after inoculation
Other issues	That put participant or their contacts at risk because of the study
	That would adversely affect the interpretation of the results
	That would impair the participant's ability to participate
	Study site staff or a partner are not allowed to enrol in the study

Table 3 Temporary exclusion criteria

Inoculation	Current illness or acute illness within 14 days
	Positive COVID-19 swab within 14 days
	Antibiotic use within 28 days
	Vaccination with an approved COVID-19 vaccine in the 14 days preceding inoculation
Vaccination	Vaccination with an inactivated vaccine/mRNA in the 14 days or with a live vaccine in the 28 days
Nasal biopsy	Antibiotic use: delay nasal biopsy for at least 1 week from the last date
	Dental infections: delay nasal biopsy for at least 2 weeks after the last day of illness

and perform blood draws as per the study schedule of events.

Antibiotics

Participants under certain criteria will be asked to take the antibiotic treatment:

- SPN3 carrier in at least one nasal wash (NW) without two posterior consecutive negative nasal washes.
- If unwell, as instructed by the study doctor.
- If you are unwell and unable to contact the research team.
- Under the investigator's discretion.

The antibiotic is oral amoxicillin 500 mg, taken three times daily for 5 days, and will be supplied to participants at the inoculation visit (D28).

Nasal biopsy

Participants in the nasal biopsy cohort will consent to have this procedure 21 days prior to vaccination and 28 days after vaccination. The procedure is carried out by an Ear, Nose and Throat consultant following the UK NHS guidelines in an outpatient setting. The tissue is recovered from the inferior turbinate and/or the postnasal space under local anaesthesia.

Measurements

Microbiological

Colonisation will be defined by the result of NW taken at 2, 7, 14-days and 28-days post-inoculation. NW will be plated onto culture media and incubated overnight at 37°C in 5% carbon dioxide. Colonies will be confirmed as pneumococcus using classical microbiological techniques. Typing by latex agglutination will be performed using a commercial kit to confirm pneumococcal serogroup. Results from the cultured NW will also be confirmed using PCR-based methods of bacterial detection. SPN3 isolates will be frozen at −80°C for storage to allow for confirmation of experimental colonisation by sequencing when required. Colonisation will be assessed at both study sites. DNA will be extracted from NW for PCR multiplex to allow for the detection of carriers with very low bacterial density.

Respiratory pathogens multiplex PCR for detection and quantification will be performed on DNA and RNA of stored swab and/or NW to detect all common respiratory pathogens. Several parameters of humoral and cellular responses will be analysed against the acquisition and clearance of colonisation.

Immune and genetic

Vaccine and challenge-induced mucosal immune responses will be analysed in the nasal cells collected using minimally-invasive superficial nasal scrape and a flocked swab. Blood samples will be assessed for immunoglobulins, inflammatory markers, peripheral blood mononuclear cell populations (cellular and transcriptome), and host RNA expression for gene induction and regulation. The nasal biopsy samples will be analysed for spatial microenvironment and transcriptomics.

Sample size

We will aim to enrol 84 evaluable participants with a primary endpoint available, 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, based on rates observed in previous studies.[39 42] 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 an 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 included in the primary outcome analysis.

Statistical analysis

The primary outcome of reduction in colonisation rate determined by the result of the NW samples will be assessed in the Per Protocol population, including participants who received the allocated vaccine, were inoculated with SPN3 and were not naturally colonised with pneumococcus. Colonisation rates between the two arms will be compared using a generalised linear model adjusting for site and sex as randomisation stratification variables. Relative risk (RR) and 95% CIs will be reported and VE will be calculated as (1-RR).

A secondary analysis of the primary endpoint will be conducted using the Kaplan-Meier method in the full analysis set population including all randomised participants who received the study IMP or control.

Immunogenicity data are expected to be highly skewed and will be log-transformed prior to analysis. 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 using a linear mixed model. The log of area under the curve of density of experimental pneumococcal colonisation will be analysed using a linear model with a single factor of vaccine arm.

ETHICS AND DISSEMINATION

Ethical approval, regulation and governance

This protocol has been reviewed by the sponsor, funder and external peer reviewers. The study is approved by the NHS Research and Ethics Committee (Reference: 24/SC/0388) and by the Medicines and Healthcare Products Regulatory Agency (Reference: CTA 21584/0485/001-0001). The study will be conducted following the ethical principles of the Declaration of Helsinki and in line with Good Clinical Practice (GCP) guidelines. The Investigator will submit and obtain approval from the above parties for all substantial amendments to the original approved documents.

The study sponsor has a specialist insurance policy in place, which would operate in the event of any participant suffering harm because of their involvement in the research.

The CI, PI and study site investigators will form the trial management group (TMG and will provide ongoing management of the trial.

An independent Data and Safety Monitoring Committee (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.

Risks

The study risks to participants are associated with the pneumococcal inoculation and to a minor extent to unexpected adverse reactions to the PCV15 vaccine or to amoxicillin. Some study participants will develop mild infection symptoms following inoculation, with a very low risk of IPD. The selection of the challenge strain, participant selection and education, daily monitoring of symptoms and provision of standby antibiotics to reduce time-to-treatment aim to greatly minimise these risks. Participants who remain SPN3 carriers without two consecutive negative NW samples will also be asked to take the antibiotics.

Participants in the nasal biopsy cohort have risks associated with the procedure. Those are usually local symptoms resolving in the first 24 hours. There is a small risk (<1%) of bleeding that would require procedures or even surgical interventions for epistaxis.

Table 4 Adverse event grading

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

Safety reporting

All AEs occurring from inoculation visit until 28 days post-challenge will be recorded and graded (table 4). Because PCV15 is a licensed vaccine, AE following vaccination visit will not be recorded, only severe AEs and severe adverse reactions (online supplemental material 8).

All solicited AEs will be automatically assumed to be related to challenge, whereas all unsolicited AEs and SAEs will undergo causality assessment in relation to the study intervention. Unsolicited AEs 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 AEs recorded in the e-diary will be severity graded by the participant using the same Likert scale (0–3).

The following events will be considered adverse events of special interest: IPD, otitis media, periorbital cellulitis, sore throat and AEs requiring a physician visit or Emergency Department visit related to the challenge.

An independent DSMC will be appointed and operate in accordance with a charter, which will be established before recruitment starts.

Auditing

A Monitoring Plan will be developed by the OVG and agreed on by the study team and CI, and it will be based on the trial risk assessment. Internal monitors from the OVG will verify that the clinical trial is conducted in accordance with the current approved protocol, GCP, relevant regulations and Standard Operating Procedures.

Data access and management

PI-delegated staff will populate the content of participants' Clinical Reseach Forms (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. Laboratory data for secondary and exploratory endpoints will be stored on secure servers on the University of Oxford MSDIT network.

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.

At the completion of the study, unless participants consent otherwise (eg, requesting to be informed of other studies), participants' personal details will not be used to contact them other than for 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.

Dissemination

Results will be published in peer-reviewed journals and at scientific conferences. In addition, we will produce a lay report of our findings, which will be made available to all participants. Authorship for the publications will include those who contribute to the design, delivery and analysis of the trial. Authorship will be defined on study completion in line with the International Committee of Medical Journal Editors guidelines.

Public and patient involvement and engagement (PPIE)

The Oxford Vaccine Centre Patient and Public Involvement and Engagement group was consulted for this study. A focus group was organised with three PPIE members to discuss the study design and objectives. This group reviewed participant-facing documents such as participant information sheets, informed consent forms and recruitment material, and their feedback was considered. PPIE members also input on recruitment strategies and how to disseminate findings to the wider community.

Limitations

This is a study of UK healthy young adults in a controlled infection model, meaning that results may not be fully generalisable to at-risk populations (older adults, adults with comorbidities and young children), and the conditions may not reflect natural exposure. Challenge occurs at only one specific time point (28 days after vaccination, when antibody levels are likely to be at or near a peak) and thus cannot assess the impact of PCV15 on carriage on earlier or later challenge as would often be the case with natural exposure. In addition, SPN3 strains exhibit a high degree of genetic diversity with distinct lineages, and this study is assessing VE and immunological mechanisms of only one strain of this serotype. Therefore, results may not translate to other SPN3 strains.

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Acknowledgements We would like to acknowledge the members of the DSMC: Professor Robert Read (chair), Dr Malick Gibani and Professor Maia Lesosky for their oversight. We would like to acknowledge the support offered by the Northwest Coast Clinical Research Network (National Institute for Health Research) with staffing.

Contributors Study design set-up: BRM, CS, AH-W, JLM, HR, SB-M, BCU, TC, FE, EM, AH, DES, KT, XL, OM, MNR, AC, DMF and SBD. Statistics: KT and XL. Ethics submission: BRM, CS, AH-W and SBD. Study coordination: BRM, CS, AH-W, JLM, HR, SB-M, MNR, BCU, TC, FE, AH and DES. Writing the protocol: BRM, CS, AH-W, HR, KT, AC, DMF and SBD. Bacterial selection, bacterial inoculum preparation and laboratory set-up: CS, BCU, TC, FE, EM and DMF. Manuscript writing: BRM, CS, KT, DMF and SBD. BRM and CS are joint first authors. AC, DMF and SBD are the joint last authors. DMF is the guarantor of this contributorship statement.

Funding This study will be conducted in collaboration between the University of Oxford and the Liverpool School of Tropical Medicine. The University of Oxford is the study sponsor. This study is funded by a research grant from the Investigator-Initiated Studies Programme of Merck Sharp & Dohme LLC (MISP101865). The opinions expressed in this paper are those of the authors and do not necessarily represent those of Merck Sharp and Dohme LLC.

Competing interests This study received funding from MSD, which manufactures VAXNEUVANCE. There are no competing interests for any author.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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