Study title: Experimental Human Pneumococcal Challenge in Older Adults

Study Code: AGES-2

Research Ethics Reference: 24/EE/0038
IRAS Project ID: 340045
Date and Version number: 8th July 2025, V4.0

Chief Investigator: Dr Ben Morton
Lead Scientific Investigator: Prof Daniela Ferreira
Principal Investigator: Angela Hyder Wright
Sponsor: Liverpool School of Tropical Medicine
Funder: Medical Research Council
ISRCTN number: 71362981

Chief Investigator Signature

None of the investigators declare any conflicts or potential conflicts of interest.

Confidentiality Statement

Protocol Signature Page

Study Name

Experimental Human Pneumococcal Challenge in Older Adults (Ages-2)

Version 4.0, Date 8th July 2025

The undersigned has read and understood the trial protocol detailed above and agrees to conduct the trial in compliance with the protocol.

Dr Ben Morton		Liverpool School of Tropical Medicine	
Chief Investigator (Please print name)	**Signature**	**Site name**	**Date**
Angela Hyder Wright		Liverpool School of Tropical Medicine	
Principal Investigator	**Signature**	**Site name**	**Date**

Following any amendments to the protocol, this page must be updated with the new protocol version number and date and re-signed by the site PI.

1. Key contacts

Chief Investigator and TMG	**Dr Ben Morton** Senior Clinical Lecturer Liverpool Vaccine Group, Liverpool School of Tropical Medicine, Accelerator Building, 1 Daulby Street, Liverpool, L7 8XZ Consultant Physician, Liverpool University Hospital NHS Foundation Trust. Ben.morton@lstmed.ac.uk
Lead Scientific Investigator and TMG	**Prof Daniela Ferreira** Oxford Vaccine Group, University of Oxford, Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital, Oxford, OX3 7LE, United Kingdom Liverpool Vaccine Group, Liverpool School of Tropical Medicine, Accelerator Building, 1 Daulby Street, Liverpool, L7 8XZ Daniela.ferreira@paediatrics.ox.ac.uk
Principle Investigator and TMG	**Angela Hyder-Wright** **Senior Research Nurse** Liverpool Vaccine Group, Liverpool School of Tropical Medicine, Accelerator Building, 1 Daulby Street, Liverpool, L7 8XZ, United Kingdom **Angie.hyder-wright@lstmed.ac.uk**
Laboratory Lead and TMG	**Dima ElSafadi** Liverpool Vaccine Group, Liverpool School of Tropical Medicine, Accelerator Building, 1 Daulby Street, Liverpool, L7 8XZ dima.elsafadi@lstmed.ac.uk
Laboratory Study Co-ordinator	**James Court** Liverpool Vaccine Group, Liverpool School of Tropical Medicine, Accelerator Building, 1 Daulby Street, Liverpool, L7 8XZ James.court@lstmed.ac.uk
Co-Investigator (Clinical) and TMG	**Dr Andrea Collins** Senior Clinical Lecturer Liverpool Vaccine Group, Liverpool School of Tropical Medicine, Accelerator Building, 1 Daulby Street, Liverpool, L7 8XZ

	Andrea.Collins@lstmed.ac.uk **Dr Oliver Hamilton** Clinical Research Fellow Liverpool Vaccine Group, Liverpool School of Tropical Medicine, Accelerator Building, 1 Daulby Street, Liverpool, L7 8XZ, United Kingdom Oliver.hamilton@lstmed.ac.uk
Trial Manager and TMG	**Paula Saunderson** Liverpool Vaccine Group, Liverpool School of Tropical Medicine, Accelerator Building, 1 Daulby Street, Liverpool, L7 8XZ, United Kingdom Paula.saunderston@lstmed.ac.uk
Collaborators	**Prof Robert Heyderman** Division of Infection and Immunity, University College London, Rayne Building, 5 University Street, London WC1E 6JF r.heyderman@ucl.ac.uk
Sponsor	Liverpool School of Tropical Medicine Pembroke Pl, Liverpool L3 5QA
Funder	Medical Research Council
Site	Liverpool Vaccine Group, Liverpool School of Tropical Medicine (LSTM), Liverpool Life Sciences Accelerator Building, 1 Daulby Street, Liverpool, L7 8XZ, United Kingdom

2. Lay Summary

The germ 'pneumococcus' (*Streptococcus pneumoniae,* a bacteria) is a major cause of pneumonia, meningitis and sepsis around the world leading to over a million deaths per year. The biggest impact is on young children and older adults, especially in low-income countries. However, most of the information that we have about how the body responds to this infection comes from younger adults. These younger adults are less likely to develop severe infection and how they respond to germs (bacteria and viruses) is different to how older adults respond.

In this study, we will look at how pneumococcus lives in the noses of older adults and causes infection. We will be looking at different strains of the germ to see if there are differences in how this happens. The aim is to better understand how this germ/bacteria affects older people, which will hopefully help us better design medication and vaccinations for this age group.

Over the past decade, our group has developed innovative methods to study pneumococcus infection in healthy volunteers. People are carefully screened for safety and then infected ("challenged") with small amounts of bacteria in their nose, which allows us to understand why some people who are exposed to the bacteria develop infection whilst others don't. This kind of study (a "human challenge" study) can improve understanding about how germs cause disease and is a cost-efficient way of designing and testing drugs and vaccinations. To date we have challenged over 2000 participants with pneumococcus, including older people, and demonstrated these studies can be done safely without harm to participants. In this study, older adults will be given pneumococcus in their nose so we can run tests to see how the germ affects them and sticks to their nose. We will take samples throughout the study so that we can understand how the immune system responds to it.

Safety will be paramount in this study. Participants will have access to a study doctor 24/7 and be given back-up antibiotics to take if needed. We will work closely with participants so that they understand study requirements and will address their questions and concerns.

3. Synopsis

Study Title	Experimental Human Pneumococcal Challenge in Older Adults
Study Code	Ages-2
Chief Investigator	Dr Ben Morton
Sponsor	Liverpool School of Tropical Medicine
Funder	Medical Research Council
Trial Design	Open label human challenge study with two phases
Study Centres	Liverpool School of Tropical Medicine
Study Participants	Healthy adults aged 50-84 (inclusive)
Planned study size	Phase A (Serotype 6B): 10-15 participants Phase B (Serotype 3): 10-15 participants
Study agents	*Streptococcus pneumoniae* serotype 6B (Spn6B) *Streptococcus pneumoniae* serotype 3 (Spn3)
Doses	Spn6B: 80,000 colony forming units (CFU)/naris Spn3: 80, 000 CFU/naris
Follow-up duration	28 days
Sampling	See visit schedule (Table 3)
Study interventions	Intranasal inoculation with Spn6B and Spn3 suspension
Primary objective	To assess the presence of pneumococcus in the nasal epithelium following experimental inoculation
Outcome measures	Detection of Spn6B (Phase A) or Spn3 (Phase B) in nasal samples by confocal microscopy and molecular methods
Secondary objectives	1. Rates of colonisation by pneumococcus and its density and duration by detection of Spn6B (Phase A) or Spn3 (Phase B) from one or more nasal wash sample in the 28 days following initial pneumococcal challenge. 2. Changes in the population of immune/inflammatory cells in participants' nasopharynges in response to challenge and/or colonisation. 3. Quantification of a systemic and mucosal humoral immune response to nasopharyngeal (NP) carriage 4. Quantification of a systemic and mucosal cellular immune response to NP carriage 5. Demonstration of functional activity of the above antibodies, as measured by assays including opsonophagocytic killing assays.
Exploratory objectives	1. Examine the epithelial innate-inflammatory response to colonisation in older adults 2. Identify transcriptomic signatures associated with micro-invasion and impaired pneumococcal clearance

4. Abbreviations

AE	Adverse Event
AUC	Area Under the Curve
CAP	Community Acquired Pneumonia
CFU	Colony Forming Units
CHIM	Controlled Human Infection Model
CRF	Case Report Form
DBRCT	Double blinded randomized-controlled trial
DSMC	Data, Safety and Monitoring Committee
EHPC	Experimental Human Pneumococcal Challenge
ELISA	Enzyme-Linked Immunosorbent Assay
ELISpot	Enzyme-Linked Immune Absorbent Spot
EPR	Electronic Patient Record
FBC	Full Blood Count
GCP	Good Clinical Practice
GEE	Generalised estimating equations
GI	Gastrointestinal
GLMM	Generalised linear mixed models
GPQ	General practitioner questionnaires
hCoV	Human Coronavirus
ICF	Informed Consent Form
ICH-GCP	International Conference on Harmonisation of Good Clinical Practice
LAIV	Live attenuated influenza vaccine
LRTI	Lower respiratory tract infection
LSTM	Liverpool School of Tropical Medicine
MHRA	Medicines and Healthcare products Regulatory Authority (UK Regulator)
mRNA	Messenger ribonucleic acid
MTA	Material Transfer Agreement
NAAT	Nucleic Acid Amplification Test
NW	Nasal wash
OM	Otitis Media
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
PCV	Pneumococcal conjugate vaccine
PFU	Plaque-forming units
PHE	Public Health England
PIC	Participant Identification Centre
PIS	Participant Information Sheet
PPSV23	Pneumococcal polysaccharide vaccine 23
qPCR	Quantitative Polymerase Chain Reaction

RCT	Randomised-controlled trial
RNA	Ribonucleic Acid
RT-qPCR	Real-time qPCR
SAE	Serious Adverse Event
SOP	Standardised Operating Procedure
Spn	*Streptococcus pneumoniae*
TDS	Three times a day
TSC	Trial Steering Committee
UKHSA	UK Health Security Agency
URTI	Upper Respiratory Tract Infection

5. Background and rationale

The *Streptococcus pneumoniae* (Spn, pneumoccocus) bacterium is a major cause of pneumonia, sepsis and meningitis worldwide, particularly in children and older adults, causing almost half a million deaths in those over 70 per year (1). Whilst vaccination against pneumococcus in the UK is routine in older adults (≥65 old) and likely prevents the most severe invasive forms of the disease, this has limited impact on pneumonia rates or death from pneumonia itself (2). Better vaccinations are urgently needed, which requires an improved understanding of the immune response to pneumococcus, particularly in older adults as they are a vulnerable group to infection in whom data obtained from younger participants is not always applicable.

It is known that the immune response to pneumococcus of younger and older adults are not the same (3). A previous Experimental Human Pneumococcal Colonisation (EHPC) study performed by our group in 64 older adults aged 50-84 (using pneumococcal serotype 6B [Spn6B]), showed that serum antibody concentrations did not change after pneumococcal colonisation and even fell in non-colonised participants, in contrast to younger adults, in whom antibody concentrations increase after colonisation (4). Again, unlike in younger adults, this study also demonstrated that experimental colonisation was not protective against homologous serotype re-challenge. This study established that this Spn6B challenge model is safe in this age group.

Nasal colonisation with pneumococcus is an essential pre-cursor to invasive disease (5). The epithelial transcriptomic signature associated with pneumococcal clearance is altered with ageing, potentially leading to increased micro-invasion of bacteria and increased risk of invasive disease (6). Further study of the nasal epithelium-pneumococcus interface is required to help determine how the bacteria bypass the immune response and colonise the nose of the older person.

There are over 100 different serotypes of pneumococcus, with varying degrees of virulence, invasive potential and clinical outcomes (7-9). In this study, we aim to first investigate the host response to carriage in a previously established, safe serotype model (Phase A - Spn6B) in this older age group (4), before progressing to using a serotype that is established and safe in a younger cohort but not yet used in this age group (Phase B – pneumococcus serotype 3 [Spn3]) (10).

Spn3 is an important pathogen, it is associated with high-rates of severe disease including sepsis and death, particularly in older adults (9, 11, 12) and children. There is concern that Spn3 prevalence is increasing despite inclusion of this serotype in pneumococcal conjugate vaccines (PCV-13) (11-13), which has led to an increasing focus on studies to investigate why there is apparent vaccine escape (14).

This study will drive development of important scientific knowledge on the mechanisms by which pneumococcus invades the nasal epithelium and modulates host immune responses in the older adult, investigating this across clinically important serotypes. This knowledge will improve vaccination strategies for this age-cohort who are at particular risk of developing severe pneumococcal disease. Better understanding of nasal immunity can unlock the potential for protection by vaccination to reduce mortality, morbidity and healthcare costs associated with pneumonia in at-risk groups in the UK and globally.

5.1 Controlled Human Infection Models (CHIM)

Controlled human infection models (CHIMs) have enormous potential to study the pathogenesis of a disease and accelerate development of therapeutics or vaccinations. CHIMs involve the deliberate exposure of volunteers, who are carefully selected and exposed under rigorously controlled conditions, to an infectious dose of human pathogens (15). CHIM trials have informed decision-making and vaccine policy worldwide. Two examples are the Vaxchora Cholera vaccine recently licensed by the FDA for travellers and the WHO recommendation of the Vi-tetanus toxoid conjugated *Salmonella typhi* vaccine which has now been given to millions of children worldwide.

Over the past decade we have developed a unique pneumococcal CHIM in which 40-70% of the participants nasally inoculated with pneumococcus develop nasal colonisation for 1-4 weeks (16-18). Over 2000 participants, including adults with moderate asthma and healthy adults aged 50-84, with a number of different serotypes (4, 16), have been inoculated to date without safety incidents. The study team have gained extensive knowledge in pneumococcal infection responses, correlates of protection and mucosal immunity (19-24), as well as how to ensure participant safety during challenge.

5.2 *Streptococcus pneumoniae* serotype selection

There are over 100 different serotypes of pneumococcus, with varying degrees of virulence, invasive potential and clinical outcomes (7-9). For Phase A of this study, we have selected Spn6B – we have vast experience with this serotype and it is associated with lower invasive potential. Our group has previously established a safe and reproducible controlled human infection model with Spn6B, demonstrating 50% carriage rate with a single dose of 80,000 CFUs/naris (25). This model has also been applied to 64 older adults (aged 50-84) with an experimental carriage rate of 39% and no serious adverse events and no cases of pneumococcal disease recorded with only three participants requiring additional clinic visits for respiratory symptoms, which all resolved with minimal intervention (4).

For Phase B, we will use Spn3 as it is an important pathogenic serotype which shows evidence of vaccine escape. In our group, we have performed three CHIM studies using Spn3 without any serious adverse events, inoculating over 500 participants aged 18-50 (10, 14, 26).

Colonisation varies between 30-70% and around 5-10% of participants require early antibiotic therapy (10). Symptoms are more frequent with Spn3 compared to Spn6B challenge, predominantly pharyngitis, seen in around 25-50% of participants (manuscript in preparation).

In clinical practice, Spn3 has been associated with high-rates of severe disease including septic shock, empyema and death, particularly in older adults (9, 11, 12). There is concern that Spn3 prevalence is increasing despite inclusion of this serotype in pneumococcal conjugate vaccines (PCV-13) (11-13). Unlike other serotypes, the capsule of Spn3 is bonded non-covalently to the bacterium, which means that it can be rapidly released, causing antibodies to remain bound to shed capsule and therefore unable to opsonise the bacterium itself (11). This means that much higher antibody titres are required to neutralise SPN3, helping this serotype to evade vaccine-induced immune responses. Improved understanding of the immune response to Spn3 in older adults is required to inform and improve vaccination strategies.
For the Spn3 model (Phase B), we will add increased safety measures including solicitation for signs and symptoms of respiratory tract infection, which will be reported as AESIs in Phase B, and increase the duration of antibiotics from three to five days, as per our previous Spn3 studies (14, 26).

6. Objectives and outcome measures

In this study we propose to further explore the systemic and mucosal immune responses over time following experimental human pneumococcal challenge and carriage in older adults, as measured by specific antibody levels and function from serum and nasal washes as well as inflammatory cell populations and cytokine profiles at the nasal mucosal surface, and compare this with previous results seen in younger participants (banked samples from a previously completed study with existing ethical approvals [15/NW/0146 and 14/NW/1460]). We also wish to explore whether, in comparison to younger individuals, pneumococcal micro-invasion in older adults is enhanced during colonisation and if the resulting innate-inflammatory response is dysregulated and bacterial clearance impaired.

6.1 Primary aim

The detection of pneumococcus (Spn6B in Phase A, Spn3 in Phase B) within nasal cells using confocal microscopy and association with nasal colonisation outcome following pneumococcal inoculation.

6.2 Secondary aims

1. Rates of colonisation by pneumococcus and its density and duration by detection of pneumococcal serotype 6B (Phase A) or serotype 3 (Phase B)

from one or more nasal wash sample in the 28 days following initial pneumococcal challenge.

2. Changes in the population of immune/inflammatory cells within the nose in response to challenge and/or colonisation.
3. Quantification of a systemic and mucosal humoral immune response to nasopharyngeal (NP) carriage
4. Quantification of a systemic and mucosal cellular immune response to NP carriage
5. Demonstration of functional activity of participant anti-pneumococcal antibodies, as measured by assays including opsonophagocytic killing assays.

6.3 Exploratory aims

1. Examine the epithelial innate-inflammatory response to colonisation in older adults
2. Identify transcriptomic signatures associated with micro-invasion and impaired pneumococcal clearance

6.4 Definitions

The following have been included to provide a better understanding of the objectives of the study:

- Spn6B challenge: individuals experimentally nasally inoculated with pneumococcus serotype 6B (Phase A)
- Spn3 challenge: individuals experimentally nasally inoculated with pneumococcus serotype 3 (Phase B)
- Spn6B carriage: individuals experimentally nasally inoculated and who become colonised with pneumococcus serotype 6B (Phase A) (positive result at any day using nasal washes [NW] detected by microbiological culture)
- Spn3 carriage: individuals experimentally nasally inoculated and who become colonised with pneumococcus serotype 3 (Phase B) (positive result at any day using NW detected by microbiological culture)

6.5 Objectives

Objectives	**Outcome Measures**
Primary Outcome	
The detection of pneumococcus within nasal cells using confocal microscopy and association with nasal colonisation outcome following pneumococcal inoculation.	Rates of colonisation determined by visualisation of the bacteria using confocal microscopy

Secondary Outcomes	
Rates of colonisation by pneumococcus and its density and duration by detection of pneumococcus serotype 6B (Phase A) or serotype 3 (Phase B) from one or more nasal wash sample in the 28 days following initial pneumococcal challenge.	Detection of pneumococcus serotype 6B (Phase A) or serotype 3 (Phase B) by classical bacteria culture and molecular methods from one or more nasal wash sample in the first 28 days following initial pneumococcal challenge.
Changes in the population of immune/inflammatory cells within the nose in response to challenge and/or colonisation.	Assessment of immune responses including nasal cytokines and cell populations and cellular immunity before and after challenge
Quantification of a systemic and mucosal humoral immune response to nasopharyngeal (NP) carriage	Measuring specific antibody levels in blood and nasal samples
Quantification of a systemic and mucosal cellular immune response to NP carriage	Measuring specific antibody levels in blood and nasal samples
Demonstration of functional activity of participant anti-pneumococcal antibodies, as measured by assays including opsonophagocytic killing assays.	Measured by assays including opsonophagocytic killing assays
Exploratory Outcomes	
Examine the epithelial innate-inflammatory response to colonisation in older adults	Assessed by confocal microscopy and electron microscopy
Identify transcriptomic signatures associated with micro-invasion and impaired pneumococcal clearance	RNAseq analysis to determine gene induction and regulation to identify gene signatures (cell specific where possible) that correlate with susceptibility to infection, as well as alterations in immune responses and symptoms during infection

Table 1: Objectives and outcome measures

The sample type schedule is also outlined in Table 3.

7. Study Design

This is a single-centre, open-label controlled human infection study of older participants using a previously established and safe model of pneumococcal challenge with two phases. Participants will be older adults aged 50-84 (inclusive) who are non-smokers and not allergic to penicillin who provide informed consent to the study. Participants must be generally healthy although certain stable co-morbidities may be permissible, as outlined in the inclusion/exclusion criteria.

Phase A will consist of a minimum of 10-15 participants being challenged with Spn6B, increasing to 15 if colonisation rates are lower than expected (40-50%). This is to be able to perform expanded exploratory analysis of nasal cells micro-invasion by the bacteria and immune responses to carriage following on from our successful and safe study in older adults

(4). Phase B will again consist of 10-15 participants (as above), challenged with Spn3. Participants enrolled to phase A cannot be enrolled to phase B.

Participants and study staff will not be blinded to the inoculation or colonisation status.

8. Participant identification

8.1 Study participants

Eligibility assessment will be completed in stages:

- Screening: study research staff highlights to participants the general screening criteria (including medical history) as part of the study information process before consent is taken.
- Eligibility is confirmed on the formal screening, inoculation and follow-up visits (Table 3). Final eligibility will be signed off by a medical doctor prior to the inoculation.

Participants will be considered enrolled after inoculation.

8.2 Inclusion criteria

- Healthy adults aged 50-84 (inclusive, at the time of consent and inoculation)
- World Health Organisation performance status 0 (able to carry out all normal activity without restriction) or 1 (restricted in strenuous activity but ambulatory and able to carry out light work)
- Access to telephone and e-mail
- Fluent spoken English – to ensure a comprehensive understanding of the research project
- Capacity to provide written informed consent in English

8.3 Exclusion criteria

Exclusion criteria will be self-reported and confirmed from GP questionnaire (GPQ) or medical summary. Individuals may not participate in the study if they are/ have:

• Research participant:

- Currently involved in another study unless observational or non-interventional, excluding the EHPC bronchoscopy study or in follow-up (at the discretion of the study team) and exceptions may be applied at the discretion of the CI/PI to ensure no harm comes to the participants (e.g. excessive blood or nasal sampling)
- Participated in a previous Spn6B/Spn3 EHPC study within 3 years

• Nasal carriage

- Participants who have natural pneumococcus identified at screening will be excluded (but can be re-screened)

• Vaccination:
  - Had any vaccination within 28 days of enrolment (defined as time of inoculation) other than against influenza or COVID-19 which are permissible up to 14 days prior to inoculation
  - Had a pneumococcal **conjugate** vaccine*

• Allergy:
  - Allergy to beta-lactam antibiotics (including penicillin and amoxicillin)

• Medical history leading to increased risk of severe infection, illness including but not limited to:
  - Asplenia or dysfunction of the spleen
  - Chronic respiratory disease (e.g. asthma [requiring medication (including salbutamol inhaler) within last 12 months], COPD, bronchiectasis) that may place the participant at increased risk of infection post inoculation
  - Chronic heart disease (e.g. angina, ischaemic heart disease, chronic heart failure) - controlled and stable hypertension may be included
  - Chronic kidney disease (e.g. kidney transplant, regular dialysis, CKD3-5)
  - Chronic liver disease (e.g. cirrhosis, hepatitis)
  - Chronic neurological disease that limits mobility, bulbar or respiratory function (including stroke, Parkinson's disease, dementia and multiple sclerosis)
  - Diabetes mellitus (including diet controlled)
  - Cancer within the past 5 years (except for basal cell carcinoma of the skin, melanoma in situ and cervical carcinoma in situ)
  - Individuals with cochlear ear implants
  - Individuals with major cerebrospinal fluid leaks (e.g. following traumatic, major skull surgery, or requiring CSF shunts)
  - Individuals with known or suspected immune deficiency (e.g. HIV, known IgA deficiency, immotile cilia syndrome, or Kartagener's syndrome)
  - History of frequent nose bleeds within the last two years
  - Bleeding disorders
  - Significant mental health disorders
  - Other uncontrolled co-morbidities, as determined by the clinical investigator, which would be expected to increase the risk of pneumococcal disease
  - Any major pneumococcal illness or pneumonia requiring hospitalisation in the last 10 years
  - Meeting STOP criteria (section 8.4)

• Medication

- Any medication that may affect the immune system for more than 7 consecutive days within the last 3 months (e.g. systemic steroids [IM/IV], steroid nasal spray, Roaccutane, disease modifying anti-rheumatoid drugs)
- Receipt of anti-cancer immunotherapy, chemotherapy or radiation therapy within the preceding 5 years
- Long-term antibiotic use or any antibiotics (other than topical) in the past 28 days
- Recipient of monoclonal antibodies in the last 6 months for any indication
- Recipient of blood transfusion products within the last year
- Any medication that may affect the coagulation system in the last 3 months (excluding low-dose aspirin)
- Use of any medication or other product (prescription or over-the-counter) for symptoms of rhinitis or nasal congestion within the last 1 month, except for anti-histamines for hayfever, which are permissible.

• Maternity:
- Female participants who are pregnant
- Female participants who are lactating
- Female participants who intend to become pregnant during the study
- Female participants of child-bearing potential** unable to take effective contraception measures***

• Direct caring role or share living accommodation with individuals at higher-risk from infection
- Children ≤5 years of age
- Adults with immunosuppression
- Adults at high risk of invasive pneumococcal disease at the discretion of the investigator
- Adults classified as clinically extremely vulnerable by the National Health Service
- Health-care worker (unless willing to wear a surgical facemask at all times with patients in Phase A only, health-care workers are excluded from Phase B)

• Smoking
- Current or ex-smoker (regular cigarettes [≥5 per week] /cigars/e-cigarette/vaping/smoking of recreational drugs) in the last 6 months
- Previous significant smoking history (>20 pack-year history of smoking OR 5-20 pack-year history of smoking but quit less than five years ago [One pack-year is defined as smoking 20 cigarettes per day for one year])

• Current alcohol and/or recreational drug use
- Regularly drinks >21 units alcohol (men) or >14 units alcohol (women) per week
- Regularly uses recreational drugs

• Overseas travel planned within the study period (28 days after primary challenge)

• Any other issue which, in the opinion of the study staff, may:
  - Put the participant or their contacts at risk because of participation in the study
  - Adversely affect the interpretation of the study results, or
  - Impair the participant's ability to participate in the study

*Previous pneumococcal polysaccharide vaccination (PPV) will *not* be an exclusion criterion as this vaccine does not confer protection against mucosal carriage (4) and will therefore not impact on our primary outcome measure. We will endeavour to document vaccination status for all participants to explore the potential for confounding within the study.

**A woman is considered of childbearing potential, i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A post-menopausal state is defined as 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.

*** Acceptable effective forms of contraception for female participants include:
  - Established use of oral, injected, topical patch or implanted hormonal methods of contraception.
  - Placement of an intrauterine device (IUD) or intrauterine system (IUS).
  - Total abdominal hysterectomy.
  - Bilateral tubal occlusion.
  - Barrier methods of contraception (condom or occlusive cap with spermicide).
  - Male sterilisation, if the vasectomised partner is the sole partner for the participant.
  - Sexual abstinence defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments (for this trial from inoculation until D28). The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the participant.

8.4 Individual STOP criteria

	STOP criteria
Age	STOP if <50 years or >84 years
Clinical history + examination	STOP if unexplained or concerning findings on history or examination
Engagement with research team	STOP if the research team have concerns about volunteer's ability to commit to frequent communication and safety checks
Full blood count (FBC)	STOP if Hb <95 g/l STOP if total WCC <1.5 $x10^9$/l STOP if total WCC >12 $x10^9$/l STOP if platelets <75 $x10^9$/l
Renal function	STOP if creatinine is ≥132 µmol/L
Resting SpO_2	STOP if < 94%

Table 2: Individual STOP criteria

8.5 Temporary exclusion criteria

The following are temporary exclusion criteria to inoculation:

- Current acute infective illness – delay inoculation by 14 days
- Recent /current URTI – delay inoculation by 14 days after last day of illness
- Positive COVID-19* or confirmed influenza – delay inoculation by 21 days
- Antimicrobial (including antiviral, excluding topical or antifungal use) use – delay inoculation by 28 days from last date of antimicrobial therapy
- Significant other concern from the investigatory team (reason will be documented)

Potential participants who are temporarily excluded at screening or prior to inoculation may be re-screened at a later date to assess study inclusion if required. There is no time limit to re-screen a potential participant. In this scenario, the entire initial visit would not need repeating, however, participants would be re-consented if the time since initial written informed consent is greater than 3 months.
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 the analysis.

*Participants that have been temporarily excluded due to a positive COVID-19 swab will require negative lateral flow test prior to subsequent inoculation.

9. Study procedures

9.1 Recruitment

Ten to fifteen healthy adults aged between 50 and 84 years (inclusive) will be recruited for each phase of the study. Participants who drop out or require antibiotics before Day 2 post-challenge will be included in the safety and secondary endpoint analysis, but will be excluded from the primary endpoint and will be replaced.

All potential participants may be contacted by methods including but not limited to email, telephone, posters, leaflets, websites, advertisements in newspaper, radio and on social media, public engagement events and/or mail using a REC approved invitation letter or other advertising material using wording from REC approved study documents in the first instance to invite them to participate in the study.
Where mail-outs are used, participants may be identified via the electoral open register, or through National Health Service databases using data extracts. For the NHS databases initial contact to potential participants will not be made by the study team. Instead, study invitation material will be sent out on behalf of the study team by an external company. For mail-outs via the electoral register, the study team will obtain access to the names and addresses of individuals who are on the open electoral register (only contains the names of registered voters who have not opted out). In this instance, the study team will upload the mailing list to the external company, and the study invitation pack will be sent out by the external company.

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

1. Email/Poster campaign: We will contact representatives of local tertiary education establishments and local employers and ask them to circulate posters and link to study website by email or hard copy.

2. Liverpool Vaccine Group database for healthy volunteers/other databases: The study may be advertised to potential participants signed up to the Liverpool Vaccine Group's Healthy Volunteers Database, Be Part of Research or similar databases (where members of the public have given their consent to be contacted when studies open for recruitment and understand that this is not a commitment to participate), or to a group or list only with the express agreement of the network administrator or with equivalent authorisation.

3. Media advertising: Local media, newspaper and website advertisement placed in locations relevant for the target age group with brief details of the study and contact details for further information.

4. Website advertising: Description of the study and copy of information booklet on study team websites and other appropriate platforms for vaccine trial advertising.

5. Social media: Advertisements placed on trial site media accounts or targeted social media platform advertisements including, but not restricted to, X/Twitter, Facebook and Instagram

6. Exhibitions: Advertising material and/or persons providing information relating to the study will exhibit using stalls or stands at exhibitions and/or fairs, such as University Fresher's Fairs.

7. Royal Mail Leaflet: Royal Mail door-to-door service with delivery of invitation letters in site envelopes to every household within certain postcode areas.

8. PIC Sites: We will use health professionals based in GP practices, walk-in centres and community respiratory clinic as Patient Identification Centres. PIC agreements will be in place with the practice. They will search their patient database to identify potential patients, send texts/ letters inviting their patients to directly contact the team for further information.

Research ambassadors and research staff will attend public engagement events as stated above to promote our research and engage the public.
Potential participants who are interested in study participation will be able to contact the sites by telephone, email, online or a reply slip.

9.2 Informed Consent

Once an expression of interest has been received, an information sheet will be downloaded from the study website by the potential participant, and/or sent to them via mail or email. Following this information, if participants are willing to proceed, they are invited for a consent and screening visit.

Potential participants will be invited to discuss the study at the study site. Written and verbal versions of the Participant Information sheet and Informed Consent will be presented to the participants detailing no less than: the exact nature of the study; 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 study 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. Groups of participants may be invited for a study presentation and discussion (either remotely or in person) prior to the screening visit.

The participant will be allowed as much time as needed to consider the information and will have the opportunity to question the Investigator (in private), GP or other independent parties to decide whether they would like to participate in the study. Participants will be asked to complete a consent quiz as part of the informed consent process to ensure they have properly understood the study and provide an opportunity to review any areas that the participant may require further information before consent is taken.

Written informed consent will then be received by means of participant-dated signature and dated signature of the person receiving the Informed Consent. The person who received the consent must be suitably qualified and experienced and have been delegated 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 study site. The consent form includes an option for participants to allow the DNA/RNA from these blood samples to be studied.

A continuous consent approach will be used throughout the study as participants will be asked at each visit if they are willing to continue.

In line with recommended practice (MRC tissue and biological samples for use in research), participants will be asked to consent to gift their anonymised samples for use in future studies and shared with research collaborators internationally and stored for any future commercial or academic partnerships.

9.3 Screening and eligibility assessment

After written informed consent is received, a clinical assessment will be made where a participant's eligibility will be assessed by member of the clinical research team who are formally delegated by the CI, trained in GCP and the trial protocol. This is to ensure that they meet the inclusion criteria and do not meet any of the exclusion criteria. Any queries about possible eligibility will be discussed with the CI/PI.
During eligibility screening, we will obtain consent from all participants to review their GP records, either via a GPQ, a short summary from the GP or reviewing the electronic GP records.

This screening visit will include a medical history and clinical exam by a medical doctor as well as baseline laboratory tests. Procedures will take place as listed on Table 3. Other tests, such as electrocardiogram, spirometry or hepatitis/HIV testing may be done, with consent, at the investigator's discretion.
Decisions to exclude potential participants from enrolling in the trial or to withdraw a participant from the trial will be at the discretion of the CI/PI and Co-Investigators.

Participants may be re-screened at a later date to determine whether they meet eligibility criteria. They will be reimbursed for the additional study visit.

If a previously undiagnosed abnormality is detected during this process, this will be explained to the individual and all relevant results will be forwarded to their GP with participant permission, so that appropriate management and follow-up within the primary care team can be arranged. Further participation will be determined at the discretion of the study doctor and team.

9.4 Study interventions, comparators and study procedures

The study procedures by visit are outlined below in Table 3.

Days post inoculation	Initial visit[%]	Screen	D0	D2	D6	D9	D14	D28
Visit Window	-60[!] to -2	-7 to -2		+/-1	+/-1	+/-1	+/-2	+/- 5
Study information	X							
Consent (written)	X							
Consent (verbal)	X	X	X	X	X	X	X	X
GPQ/GP records/summary	X							
Clinical exam		X	X					
Vital signs		X	X	X	X	X	X	X
Medical history	X[€]	X						
Pregnancy test[$]		X	X					
*Screen for AEs**			X	X	X	X	X	X[^]
*Inoculation***			X					
Cough Sample				X	X	X	X[$]	X[$]
Nose to hand swab				X	X	X	X[$]	X[$]
Nose/Throat swab		X	X	X	X	X	X	X
Nasosorption			X	X	X	X	X	X
Nasal wash		X		X	X	X	X	X
Nasal cells		X		X	X	X	X	X
Saliva sample		X		X	X	X	X	X
FBC, U&E	X[α]	X[α]						
PBMCs		X[α]	X[α]				X	X
Serum		X[α]	X[α]		X		X	X
Blood for RNA			X	X	X	X		

Table 3 – Study procedure overview

GPQ = General Practitioner's Questionnaire, AE = adverse events, FBC= Full Blood Count, U&E = Urea & Electrolytes, PBMCs = Peripheral Blood Mononuclear Cell, RNA = Ribonucleic Acid

* - participants will also be expected to check a temperature daily for 3 days (Phase A) or 5 days (Phase B) after inoculation and they will keep a symptom e-diary for 7 days which will be monitored for AEs. ** - Safety pack explained and given at this time-point. ^ - Colonised participants will be advised to take their course of antibiotics, if not already done. $ - if indicated. €- details about medical history may be gathered at initial visit and will be repeated at screen. % - α This visit may be combined with the screening visit. Part of this visit may be conducted over the telephone. The participant may also leave after receiving study information and return after consideration to complete consent process. X[α] these samples can be taken at either visit. ! - -60 day window not applicable if temporary exclusion criteria develops (see section 8.5) $ If positive on day 2, 6 or 9

The following samples will be obtained during the study as detailed in Table 3.

1. **Urine** – Women of childbearing potential (WOCBP) as defined in exclusion criteria will be asked to provide a urine sample at the screening visit for pregnancy testing
2. **Throat swabs** will be obtained for detection of viral and bacterial pathogens by microbiological and molecular techniques. Swabs may also be taken at 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. Up to 2 swabs may be taken at each time point (unless further clinical swabs are required for symptomatic participants), as indicated.
3. **Nasosorption** will be obtained before the nasal wash. Sample strips collect concentrated nasal lining fluid to measure inflammatory responses. Blotting paper will be held inside the nostril for two minutes. These will then be removed and placed in a microcentrifuge tube for storage.
4. **Nasopharyngeal or nasal swabs and saliva** will be obtained for detection of viral (including SARS-CoV2) and bacterial pathogens. Regular nasopharyngeal or nasal samples will be taken. Participants will provide saliva samples by spitting into a container.
5. **Nasal wash** will be performed using our established SOP to collect nasal flora/ pathogen specimens and soluble biomarkers (27). 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 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).
6. **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 biopsies (rhinoprobe). Participants can be biopsied multiple times with no significant side effects (28, 29). Up to 5 samples will be obtained at each nasal sampling visit. If no cells are visible on the rhinoprobe following sampling, the sample will be repeated.
7. **Blood sampling** will be performed by trained, experienced staff. Up to 74ml of blood will be collected at a single visit to measure full blood count and renal function (for safety), and laboratory measures including, but not limited to serum immunoglobulins, PBMC populations, and host RNA expression.
8. **Shedding samples,** hand to nose swabs and cough plates will be taken to determine if the bacteria is shed following colonisation. Participants will rub the anatomical snuff box with their nose repeatedly, a swab will be taken of the area to test for pneumococcal bacteria. Participants will hold an agar plate in front of their face and cough onto the plate to check for shedding of the bacteria. If they test negative for Spn at day 2, 6 and 9, the shedding samples will not be taken at the remaining follow up visits.

All laboratory results will be reviewed and collated by the study team who will record these in the CRF. If a test result is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the participant will be informed. Depending on the nature of the result the participant may be asked to see their GP and be given the relevant information from any test results from the trial. Alternatively, if consent provided, the participant's GP may be contacted to discuss a particular result or finding.

9.5 Preparation of the challenge agents

The dilution of the inoculum will be prepared as per local SOPs in a room dedicated for challenge agent preparation. A mid-log broth culture of pneumococcus (Spn6B or Spn3) 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. These checks will first be carried out in research laboratories and then identification, purity by whole genome sequencing and penicillin sensitivity will be confirmed in a reference laboratory (UKHSE). On experimental inoculation days, aliquots will be thawed, washed twice, and re-suspended in 0.9% normal saline at the correct density. Once inoculum is prepared it will be administered within 30 minutes to avoid degradation. Prepared inoculum is transported to the site clinical facilities in a clearly labelled vial and transportation bag and kept closed until they reach the inoculation room.

We will aim for a dose of 80,000 CFU/100µl per naris of the inoculum (inoculum dose determined from previous challenge studies). We will allow a variation of half or double of this dose and have previously demonstrated in our dose-ranging studies that this range is safe and leads to similar colonisation outcome (rates and density recovered from the nasopharynx) (16).

Supply and accountability

Accurate records of receipt and condition of all Challenge stock agents will be available for verification by the Study Monitor. Trained site staff will be responsible for adequate and accurate accounting of all Challenge Agents inoculum. Any deviation from the protocol-dispensing regimen will be fully documented.

9.6 Inoculation

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. This will be done slowly with sufficient interval between each inoculation to ensure maximum contact time between the nasal and pharyngeal mucosa. After inoculation, the participant will remain in this position for up to 15 minutes. Participants will be asked not to wash or blow their noses for at least one hour.

Post inoculation

After the inoculation participants will be given a safety pack containing:

- Thermometer
- Safety information leaflet (including how to take their temperature and symptoms of pneumococcal infection)
- Medical alert card with study team contact details
- Amoxicillin 500mg TDS 3-day (Phase A) or 5-day (Phase B) supply (including antibiotic patient information sheet). Participants will be asked to return any remaining antibiotic doses to reconcile and discard.

Written and verbal instructions are given to the participant describing potential mild, moderate and severe symptoms and the instances when antibiotics can be taken. These include:

- At an investigator's discretion at any point
- If unwell and/ or symptomatic and instructed to take by the research team
- If unwell and unable to contact the research team
- At D28 if they have been positive for pneumococcus, at any timepoint following inoculation, without having had two consecutive negative NWs before the visit

Participants will be instructed to monitor the development of any symptoms at home and complete diaries. Home monitoring of symptoms will include a clear flow chart of the necessary intervention should any symptoms develop (see participant safety information leaflet). They will be requested to complete electronic diaries for 7 days, detailing their temperature and any symptoms.

A member of the research team will review diaries and assessments daily in the first week following inoculation and attempt to contact the participants should they not make contact or fill in the diary by the specified time. If no contact is possible, 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. Participants reporting symptoms potentially consistent with pneumococcal disease (for example, ear pain, sore throat, cough and fever) will be seen in person for medical assessment and may begin the course of amoxicillin if the research medical staff feel treatment is warranted, with reference to a pre-defined algorithm (see section 11.1).

9.7 Subsequent visits

Subsequent visits will continue as outlined in Table 3. Flexibility around visit windows is described.

Monitoring of colonisation

Colonisation will be defined by the result of nasal washes taken at D2, D6, D9, D14 and D28 as per visit schedule (Table 3).

Confirmation of pneumococcal colonisation

Nasal washes will be plated onto culture media, incubated overnight and colonies will be confirmed as *S. pneumoniae* using classical microbiological techniques. If and when microbiological culture confirms *S. pneumoniae* experimental colonisation in the laboratory, the clinical team will be informed to ensure participants are counselled appropriately. A participant is considered positive for colonisation if they test positive for pneumococcus at any nasal wash sample by either classical microbiology or molecular methods. If study participants persistently carry pneumococcus at D28, they will be instructed to take oral Amoxicillin 500mg three times daily for 3 days (Phase A) or 5 days (Phase B) to clear or reduce the colonisation. DNA will be extracted from nasal wash (NW) samples using our well-defined protocols (30). *S. pneumoniae* serotype 6B detection will be done by multiplex qPCR for *lytA* and *6A/Bcps genes*, respectively. Serotype 3 detection will be done by multiplex qPCR for *lytA* and 3*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 laboratories.

Antibody measurement

As per our previous published work we will measure pneumococcal serotype-specific antibody both systemically and at the respiratory mucosa. Response measurements will include bacterial neutralisation assays *in vitro*. Antibody titres and function will be compared between participants. We will measure changes in responses post inoculation to baseline.

Cellular responses

We will also assess cellular responses from PBMCs and at the respiratory mucosa. Pneumococcal specific T-cell recall responses (both CD4+ and CD8+) will be quantified post challenge and we will also measure levels of memory B cells to Spn6B and Spn3 respectively. We will measure changes in the responses post challenge to baseline. We will also evaluate changes in the innate immune cell dynamics, activation and functionality in a kinetic fashion in the nasal mucosa and correlate those findings with susceptibility to infection or ability to clear infection.

Genetic responses

As per our previously published work, we will use mRNA sequencing to perform in depth investigation of transcriptome changes in both peripheral blood and respiratory mucosal cells as a response to bacterial infection. Such gene signatures will be paired with immunophenotyping data. This type of analyses will enable us to identify correlates of

protection or susceptibility to pneumococcal infection and mechanisms of host-pathogen interaction and how this affects the downstream immune responses.

Clinical Symptom Scores

Individual symptom scores will be collected for 7 days after the challenge (D0).

Upper respiratory tract symptoms

A total 'upper respiratory clinical symptom score' will be derived using a four-point scale (0-4 for absent, mild, moderate, severe and requiring emergency department visit or hospitalisation) for each of the following eight respiratory symptoms: sneezing, headache, malaise, fever (37.6^{o}C or above/chills), nasal discharge, nasal obstruction, sore throat and cough according to established methods, giving a maximum clinical severity score of 24. This is an established method for studies of common cold illnesses (31). Symptoms will be recorded at the same time of day and before any procedures such as nasal lavage is performed. More details are provided in Annex 1.

Lower respiratory tract symptoms

An e-diary of lower respiratory tract symptoms will also be completed with a scoring system outlined in the diary card (see Annex 1). The purpose of this exercise is to rapidly identify any participant who may be at risk of developing an SAE. These will also be used to identify AESIs in Phase B (see section 12.6).

Concomitant medications

The use of concomitant medication prescribed or over the counter, will be recorded in the participant's CRF. Prescribed medications such as antipyretics, antibiotics and immunosuppressive agents may impact the study results or may impact the safety of the participant (particularly during the challenge period). It is at the discretion of the study investigators to determine whether withdrawal or temporary exclusion of the participant is required.

9.8 Symptomatic participants

Participants reporting fever or symptoms consistent with pneumococcal infection (including upper or lower respiratory tract symptoms), either directly or via e-diaries, will be assessed by a clinician for safety and to ensure their symptoms are not due to concurrent infection. A nasopharyngeal swab may be sent for detection for bacterial and viral pathogens. Participants will be directed to commence their antibiotics if appropriate.

9.9 Termination of colonisation

At the end of the study (D28), study participants who have been positive for Spn6B or Spn3 colonisation at any time point, and who have not subsequently had two consecutive negative nasal wash samples, will be asked to take oral amoxicillin 500mg three times daily for 3 days

(Phase A) or 5 days (Phase B). The aim of this is to reduce the colonisation density and therefore the risk of pneumococcal disease.

9.10 Early discontinuation / withdrawal of participants

A participant may be withdrawn from challenge for any of the following reasons:

- Withdrawal of consent to continue in the study (a participant may withdraw their consent at any time)
- The study Investigator or Sponsor, for any reason, decides the participant should be withdrawn from the study
- Adverse events, which cannot be tolerated by the participant
- Develops an exclusion criterion
- Pregnancy
- Significant non-compliance with the protocol, as determined by the medical monitor(s)
- Sponsor decides to discontinue the participant's participation in the study or to terminate the study

If a participant is withdrawn from the study, where possible, they should continue to attend the safety follow-up visits so that data can be collected for the Intention-to-Treat Population. If participants do not agree to this, then the clinical team will work with them to ensure safe withdrawal from the study, including administration of antibiotics as appropriate.

9.11 End of Trial definition

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 12 months. An additional 6 months will be required for data analysis and publishing results.
The end-of-study is completed when the last laboratory assay is analysed on the last participant sample.

10. Study interventions

10.1 Investigational Medicinal Products (IMP)

No investigational medicinal products will be used during this study

10.2 Assessment of compliance

Issues related to compliance will be assessed by the research team. Any issues with compliance will be recorded and documented according to GCP guidelines.

10.3 Post study interventions

Participants' GPs will be informed of their challenge and antibiotics if required.

11. Risk Assessment and Mitigation

This study is designed to ensure minimal risk by applying established safety procedures from the EHPC model. These safety procedures are described throughout the study protocol but are summarised in Table 4 below. Safety reporting will be consistent with NHS National Research Ethics Service (NRES) and MHRA guidance.

Screening	Exclude adults with potential risk factors for invasive pneumococcal infection based on history, vital signs, clinical assessment and safety laboratory samples. Exclude adults who have contacts with risk factors for invasive or severe infection.
Participant safety	Safety guidance is presented to the participant during consent and on the days of inoculation. Participants are provided a safety information leaflet detailing: • contact details for the research team available 24/7 • symptoms of infection • advice to report early signs of infection or to seek urgent health care if concerned • if/when to take the antibiotics provided • to report adverse events including unrelated hospital admissions for the duration of the follow up period until their last visit Close friend or family: participants are encouraged to inform a close contact that they are taking part in a study and a copy of the safety leaflet is provided for them including contact details for the research team. We advise participants that if they are unwell, they should contact the clinical research team and inform their identified close contact. The close contact's contact details will be provided to the study team should the site be unable to locate the participant. Attempts will be made to contact the close contact instead if there are concerns.
Symptoms and access to healthcare	**Urgent Care:** to avoid any delay in diagnosis, participants are advised to attend their usual health care facility or dial 111/999 if seriously concerned about their health, as their condition may not necessarily be related to the inoculation. They will also be advised to inform the research team. **Daily checks:** following primary inoculation participants will complete an electronic or paper diary to report any symptoms for a total of 7 days. Thermometers are provided for daily temperature measurement. **Symptoms:** will be monitored and recorded systematically at each visit by the clinical research team. **Triggered clinician assessment:** participants with respiratory/ear symptoms, fever, and other symptoms potentially associated with pneumococcal infection may attend the clinic for a triggered assessment by the research nurse/doctor available weekdays 0800 to 1600. They may be advised to seek another healthcare route if a visit with the research nurse/doctor is not possible or the

	symptoms are severe. A research doctor is available for telephone advice 24/7 for participants. **General Practitioner:** will be routinely notified of participants' involvement in the study.
Antibiotics	**Sensitivity:** the bacterial inoculum will be tested to confirm sensitivity to the protocol antibiotics. **Supply of antibiotics:** Oral Amoxicillin 500mg TDS for 3 days (Phase A) or 5 days (Phase B) is provided to each participant to avoid delay in treatment if the participant has symptoms of potential pneumococcal infection (preferably taken upon discussion with the clinical team). **Termination of colonisation:** participants who remain bacterial colonisation positive at D28 visit will be advised to take a 3-day (Phase A) or 5-day (Phase B) course of amoxicillin 500mg TDS antibiotics to clear or reduce colonisation post inoculation.
Monitoring colonisation	**Nasal Wash:** results of colonisation are reported to the clinical team after each follow-up visit following inoculation. Colonisation and safety data are communicated weekly for discussion by the TMG.
Withdrawal	If a participant or their close contacts develop potential risk factors for invasive bacterial then they may be withdrawn from the study at any time and commence antibiotics to clear/reduce bacterial colonisation when required.
Staff Safety	Use of personal protective equipment, assessment by Occupational Health, management of high-risk staff members (e.g. pregnant or immunocompromised individuals)
Safety monitoring	**Trial Oversight:** An established DSMC and TMG will review infection/colonisation rates and adverse events.

Table 4: Risks and mitigations

11.1 Risk to participant safety

Bacterial inoculation and colonisation

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. While the risk to individuals of developing any infection is very low, the study is designed to ensure that any risk is minimal.

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

- The research team has over 14 years of experience in human challenge studies, following very similar protocols and facing similar risks as previous studies, including in older adults (4).

- For Phase A, the selected pneumococcal serotype (6B) is fully antibiotic sensitive and has a lower risk of invasive disease compared to other serotypes. For Phase B, the selected serotype Spn3 will be fully antibiotic sensitive and there is strong institutional experience of using this serotype in CHIMs.
- 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/or fever.
- 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.

We have experience of inoculating and studying over 2000 participants (32) using several serotypes, in different age cohorts and at a range of doses (20,000-320,000 CFU/100µL), 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.
This model has been applied to 64 older adults (aged 50-84) using Spn6B (4) with an experimental carriage rate of 39% and no SAEs recorded, no cases of pneumococcal disease and only three participants requiring additional clinic visits for respiratory symptoms, which all resolved with minimal intervention.

Spn3 safety dataRegarding Phase B, we have performed three CHIM studies using Spn3 without SAEs, inoculating over 500 participants aged 18-50 (10, 14, 26). Symptoms are more frequent with Spn3 compared to Spn6B challenge, especially pharyngitis, seen in around 25% of participants (data in preparation). For further information on safety data in Spn3 please refer to section 5.2. For Phase B, we will add increased safety measures including solicitation for signs and symptoms of respiratory tract infection, which will be reported as AESIs in Phase B, and increase the duration of antibiotics from three to five days, as per our previous Spn3 studies (14, 26).

In the event that symptoms occur during the inoculation or follow up period, participants will be contacted on the day that the symptoms are reported, and the clinical team may advise participants to commence amoxicillin based on a pre-defined algorithm (available in the Clinical Study Manual) and irrespective of the colonisation status at that point. Participants may be reviewed in study clinic or asked to attend an NHS healthcare treatment facility directly at investigator discretion.

Sample collection

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

- Nasal Cells: collecting nasal cells may cause discomfort, eye watering or minor local bleeding.
- Nasal Wash: participants may swallow saline which may taste salty.
- Venepuncture: taking blood samples may cause some discomfort or result in a bruise. Very rarely participants experience light-headedness or fainting. Clinical staff are trained to assess and deal with such occurrences. The amount of blood collected during the study will be within the NHS Blood and Transplant guidelines for blood donation
- Throat Swab: participants may gag when the sample is taken.
- Nasopharyngeal/nasal Swab and nasosorption: this may cause some discomfort, eye watering or a minor local bleeding.

Risks associated with the COVID-19 pandemic

To protect participants, infection control procedures in line with the latest UKHSA guidance will be used throughout the study. Participants who test positive for COVID-19 at the screening visit will be temporarily suspended from the study to reduce the risk of severe disease or onwards transmission. Samples provided up to that point will be retained and participation will be continued after at least 28 days or longer (if participant remains unwell).

If a participant does develop symptoms suggestive of COVID-19 infection they will be advised to follow the latest UKHSA guidance with regards to self-isolation. A clinical review will be conducted by medical staff with appropriate PPE (for both staff and participant) to clarify whether their symptoms are related to COVID-19 infection or pneumococcal challenge. This applies to the post inoculation period only. A COVID-19 lateral flow and/or PCR test will be performed at this visit. In the event of a study participant becomes acutely unwell with COVID-19 symptoms, they will be advised to seek urgent medical attention via normal routes of healthcare.

11.2 Risk to participant contacts

To mitigate any potential risk of spreading pneumococcus to vulnerable groups in the community, we will discuss these risks with the participants and exclude anyone with close physical contact with at risk individuals (i.e. children ≤5 years of age, children/adults with immunosuppression or severe chronic ill health) during the trial period. There will not be an upper age limit for individuals at home but we will explore functional and health status to ensure that individuals are not at risk of invasive disease. We will prescribe oral amoxicillin 500mg TDS for 3 days (Phase A) or 5 days (Phase B) to all participants that are positive for pneumococcus to terminate or reduce colonisation at the end of the participants study

period, which should reduce transmissibility. Finally, it has been shown in previous respiratory CHIM studies that transmission is rare (33).

11.3 Risk to researchers

Possible risks to researchers include:

- Needle stick injury during venepuncture,
- Biological and chemical hazards within the laboratory,
- Infection from colonisation positive participants or inoculum.

Experienced staff will carry out procedures that are within their competencies, as delegated by the Chief/Principal Investigator, and which are in accordance with the relevant SOPs. Appropriate risk assessments are in place for clinical and laboratory procedures. All laboratory work will be conducted in an appropriately rated laboratory in line with health and safety regulations for research with human tissues/infectious agents. Personal protective equipment including gloves, aprons and fluid resistant surgical masks will be used at all study visits according to the established procedures.

12. Safety reporting

12.1 Definitions

Our safety reporting terms and definitions are described in Table 5 below.

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant to whom an inoculum has been administered, including occurrences, which are not necessarily caused by or related to that product.
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 inoculation, 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 serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. 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. * A&E assessment in itself does not constitute a SAE.

Table 5: Safety reporting terms. NB: to avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Serious" is the regulatory definition supplied above.

12.2 Grading

The labelling of an AE will be defined by the severity threshold described below in Table 6. Severity grading criteria for local and systemic AEs. Refer to section 12.1 for SAE definition.

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.
GRADE 4	Potentially Life-threatening: requires assessment in A&E or hospitalisation

Table 6: Grading of adverse events. Note: A&E assessment in itself does not constitute a SAE.

12.3 Causality

The relationship of each adverse event to inoculation must be determined by a medically qualified individual within the site study team according to the following definitions:

Not related	• No temporal relationship to *S. pneumoniae inoculation* ***and*** • Alternative aetiology (clinical, environmental or other intervention), ***and*** • Does not follow pattern of recognised response to or *S. pneumoniae* inoculation or other study procedure.
Possible	• Reasonable temporal relationship to or *S. pneumoniae* inoculation, ***or*** • Event not readily explained by alternative aetiology (clinical, environmental or other interventions), ***or*** • Similar pattern of response to that seen to or *S. pneumoniae*.

Probable	• Reasonable temporal relationship to *S. pneumoniae* inoculation or other study procedure, ***and*** • Event not readily produced by alternative aetiology (clinical, environment, or other interventions), ***or*** • Known pattern of response with *S. pneumoniae* or other study procedure.
Definite	• Reasonable temporal relationship to *S .pneumoniae inoculation* or other study procedure, ***and*** • Event not readily produced by alternative aetiology (clinical, environment, or other interventions), ***and*** • Known pattern of response to *S. pneumoniae or other study procedure*.

Table 7: Causality assessment of adverse events

12.4 Procedure for collecting and recording of adverse events

We will record AEs/SAEs occurring from the first challenge visit (D0) until the final study visit that are observed by the investigator or reported by the participant (either verbally or in an eDiary) or until completion of antibiotics. Severity gradings will be in accordance with Annex 2.

All AEs that result in a participant's withdrawal from the study will, subject to participant consent, be followed up, where possible until a satisfactory resolution occurs, or until a non-study related causality is assigned.

AEs will be recorded using the following guidance:

- Pre-existing medical conditions or abnormal vital signs (present before the study start) are considered medical history and should not be recorded as AEs. However, if the participant experiences a worsening or complication of such a condition, the worsening or complication should be recorded as an AE. Investigators should ensure that the AE term recorded captures the change in the condition (e.g., "worsening of")
- Each AE should be recorded to represent a single diagnosis. Accompanying signs or symptoms (including abnormal laboratory values) should not be recorded as additional AEs.
- Changes in laboratory values are only considered to be AEs if they are judged to be clinically significant, for example, if some action or intervention is required. If abnormal laboratory values are the result of pathology for which there is an overall diagnosis, the diagnosis only should be reported as one AE.

The following information will be recorded in the CRF:

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

- Action taken.

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. If either of these occurs, the participant should undergo an end of study assessment 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 warrants it.

12.4.1 E-diary AEs

Solicited adverse events will be recorded by the participant in an electronic diary graded by the participant alone (Appendix A). Participants will be asked to complete an electronic diary during their challenge visit for 7 days, which will be checked daily by study doctors. Causality will be assigned by a member of the clinical team.
Solicited adverse events will be reviewed daily during the periods of recording as detailed above by the clinical study team. If the clinical team have concerns about the severity or frequency of an event, or a diary is not completed, this will be followed up with the participant by phone or at a scheduled visit. All $\geq$ grade 3 solicited adverse events recorded in the challenge diary will be followed up with the participant by the clinical team. Participants will have access to the study team 24 hours a day via the study mobile number, should they have concerns.

12.4.2 Unsolicited AEs

These may be recorded by the participant in an electronic diary for the same period as specified above. 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. Causality will be assigned as for solicited AEs.
In-person clinical reviews may be arranged at the investigator's discretion if there is sufficient clinical concern.

12.4.3 Vital sign AEs

At all visits vital signs are taken. These will be recorded directly into the eCRF at the time of review and severity grading will be assigned as per Annex 3. Where a new moderate or severe (grade 2-4) AE is identified a clinician should review the participant in clinic and document the clinical assessment carried out. Changes in vital signs that are deemed clinically significant by a PI-delegated clinician will be causality assessed.
Vital signs that were also abnormal on screening (e.g. hypertension, bradycardia) do not need to be recorded as an AE if stable and not deemed to have changed significantly during the study.

12.4.4 Laboratory AEs

All laboratory tests will be recorded onto a results eCRF and graded (Annex 4). If a test is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the participant will be informed and advised with regards to appropriate medical care. Laboratory results can be out of normal range for a number of reasons other than physiological disturbance (e.g. delayed transit to processing laboratory). If judged to be clinically significant these will undergo causality assessment. All laboratory AEs Grade 3 or 4 will be recorded as AEs and appropriate treatment or follow-up will be arranged.

12.5 Recording and reporting of serious adverse events (SAEs)

Serious adverse reactions/events (SARs/SAEs) will be reported from the time of enrolment until the completion of the study (D28 [+3/5 days if starting antibiotics on D28]).

All SAEs will be recorded on an SAE form and reported to the DSMC chair, Sponsor and the funder within 24 hours of discovery or notification of the event. SAEs will be monitored and reported until the end of the participants last study visit or until resolution/stabilisation. Additional information received for a case (follow-up or corrections to the original case) need to be detailed on an SAE update form and sent to the Sponsor and the DSMC within 24 hours of new information becoming available. The DSMC will perform an independent review of the SAEs and request any further information required. Documentation of any review will be kept in the site file.

The TMG may make the decision to terminate the trial early in the event of serious safety concerns, informed by the DSMC.

12.6 Adverse Events of Special Interest (AESI)

An adverse event of special interest is one of scientific and medical concern specific to a product or trial, for which ongoing monitoring and rapid communication by the investigator to the safety committee or Sponsor may be appropriate.

Due to the additional study procedures the following events will be considered AESIs for both phases.

- Pneumococcal pneumonia, septicaemia, OM or pneumococcal meningitis
- AEs requiring a physician visit or Emergency Department visit which, in the opinion of the study staff, are related to the challenge with Spn6B

The following will also be AESIs for Phase B if deemed clinically significant or graded as moderate by the participant:

- Lower respiratory tract symptoms: productive cough, wheeze, chest pain, difficulty breathing
- Headache: headache only, with neck stiffness, with pyrexia >38^{0}C, with nasal congestion/ sinus pain, with changes in vision/ photophobia
- Earache: earache only, with discharge, with pyrexia >38^{0}C

- Sore throat: sore throat only, with pyrexia >38^{0}C
- Fever > 38°C

All SAEs and AESIs will be followed until resolution, until the event is considered stable or until a non-study causality is assigned.

12.8 Data and Safety Monitoring Committee (DSMC)

A DSMC is an independent committee which 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. The specific role of the committee will be:

- To independently review AEs, SAEs and AESIs regardless of relatedness to any of the study procedures throughout the study.
- To perform unscheduled reviews on request of the study team at a demand and frequency determined by the severity of reported adverse events.

The DSMC will be supplied with a safety report weekly during the study, at completion of the study, in the event of an SAE, or if requested at any time by the CI or DSMC members.
The outcome of each DSMC review will be communicated directly to the CI 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.

13. Statistics and Analysis

13.1 Statistical analysis

Statistical Analysis will be performed by statisticians at either LSTM or a collaborator at University of Oxford (Associate Prof Xinxue Liu).
As an exploratory study, no power calculations have been performed.

13.2 Populations for analysis of primary and secondary endpoints

The per-protocol population will be used for evaluation of primary and secondary outcomes. For primary outcome evaluation, the per-protocol population is defined as study participants who:

1. Have consented to the study and met all the inclusion and exclusion criteria;
2. Have been successfully challenged with pneumococcus
3. Have had at least one nasal wash and nasal cells data point post-inoculation

13.3 Analysis of demographics and baseline characteristics

Descriptive statistics relating to participant characteristics at baseline will be calculated overall.

13.4 Data summaries

Continuous variables such as the density of pneumococcal colonisation summarised according to number of participants with non- missing data (n), mean, standard deviation (SD), median, minimum, and maximum.

Categorical variables such as presence/absence and duration of pneumococcal colonisation, AEs and SAEs will be summarised according to the absolute frequency and percentage of participants (%) in each category level. The denominator for the percentages is the number of participants with data available, unless noted otherwise.

13.5 Analysis of primary outcome

Primary outcome (presence/absence of experimental *Streptococcus pneumoniae* in nasal cells using confocal microscopy at any time point during 28 days post challenge) will be summarised by frequency and proportion. Natural carriers will be included in the above final analysis. The analysis strategy will follow the procedure above.

13.6 Analysis of secondary outcomes

Pneumococcal density: Density of pneumococcal colonisation at different time points will be available for those who have a recorded positive value and will be analysed in two ways. We will use longitudinal data analysis methods, specifically generalised linear mixed models (GLMM) or generalised estimating equations (GEE), to analyse pneumococcal colonisation status and density at individual time points with treatment, time and interaction between time and treatment as fixed effects and study participant as random effect / clustering variable. The density will be summarised using number, mean, geometric mean, standard deviation, median, minimum and maximum at each time point. Exchangeable covariance structure will be used. The area under the curve (AUC) of density of experimental pneumococcal colonisation over time 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 treatment.

The immune responses data are expected to be highly skewed, and the data will be log-transformed prior to analysis. The geometric mean concentration (GMC) and associated 95% confidence interval (CI) will be summarised by computing the anti-log of the mean of the log-transformed data. Data will be summarised at different time points.

13.7 The Level of Statistical Significance

Statistical significance will be assumed if $p < 0.05$ for primary and secondary outcome measures after appropriate adjustment if multiple tests are used.

13.8 Procedure(s) to Account for Missing or Spurious Data

Reasons for missing data (including withdrawal of consent, loss to follow-up, removal from study due to serious side effects, death, or inability to obtain any laboratory results) will be indicated, but missing data will not be imputed for primary endpoints. Missing data for colonisation density or immune responses will be re-sampled where possible, otherwise (as when participants are excluded at an early timepoint within the study), removed from the analysis.

14. Data Management

Professor Daniela Ferreira will act as custodian for all samples and data collected during this study.

14.1 Data Collection Tools and Source Document Identification

Source Data: Source data is defined as all information in original records and certified copies of original records or clinical findings, observations, or other activities in a study necessary for the reconstruction and evaluation of the study. All source data are contained in source documents. All source data produced in this study will be maintained by the Investigator and made available for inspection by the Sponsor's representatives, the REC, and any applicable regulatory authorities.

Source Documents: Source documents are defined as the results of original observations and activities of a clinical investigation. Source documents may include, but are not limited to, study progress notes, e-mail correspondences, computer printouts, laboratory data, and drug accountability records. All source documents produced in this study will be maintained by the Investigator and made available for inspection by the Sponsor's representatives, the REC, and any applicable regulatory authorities

Electronic Data Capture: study data will be recorded directly into REDCap, an Electronic Data Capture (EDC) system or onto a paper source document for later entry into REDCap if it is not available. This includes safety data, laboratory data and outcome data. 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. All documents will be stored safely and securely in confidential conditions. The electronic CRF (eCRF) must be completed by designated and trained study personnel. It is the responsibility of the Investigator to ensure the eCRFs are completed and submitted to the Sponsor (or designee) in an accurate and timely manner. The processing of eCRFs will include an audit trail (to include changes made, reason for change, date of change and person making change).

14.2 Data Handling and Record Keeping

Participant data, including case report forms, colonisation and safety reports will be archived as below. A unique identification number will be used to identify each participant.

14.3. Access to data

Direct read-only access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit study-related monitoring, audits and inspections.

14.4. Archiving

Archiving will be authorised by the Sponsor following submission of the end of study report and the Sponsor will be responsible for archiving all study documents and study databases. All essential documents will be archived for a minimum of 5 years after completion of the study and no study records will be destroyed without prior authorisation from the Sponsor and the data custodian (Prof Daniela Ferreira).

15. Monitoring, Audit and Inspection

A Trial Monitoring Plan will be developed by the Sponsor and agreed by the TMG and CI based on the trial risk assessment. The frequency of monitoring will be dependent on a documented risk assessment of the trial undertaken by the Sponsor. Monitoring will be performed according to ICH Good Clinical Practice (GCP) by the Sponsor. Following written standard operating procedures, 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. The investigational site will provide direct access to all trial related source data/documents, eCRFs and reports for the purpose of monitoring and auditing by the sponsor and inspection by local and regulatory authorities.

16. Ethical and Regulatory Considerations

The CI will ensure that this study is conducted in accordance with relevant regulations and requirements outlined in Good Clinical Practice. This study is subject to approval from the Sponsor at LSTM in addition to the following regulatory and ethical bodies:

- Research Ethics Committee
- Research and Governance Office – Liverpool School of Tropical Medicine

16.1 Research Ethics Committee Review and Reports

The CI will ensure that this trial is conducted within the Ethical Principals in the Declaration of Helsinki and in line with Good Clinical Practice guidelines. Approval of the protocol, supporting documents and subsequent amendments will be submitted for approval by the

REC before the start of the trial. Amendments that require review by REC will not be implemented until the REC grants a favourable opinion.

All correspondence with the REC will be retained in the Trial Master File/Investigator Site File. An annual progress report will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended. Within one year after the end of the trial, the CI will submit a final report with the results, including any publications or abstracts, to the REC. If the trial ends prematurely, the CI will notify REC with reasons for premature termination.

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC) and host institution(s) for written approval. The CI will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

The design and conduct of the trial will include the principles of autonomy, non-maleficence, beneficence and justice. Participants may learn about clinical research from their experience and there is a possibility of detecting medical problems during clinical examination and their GP may be informed for further investigation as needed. The research is open to all individuals, but important exclusion criteria are in place, primarily to protect individuals from undue risk. We will not recruit potentially vulnerable participants to this project. All participants must demonstrate capacity to consent for themselves. This study offers the potential for both local (UK) and global impact in the development of future vaccines as part of the global effort to prevent pneumonia.

16.2 Risk assessment

A protocol risk assessment and monitoring plan is prepared before the finalisation of the protocol and will be reviewed as necessary over the course of the study to reflect significant changes to the protocol or outcomes of monitoring activities.

16.3 Study committees

16.3.1 Trial Management Group (TMG):

Includes scientists, health professionals and investigators who provide ongoing management of the trial. They conduct the study and review recruitment, safety and colonisation reports weekly.

16.3.2 Data and Safety Monitoring Committee (DSMC):

The DSMC safeguards and monitors the interests of the trial participants by assessing the safety of interventions and review the protocol according to the DSMC charter. They periodically review safety data to determine patterns and trends of events, or to identify safety issues, which would not be apparent on an individual case basis. They may review data

in the interest of safety. Members are independent to the trial, experienced in this field and the conduct of clinical trials. The DSMC will be provided with interim safety data on a weekly basis. Interim data will be provided if at any time the TMG have any concerns regarding the safety of a participant or the general public. The DMSC will advise the TMG on whether there are any ethical or safety reasons why the trial should be changed or not continue. The DSMC will meet as per the terms of reference.

16.4 Protocol deviations

Prospective, planned deviations or waivers to the protocol will not be allowed. If any accidental protocol deviations happen at any time, they will be adequately documented on the relevant forms according to relevant SOPs and reported to the CI and Sponsor immediately. Any deviations from the protocol which are found to frequently recur are not acceptable. This will require immediate action to ensure there are not ongoing protocol deviations and, if they continue to occur, may be classified as a serious breach.

16.5 Serious breaches

A “serious breach” is defined as a breach which is likely to affect to a significant degree the safety or physical or mental integrity of the participants or the scientific value of the trial.
The Sponsor will be notified immediately of any case of a serious breach where the above definition applies during the trial conduct phase. The Sponsor of a clinical trial will notify the ethics committee in writing of any serious breaches of the conditions and principles of GCP in connection with that trial; or the protocol relating to that trial, as amended from time to time, within 7 days of becoming aware of that breach.

16.6 Transparency in research

Prior to the recruitment of the first participant, the study will have been registered on a publicly accessible database. Results will be uploaded to the databases within 12 months of the end of trial declaration 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.

16.7 Participant confidentiality

The study will comply with the General Data Protection Regulation (GDPR) and Data Protection Act 2018, which require data to be anonymised as soon as it is practical to do so. The trial staff will ensure that the participants’ anonymity is maintained other than for uses (e.g. communication with the GP) about which the participants will be specifically consented for. Participants will be identified by a participant ID number on the CRF. Any electronic databases and documents with participant identifying details will be stored securely and will only be accessible by study staff and authorised personnel. Paper documentation containing personal information will be kept in a locked filing cabinet in a locked room in the LSTM.

16.8 Patient and public involvement

This study is run in conjunction with the EHPC studies, which have been studying pneumococcal colonisation for over ten years. Within this team, there are numerous opportunities for public and patient involvement: there is a newsletter that is sent out to all participants to keep them informed of the study results and further work in the area. Our research ambassadors, and previous participants, assist with recruitment events and disseminations of information about ongoing work to the public. Additionally, our social media accounts (X/Twitter, Instagram and Facebook) update followers about current studies and our ongoing work.

17. Finance and indemnity/insurance

17.1 Funding

Funding for the study has been provided by the Medical Research Council.

17.2 Indemnity

The Sponsor, Liverpool School of Tropical Medicine, will ensure insurance and/or indemnity to meet the potential legal liability for harm to participants, the research team or to equipment arising from the management of the research.

17.3 Contractual arrangements

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

17.4 Expenses and benefits

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	Approximate duration	Payment per visit
Initial visit (participant presentation, eligibility questions, consent quiz, informed consent + GP summary)	45 minutes	£0
Screen appointment (clinical exam, medical history, vital signs, throat swab, nasal wash, nasal cells, bloods)	45 minutes	£40
Inoculation with pneumococcus and nose/throat samples (clinical exam, nasal/throat samples, bloods)	45 minutes	£40
Daily contact for 7 days	-	£5 per day
Clinic visit samples on Day 2, 6, 9, 14, 28 (transmission samples, nasal samples/wash, nasal cells, bloods)	30 minutes	£30

Ad-hoc visits (e.g., symptomatic visits, repeat bloods)	-	£10

Table 8: Participant remuneration

Therefore, participants can expect a maximum of £265 for full participation (if no ad-hoc visits are required).

Participants will be provided remuneration by direct bank transfer following attendance of their final visit in the study. Remuneration is on a *pro rata* basis should a participant not complete all visits and/or study requirements. If a participant withdraws from the study early, they will be remunerated for the visits they attended and samples which were taken up until the time they withdrew.

18. Publication policy

18.1 Publications

A publication is defined as any written document intended for submission to a congress, conference, journal or other public forum, and includes abstracts, posters and full articles, pertaining to the study, with or without study results. Publications will be consistent with the Consort Guidelines and checklist http://www.consort-statement.org/ and will be based on the International Committee of Medical Journal Editors (ICJME) requirements in that all persons listed as authors must meet ICJME requirements and all persons that meet these requirements will be listed as an author, other contributors will be acknowledged.

The findings from this study will be disseminated amongst the scientific community. We intend to publish our findings in peer reviewed scientific journals and present data at appropriate local, national and international conferences. In addition, we will produce a lay report of our findings, which will be made available to all participants.

18.2 Authorship

Authorship of the final trial report and subsequent publications will include those who contribute to the design, delivery and analysis of the trial. Authorship will be defined on completion of the study in discussion with Dr Ben Morton, Angela Hyder Wright and Professor Daniela Ferreira.

19. Conflicts of Interest

Neither the CI nor any collaborator has any direct personal involvement in organisations sponsoring or funding the research that may give rise to a possible conflict of interest

20. References

1. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Infect Dis* 2017; 17: 1133-1161.

2. Latifi-Navid H, Latifi-Navid S, Mostafaiy B, Jamalkandi SA, Ahmadi A. Pneumococcal Disease and the Effectiveness of the PPV23 Vaccine in Adults: A Two-Stage Bayesian Meta-Analysis of Observational and RCT Reports. *Scientific Reports* 2018; 8: 11051.
3. Verschoor CP, Johnstone J, Loeb M, Bramson JL, Bowdish DME. Anti-pneumococcal deficits of monocyte-derived macrophages from the advanced-age, frail elderly and related impairments in PI3K-AKT signaling. *Human Immunology* 2014; 75: 1192-1196.
4. Adler H, German EL, Mitsi E, Nikolaou E, Pojar S, Hales C, Robinson R, Connor V, Hill H, Hyder-Wright AD, Lazarova L, Lowe C, Smith EL, Wheeler I, Zaidi SR, Jochems SP, Loukov D, Reiné J, Solórzano-Gonzalez C, de Gorguette d'Argoeuves P, Jones T, Goldblatt D, Chen T, Aston SJ, French N, Collins AM, Gordon SB, Ferreira DM, Rylance J. Experimental Human Pneumococcal Colonization in Older Adults Is Feasible and Safe, Not Immunogenic. *Am J Respir Crit Care Med* 2021; 203: 604-613.
5. Weight CM, Venturini C, Pojar S, Jochems SP, Reiné J, Nikolaou E, Solórzano C, Noursadeghi M, Brown JS, Ferreira DM, Heyderman RS. Microinvasion by Streptococcus pneumoniae induces epithelial innate immunity during colonisation at the human mucosal surface. *Nat Commun* 2019; 10: 3060.
6. Weight CM, Jochems SP, Adler H, Ferreira DM, Brown JS, Heyderman RS. Insights Into the Effects of Mucosal Epithelial and Innate Immune Dysfunction in Older People on Host Interactions With Streptococcus pneumoniae. *Front Cell Infect Microbiol* 2021; 11: 651474.
7. Groves N, Sheppard CL, Litt D, Rose S, Silva A, Njoku N, Rodrigues S, Amin-Chowdhury Z, Andrews N, Ladhani S, Fry NK. Evolution of Streptococcus pneumoniae Serotype 3 in England and Wales: A Major Vaccine Evader. *Genes (Basel)* 2019; 10.
8. Ahl J, Littorin N, Forsgren A, Odenholt I, Resman F, Riesbeck K. High incidence of septic shock caused by Streptococcus pneumoniae serotype 3--a retrospective epidemiological study. *BMC Infect Dis* 2013; 13: 492.
9. Weinberger DM, Harboe ZB, Sanders EA, Ndiritu M, Klugman KP, Rückinger S, Dagan R, Adegbola R, Cutts F, Johnson HL, O'Brien KL, Scott JA, Lipsitch M. Association of serotype with risk of death due to pneumococcal pneumonia: a meta-analysis. *Clin Infect Dis* 2010; 51: 692-699.
10. Robinson RE, Mitsi E, Nikolaou E, Pojar S, Chen T, Reiné J, Nyazika TK, Court J, Davies K, Farrar M, Gonzalez-Dias P, Hamilton J, Hill H, Hitchins L, Howard A, Hyder-Wright A, Lesosky M, Liatsikos K, Matope A, McLenaghan D, Myerscough C, Murphy A, Solórzano C, Wang D, Burhan H, Gautam M, Begier E, Theilacker C, Beavon R, Anderson AS, Gessner BD, Gordon SB, Collins AM, Ferreira DM. Human Infection Challenge with Serotype 3 Pneumococcus. *Am J Respir Crit Care Med* 2022; 206: 1379-1392.
11. Luck JN, Tettelin H, Orihuela CJ. Sugar-Coated Killer: Serotype 3 Pneumococcal Disease. *Front Cell Infect Microbiol* 2020; 10: 613287.
12. Amin-Chowdhury Z, Collins S, Sheppard C, Litt D, Fry NK, Andrews N, Ladhani SN. Characteristics of Invasive Pneumococcal Disease Caused by Emerging Serotypes After the Introduction of the 13-Valent Pneumococcal Conjugate Vaccine in England: A Prospective Observational Cohort Study, 2014-2018. *Clin Infect Dis* 2020; 71: e235-e243.
13. Pick H, Daniel P, Rodrigo C, Bewick T, Ashton D, Lawrence H, Baskaran V, Edwards-Pritchard RC, Sheppard C, Eletu SD, Rose S, Litt D, Fry NK, Ladhani S, Chand M,

Trotter C, McKeever TM, Lim WS. Pneumococcal serotype trends, surveillance and risk factors in UK adult pneumonia, 2013-18. *Thorax* 2020; 75: 38-49.
14. Liatsikos K, Hyder-Wright A, Pojar S, Chen T, Wang D, Davies K, Myerscough C, Reine J, Robinson RE, Urban B, Mitsi E, Solorzano C, Gordon SB, Quinn A, Pan K, Anderson AS, Theilacker C, Begier E, Gessner BD, Collins A, Ferreira DM. Protocol for a phase IV double-blind randomised controlled trial to investigate the effect of the 13-valent pneumococcal conjugate vaccine and the 23-valent pneumococcal polysaccharide vaccine on pneumococcal colonisation using the experimental human pneumococcal challenge model in healthy adults (PREVENTING PNEUMO 2). *BMJ Open* 2022; 12: e062109.
15. Pollard AJ, Savulescu J, Oxford J, Hill AV, Levine MM, Lewis DJ, Read RC, Graham DY, Sun W, Openshaw P, Gordon SB. Human microbial challenge: the ultimate animal model. *Lancet Infect Dis* 2012; 12: 903-905.
16. Ferreira DM, Neill DR, Bangert M, Gritzfeld JF, Green N, Wright AK, Pennington SH, Bricio-Moreno L, Moreno AT, Miyaji EN, Wright AD, Collins AM, Goldblatt D, Kadioglu A, Gordon SB. Controlled human infection and rechallenge with Streptococcus pneumoniae reveals the protective efficacy of carriage in healthy adults. *Am J Respir Crit Care Med* 2013; 187: 855-864.
17. Nikolaou E, Jochems SP, Mitsi E, Pojar S, Blizard A, Reiné J, Solórzano C, Negera E, Carniel B, Soares-Schanoski A, Connor V, Adler H, Zaidi SR, Hales C, Hill H, Hyder-Wright A, Gordon SB, Rylance J, Ferreira DM. Experimental Human Challenge Defines Distinct Pneumococcal Kinetic Profiles and Mucosal Responses between Colonized and Non-Colonized Adults. *mBio* 2021; 12.
18. Mitsi E, Roche AM, Reine J, Zangari T, Owugha JT, Pennington SH, Gritzfeld JF, Wright AD, Collins AM, van Selm S, de Jonge MI, Gordon SB, Weiser JN, Ferreira DM. Agglutination by anti-capsular polysaccharide antibody is associated with protection against experimental human pneumococcal carriage. *Mucosal Immunol* 2016.
19. Wright AK, Bangert M, Gritzfeld JF, Ferreira DM, Jambo KC, Wright AD, Collins AM, Gordon SB. Experimental human pneumococcal carriage augments IL-17A-dependent T-cell defence of the lung. *PLoS Pathog* 2013; 9: e1003274.
20. Mitsi E, Kamng'ona R, Rylance J, Solorzano C, Jesus Reine J, Mwandumba HC, Ferreira DM, Jambo KC. Human alveolar macrophages predominately express combined classical M1 and M2 surface markers in steady state. *Respir Res* 2018; 19: 66.
21. Carniel BF, Marcon F, Rylance J, German EL, Zaidi S, Reine J, Negera E, Nikolaou E, Pojar S, Solorzano C, Collins AM, Connor V, Bogaert D, Gordon SB, Nakaya HI, Ferreira DM, Jochems SP, Mitsi E. Pneumococcal colonization impairs mucosal immune responses to live attenuated influenza vaccine. *JCI Insight* 2021; 6.
22. Mitsi E, Carniel B, Reine J, Rylance J, Zaidi S, Soares-Schanoski A, Connor V, Collins AM, Schlitzer A, Nikolaou E, Solorzano C, Pojar S, Hill H, Hyder-Wright AD, Jambo KC, Oggioni MR, De Ste Croix M, Gordon SB, Jochems SP, Ferreira DM. Nasal Pneumococcal Density Is Associated with Microaspiration and Heightened Human Alveolar Macrophage Responsiveness to Bacterial Pathogens. *Am J Respir Crit Care Med* 2020; 201: 335-347.
23. Peno C, Banda DH, Jambo N, Kankwatira AM, Malamba RD, Allain TJ, Ferreira DM, Heyderman RS, Russell DG, Mwandumba HC, Jambo KC. Alveolar T-helper 17 responses to streptococcus pneumoniae are preserved in ART-untreated and treated HIV-infected Malawian adults. *J Infect* 2018; 76: 168-176.

24. Diniz MO, Mitsi E, Swadling L, Rylance J, Johnson M, Goldblatt D, Ferreira D, Maini MK. Airway-resident T cells from unexposed individuals cross-recognize SARS-CoV-2. *Nat Immunol* 2022; 23: 1324-1329.
25. Collins AM, Wright AD, Mitsi E, Gritzfeld JF, Hancock CA, Pennington SH, Wang D, Morton B, Ferreira DM, Gordon SB. First human challenge testing of a pneumococcal vaccine. Double-blind randomized controlled trial. *Am J Respir Crit Care Med* 2015; 192: 853-858.
26. Hazenberg P, Robinson RE, Farrar M, Solorzano C, Hyder-Wright A, Liatsikos K, Brunning J, Fleet H, Bettam A, Howard A, Kenny-Nyazika T, Urban B, Mitsi E, El Safadi D, Davies K, Lesosky M, Gordon SB, Ferreira DM, Collins AM. Serotype 3 Experimental Human Pneumococcal Challenge (EHPC) study protocol: dose ranging and reproducibility in a healthy volunteer population (challenge 3). *BMJ Open* 2024; 14: e075948.
27. Gritzfeld JF, Wright AD, Collins AM, Pennington SH, Wright AK, Kadioglu A, Ferreira DM, Gordon SB. Experimental human pneumococcal carriage. *J Vis Exp* 2013.
28. Jochems SP, Marcon F, Carniel BF, Holloway M, Mitsi E, Smith E, Gritzfeld JF, Solórzano C, Reiné J, Pojar S, Nikolaou E, German EL, Hyder-Wright A, Hill H, Hales C, de Steenhuijsen Piters WAA, Bogaert D, Adler H, Zaidi S, Connor V, Gordon SB, Rylance J, Nakaya HI, Ferreira DM. Inflammation induced by influenza virus impairs human innate immune control of pneumococcus. *Nat Immunol* 2018; 19: 1299-1308.
29. Rylance J, de Steenhuijsen Piters WAA, Mina MJ, Bogaert D, French N, Ferreira DM. Two Randomized Trials of the Effect of Live Attenuated Influenza Vaccine on Pneumococcal Colonization. *Am J Respir Crit Care Med* 2019; 199: 1160-1163.
30. German EL, Solórzano C, Sunny S, Dunne F, Gritzfeld JF, Mitsi E, Nikolaou E, Hyder-Wright AD, Collins AM, Gordon SB, Ferreira DM. Protective effect of PCV vaccine against experimental pneumococcal challenge in adults is primarily mediated by controlling colonisation density. *Vaccine* 2019; 37: 3953-3956.
31. Jackson GG, Dowling HF, Spiesman IG, Boand AV. Transmission of the common cold to volunteers under controlled conditions. I. The common cold as a clinical entity. *AMA Arch Intern Med* 1958; 101: 267-278.
32. Robinson RE, Myerscough C, He N, Hill H, Shepherd WA, Gonzalez-Dias P, Liatsikos K, Latham S, Fyles F, Doherty K, Hazenberg P, Shiham F, McLenghan D, Adler H, Randles V, Zaidi S, Hyder-Wright A, Mitsi E, Burhan H, Morton B, Rylance J, Lesosky M, Gordon SB, Collins AM, Ferreira DM. Comprehensive review of safety in Experimental Human Pneumococcal Challenge. *PLoS One* 2023; 18: e0284399.
33. Nguyen-Van-Tam JS, Killingley B, Enstone J, Hewitt M, Pantelic J, Grantham ML, Bueno de Mesquita PJ, Lambkin-Williams R, Gilbert A, Mann A, Forni J, Noakes CJ, Levine MZ, Berman L, Lindstrom S, Cauchemez S, Bischoff W, Tellier R, Milton DK. Minimal transmission in an influenza A (H3N2) human challenge-transmission model within a controlled exposure environment. *PLoS Pathog* 2020; 16: e1008704.

Annex 1: Example diary card

Example of fields for diary card: upper and lower respiratory symptoms scores

	Sneezing	Headache	Malaise	Fever / chills	Nasal discharge	Nasal obstruction	Sore throat	Cough	Total score
Day 0*									
Day 1									
Day 2									
Day 3									
Day 4									
Day 5									
Day 6									
Day 7									

***- To be collected prior to challenge**

Upper respiratory clinical symptom score. Scores are documented as 0 = absent, 1 = mild, 2 = moderate, 3 = severe, and 4 = emergency department visit or hospitalisation

Definition of a clinical cold

A clinical cold is diagnosed if two or more of the following are present:

- A cumulative clinical symptom score of 14 or greater over a six-day period
- Nasal discharge is present on three or more days
- A subjective impression of a cold developing. This latter criterion is used because there are a few participants who have had a very strong subjective impression of a clinical cold but the cumulative clinical score does not reach the arbitrary cut-off level

	Cough on waking	Wheeze on waking	Daytime cough	Daytime wheeze	Daytime SOB*	Nocturnal cough, wheeze or SOB*	Coughing up phlegm	Total score
Day 0*								
Day 1								
Day 2								
Day 3								
Day 4								
Day 5								
Day 6								
Day 7								

***- To be collected prior to challenge**

Lower respiratory clinical symptom score. Scores are documented as 0 = absent, 1 = mild, 2 = moderate, 3 = severe, and 4 = emergency department visit or hospitalisation SOB: Shortness of breath

Annex 2: Grading the severity of self-reported Adverse Events (challenge period)

Adverse event	Grade	Definition (in degrees Celsius)
Temperature	0	< 37.6

1	37.6 – 38.0
2	38.1 – 39.0
3	> 39.0

Annex 3: Grading the severity of visit-observed Adverse Events

Observation	Grade 1	Grade 2	Grade 3	Grade 4
Oral temperature (°C)	37.6 – 38.0	38.1 – 39.0	> 39.0	A&E visit or hospitalisation for hyperpyrexia
Tachycardia (beats/min)	101-115	116-130	>130	A&E visit or hospitalisation for arrhythmia
Bradycardia*/ (beats/min)**	50-54	45-49	<45	A&E visit or hospitalisation for arrhythmia
Systolic hypertension (mmHg)**	141-150	151-155	>155	A&E visit or hospitalization for malignant hypertension
Diastolic hypertension (mmHg)**	91-95	96-100	>100	A&E visit or hospitalization for malignant hypertension
Systolic hypotension (mmHg)	85-89	80-84	<80	A&E visit or hospitalization for hypotensive shock

*Clinical discretion will be applied to lower heart rates due to physical fitness

** Pre-existing bradycardia/hypertension that is unchanged from screening will not require grading

Annex 4: Grading the severity of laboratory Adverse Events

Parameter	Grade 1	Grade 2	Grade 3	Grade 4*
White cell count: elevated (10^9/L)	11–15	16–20	21–25	>25
White cell count: depressed (10^9/L)	2.5-3.5	1.5-2.4	1.0-1.4	<1.0
Neutrophil count (10^9/L)	1.5-2.0	1.0-1.4	0.5-0.9	<0.5
Platelets (10^9/L)	125-140	100-124	25-99	<25
Sodium: hyponatraemia (mmol/L)	132–134	130–131	125–129	<125
Sodium: hypernatraemia (mmol/L)	146	147	148–150	>150

Potassium: hyperkalaemia (mmol/L)	5.4 – 5.5	5.6 – 5.7	5.8 – 5.9	>5.9
Potassium: hypokalaemia (mmol/L)	3.3–3.4	3.1–3.2	3.0	<3.0
Urea (mmol/L)	8.2–8.9	9.0–11	>11	RRT
Creatinine (µmol/L)	132-150	151-176	177-221	>221 or RRT
C-reactive protein	>10-30	31-100	101-200	>200

Grade 4* Potentially life threatening