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CHIRP01 PROTOCOL

Study Title: Novel Mucosal Correlates Of RSV Protection In Older Adults
(A **C**ontrolled **H**uman **I**nfection Study with **R**SV in older **P**eople)

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Sponsor

Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

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Funder

Merck has provided the funding for this study.

This protocol describes the CHIRP-01 study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the UK Policy Frame Work for Health and Social Care Research. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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GLOSSARY OF ABBREVIATIONS

AE	Adverse Event
ANOVA	Analysis of variance
CI	Chief Investigator
CRF	Case Report Form
DBP	Diastolic Blood Pressure
DEG	Differentially expressed genes
ECG	Electrocardiogram
ELF	Epithelial Lining Fluid
ENT	Ears/Nose/Throat
FDA	Food and Drug Administration
FEV_1	Forced Expiratory Volume in One Second
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
HBV/HCV	Hepatitis B/C Virus
HIV	Human Immunodeficiency Virus
HRA	Health Research Authority
HR	Heart Rate
ICHNT	Imperial College Healthcare NHS Trust
ICRF	Imperial Clinical Research Facility
IMP	Investigational Medicinal Product
LLOQ	Lower limit of quantification
LPLV	Last Participant Last Visit
NHS	National Health Service
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
PEFR	Peak Expiratory Flow Rate
PI	Principal Investigator
PIS	Participant Information Sheet
PNIF	Peak Nasal Inspiratory Flow
PPE	Personal Protective Equipment
PRB	Protocol Review Board
REC	Research Ethics Committee
RNA	Ribonucleic acid
RR	Respiratory Rate
RSV	Respiratory Syncytial Virus
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event
SAM	Synthetic Absorptive Matrix
SBP	Systolic Blood Pressure
SOP	Standard Operating Procedure
URI	Upper Respiratory Infection
WGCNA	Weighted gene co-expression network analysis
WHO	World Health Organisation

KEYWORDS

Respiratory Syncytial Virus, RSV, immune, virus, bronchiolitis, viral challenge, viral lung disease, immunosenescence, T cells, B cells, vaccines

STUDY SUMMARY

TITLE Novel Mucosal Correlates of RSV Protection in Older Adults

DESIGN Human viral challenge study in healthy older adults

AIMS 1. To identify biomarkers at the point of RSV inoculation associated with susceptibility to or protection from infection in older adults

2. To identify biomarkers in response to RSV infection associated with differential disease severity in older adults

STUDY OBJECTIVES

Primary objective:

- To confirm safety and tolerability in older adult volunteers of inoculation with RSV Memphis 37 as measured by
  - Occurrence of adverse events (Aes) from the viral challenge (Day 0) up to Day 28 follow-up
  - Occurrence of serious adverse events (SAEs) from the viral challenge (Day 0) up to Day 28 follow-up
- To confirm the infection rate in older adult volunteers after inoculation with RSV Memphis 37, with infection defined by
  - Two or more quantifiable greater than lower limit of quantification (viral load ≥LLOQ) by RT-PCR from nasal wash, reported on 2 or more consecutive timepoints, starting from Day 2 post-inoculation and up to discharge from quarantine.

Secondary objectives:

- To assess RSV viral dynamics by RT-PCR in upper respiratory samples
  - Peak, Duration, Area Under the Curve
- To assess RSV-induced symptoms by self-reported symptom scores
  - Peak, Duration, Area Under the Curve
- To assess antibody levels in blood before and after RSV challenge infection

Exploratory objectives:

- To assess antibody levels in nasal lining fluid before and after RSV challenge infection
- To assess RSV-specific T cell frequencies in blood before, during and after RSV challenge infection
- To assess T cell frequencies in nasal mucosa before, during and after RSV challenge infection
- To assess soluble mediator levels in blood before, during and after RSV challenge infection by multiplex protein assay
- To assess differential gene expression in nasal cells before, during and after RSV challenge infection by RNA sequencing
- To assess age-related changes in nasal epithelial cells before and after RSV infection using air-liquid interface cultures
- To compare virologic, inflammatory and immune responses before and after RSV infection with other pathogens using data and samples from other studies

POPULATION Healthy, non-smoking volunteers aged 65 – 75 years. 20 participants in total.

ELIGIBILITY Healthy, non-smoking volunteers aged 65 – 75 years that fit the inclusion and exclusion criteria.

DURATION For each participant, the duration of the study will be approximately 6 months from the day of enrolment (Day 0) until their last study related follow up. The duration of the entire study is estimated at 2 years, with the study end date

being 1 year after the Last Participant’s Last Visit.

1. INTRODUCTION

BACKGROUND

Respiratory Syncytial Virus (RSV) is one of the most common causes of severe viral respiratory tract infection, second only to influenza. Despite the global burden of disease, it remains underappreciated although more detailed epidemiological studies have recently highlighted the importance of this disease in terms of incidence, morbidity and mortality. WHO estimates put RSV as the cause of around 64 million infections each year and 160,000 deaths but this is likely to be an underestimate in view of difficulties in diagnosis. Not only is RSV the leading cause of severe respiratory illness in young children, it is also a major contributor to mortality in older adults. Incidence increases with older age, such that RSV is responsible for around 20% of GP visits for acute respiratory illness and up to 5% of admissions with community acquired pneumonia[1]. This imposes a huge socioeconomic cost, with $680 million spent each year in the USA for in-patient treatment alone[1,2].

Despite decades of research, the first effective RSV vaccine[3] was only approved by the FDA in May 2023 and there is still no specific treatment. Unusually, RSV does not induce long-lasting immunity following infection and therefore recurrent infections with RSV occur in all age groups with the most severe disease occurring at either end of life. While infection with a given strain of influenza may protect against symptomatic disease caused by that same strain for up to 7 years[4], even healthy adults can be repeatedly infected with an identical RSV at as little as 2 month intervals[5]. This has hindered vaccine development as the correlates of protection that might be defined in genuinely immune individuals are not available and therefore measurements of vaccine immunogenicity remain poorly predictive of subsequent vaccine efficacy.

Antibody as a correlate of protection against RSV infection

The mechanisms underlying the incomplete immunity elicited by RSV infection remain unclear and factors that determine whether an individual will develop symptomatic infection following exposure to a respiratory pathogen remain ill defined. Serum neutralising antibody remains the most well-studied correlate of protection against RSV but the protection associated with circulating antibody is partial at best. Circulating antibody alone does have some role in preventing severe RSV disease, as evidenced by the effectiveness of prophylactic administration of the anti-RSV monoclonal antibody palivizumab (Synagis)[6]. However, few adults are able to generate sufficiently high levels to provide consistent protection. Furthermore, some individuals with low antibody titres remain resistant to infection, suggesting that antibody-independent immune mechanisms are also important[7]. Nevertheless, the first RSV vaccine to be approved, Arexvy (GSK), and all leading competitors have been developed to induce high levels of the most potent circulating antibodies. These have been shown in phase III clinical trials to be efficacious in preventing severe disease, with less efficacy against milder symptomatic disease[3,8]. However, there is currently no data about the impact of these intramuscular vaccines on asymptomatic/minimally symptomatic infection that may nevertheless contribute to protection, or duration of protection.

The importance of cell mediated immunity

T cells are believed to play an essential role in clearance of RSV from the lungs. In the BALB/c mouse, up to 40% of CD8+ T cells infiltrating the lung may be specific for an epitope from the M2 protein at the peak of infection[9]. Early studies in athymic nude or irradiated mice showed that persistent infection with prolonged RSV shedding could be cleared by adoptive transfer of memory T cells from previously primed animals[10]. In BALB/c mice depleted of CD4+ and CD8+ T cells, both T cell subsets play a role in shortening the duration of RSV shedding[11]. Conversely, stimulation of epitope-specific CD8+ T cells using peptide immunisation of HLA-A*0201 transgenic mice ameliorates disease and improves viral control[12]. Immunisation using peptide-loaded dendritic cells (DCs) from the closely related pneumonia virus of mice confers partial protection against subsequent RSV challenge[13]. Similar findings have been observed in other animal models such as RSV infection (e.g. calves) in which depletion of CD8+ T cells delays viral clearance.

In human observational studies, T cells are also implicated in viral clearance. In children with severe infection, CD8+ antigen-specific T cells accumulate in the peripheral blood and airways, peaking at around 9 days after symptom onset[14]. These display an activated phenotype, but their abundance does not correlate with disease severity. In studies of adults, numbers of activated CD4+ and CD8+ T cells do correlate with disease severity[15]. A role for T cells in viral clearance is also implied by a prospective study examining children under the age of 5, in which individuals immunocompromised due to chemotherapy or with primary immunodeficiencies affecting T cell function were shown to suffer more severe disease and shed virus at higher levels for several months, compared with 7-21 days in normal children[16]. Thus it is likely that cell-mediated immunity is important both in shortening the duration of symptomatic disease and coordinating the generation of the overall adaptive immune response.

Aging, immune senescence and its effect on RSV infections

Advancing age leads to progressive decline in immune responsiveness and alterations in immune regulation[17]. This contributes to increased susceptibility to infectious diseases and reduced responsiveness to vaccines. Immune responses against the vaccines commonly recommended for elderly adults are generally less robust in this population than in young adults in terms of magnitude, duration and functionality[18]. This usually translates to reduced vaccine efficacy[19]. However, the effect of aging on immune factors related to protection from symptomatic RSV disease has been relatively under-studied. Anti-RSV antibody levels in the blood are believed to be well maintained, as serum neutralising antibody titres are similar between young and old cohorts. However, low serum and nasal antibody levels have also been associated with greater risk of disease[20]. In addition, some groups have reported age-related reductions in antigen-specific CD8+ T cells using tetramer labelling or intracellular staining of interferon-γ following *in vitro* RSV stimulation[21–23]. None of these studies have correlated these observed reductions with increased risk of infection.

RATIONALE FOR CURRENT STUDY

Human infection challenge to investigate mucosal immunity against RSV

RSV infects via the upper respiratory tract epithelium and severe disease is characterised by exuberant inflammation in the lower airways. Viraemia is not believed to occur and it is therefore assumed that effector mechanisms that prevent and clear RSV must mainly act at and around the respiratory mucosa. In animal models, relative ease of access has allowed analysis of mucosal immune responses in RSV disease but in humans this has been difficult. Instead, human immune responses are most commonly investigated in blood with relatively few studies designed to investigate the unique features of immunity in the airway.

For >12 years, our group has conducted human infection challenge studies with RSV to better understand how immune factors affect the clinical outcome following virus exposure[24]. Challenge studies overcome the many difficulties associated with studying a naturally infected patient group where it is impossible to control for the strain or dose of virus exposure; diagnosis is often delayed; the timing is unknown; many patients have underlying medical conditions influencing their immune responses; and where they may be subject to therapeutic measures that alter those responses. Using a well-characterised, fully virulent GMP-certified challenge strain of RSV allows us to closely replicate natural infection but also provide us with the control required to answer important questions about the essential immune responses required for protection and which of these may be impaired with increasing age.

Our previous work using this system demonstrated the role of nasal IgA in protection from RSV infection in healthy young adults, where very low levels are associated with susceptibility to infection but even at moderately high levels, protection is incomplete. Furthermore, our data indicate that RSV infection fails to induce the generation of IgA-producing memory B cells despite a robust IgG memory response, in contrast with influenza, which can induce both. These studies therefore explain the short duration of protective immunity seen following RSV infection.

However, a follow-up study in older adults showed that even short-term nasal IgA responses were impaired in this age group[25]. In a pilot study of older adults challenged with the same strain and dose of RSV as young adults, little boosting of nasal IgA was seen following RSV challenge infection and there was no correlation between nasal IgA titres and protection from infection. Instead, serum neutralising and IgG antibodies showed a statistically stronger relationship with protection, suggesting that in the absence of a robust local IgA response, circulating IgG could be stimulated in some older individuals to higher protective levels.

If viral replication overcomes the innate and humoral mechanisms that function to block infection, additional lines of defence in the airway must be mobilised to minimise tissue damage and symptoms. In a number of peripheral tissues, the importance of the recently described CD69+CD103+ resident memory T (T_{RM}) cell subset is increasingly appreciated. Not only are CD8+ T_{RM} cells poised for immediate cytotoxicity but they may also exhibit sensing functions that provide early innate-like activity contributing to the first line of defence[26]. In animal models, T_{RM} cells have been shown to provide superior immune protection compared with systemic antigen-specific T cells, although their abundance in tissues may also predispose toward immunopathology[27]. Our work using experimental human infection with RSV also demonstrated the predominance of CD8+ T_{RM} cells in the lower airway[28]. Following RSV infection, antigen-specific CD8+ T_{RM} cells were dramatically enriched, persisting for longer and at higher frequencies than in blood. At rest, their phenotype suggests higher activation requirements in keeping with the relatively anti-inflammatory milieu of the lung, but their relatively high frequency suggests a role as patrolling cells for early defence, with little trafficking through peripheral blood. Further analysis of these and other tissue-resident cells in the nasal compartment under the controlled conditions of an experimental human infection trial will additionally permit their correlation with risk and severity of infection in older people.

Based on extensive preclinical and clinical research, we hypothesise that susceptibility to RSV infection and disease in the setting of older age represents a dysregulated and over-exuberant inflammatory response to infection. Steroids have limited efficacy, and identification of diagnostic, prognostic and/or druggable inflammatory pathways in severe RSV disease would be of great potential value. Observational studies of natural infection are constrained by sampling, timing and access. We therefore propose to conduct a human RSV infection challenge study focusing on the nasal response to RSV infection in older adults to identify biomarkers and signatures associated with differential disease severity that may act as targets for diagnostics and interventions.

Safety of human challenge with RSV in young and older volunteers

Since 2010, we have been conducting experimental human challenge studies with RSV, influenza virus and SARS-CoV-2 in young adult volunteers. To date, >150 individuals have been inoculated safely with RSV, with around 55% of those aged 18-55 years subsequently developing PCR-confirmed infection. Of those, around 66% develop symptoms consistent with a common cold. The infections have been uniformly mild, self-limiting and there have been no serious adverse events.

In parallel, Dr. Patrick Mallia and Professor Sebastian Johnston with whom we work closely have conducted several experimental infection studies in older subjects with chronic obstructive pulmonary disease using rhinovirus. These studies included volunteers aged 40-75 with post-bronchodilator FEV1/FVC <70% (i.e. moderate COPD) who were either current or ex-smokers of at least 20 cumulative pack years. As well as having been infected, these individuals also underwent multiple bronchoscopies during the course of their infections. Furthermore, these were conducted as out-patient studies without residential monitoring. Thus, rhinovirus challenge and bronchoscopy in this context have been shown to be safe even in elderly individuals with underlying lung disease and extensive smoking histories.

Although older adults are on average more susceptible to RSV disease, a study of natural RSV infection in adults aged 69-81 in the USA showed that only high-risk patients (i.e. with congestive heart failure or chronic pulmonary disease) ever developed disease severe enough to require hospital treatment. Of 46 healthy older adults with virologically confirmed RSV infection, only 17% required a physician consultation and none

required emergency room or hospital care. This contrasted with high-risk patients of whom 16% required hospital admission. While we recognise that RSV is generally understood to cause a more severe disease in high-risk patients than rhinovirus, these data indicated that in a carefully screened healthy non-smoking group of older volunteers there is little risk of severe disease. On this background, we extended the human RSV challenge system to older adults aged 60-75 years[25] and have now challenged 28 individuals, 18 of whom also underwent lower airway sampling by bronchoscopy. To date, there have been no serious adverse events and RSV infection has been uniformly well tolerated. By setting the upper age limit at 75 years and conducting these studies within an in-patient quarantine unit with close clinical monitoring and access to higher level clinical care, we therefore believe the risks of more severe outcomes have been mitigated.

With a global aging population and continuing difficulties in generating vaccines that can reliably induce protective immunity in the elderly, there is a critical need to more clearly understand why older people are more susceptible to respiratory viral infections. This unique controlled study will not only indicate the targets at which development of vaccines and therapeutics against RSV should be directed, but we also anticipate the discovery of generalisable biomarker signatures that will accelerate the development of interventions against respiratory viruses as a whole.

Research Hypotheses

1. Older age alone, in the absence of co-morbidities, is associated with impairment in local and/or systemic immune responses to RSV

2. Humoral and cell mediated immune responses in the airway and/or blood correlate differently with protection against RSV in older people compared to young adults

3. Immune biomarkers in the nasal mucosa are associated with differential clinical outcome following exposure to RSV

2. STUDY OBJECTIVES

Primary objective:

- To confirm safety and tolerability in older adult volunteers of inoculation with RSV Memphis 37 as measured by
    - Occurrence of adverse events (aEs) from the viral challenge (Day 0) up to Day 28 follow-up
    - Occurrence of serious adverse events (SAEs) from the viral challenge (Day 0) up to Day 28 follow-up
- To confirm the infection rate in older adult volunteers after inoculation with RSV Memphis 37, with infection defined by
    - Two or more quantifiable greater than lower limit of quantification (viral load ≥LLOQ) by RT-PCR from nasal wash, reported on 2 or more consecutive timepoints, starting from Day 2 post-inoculation and up to discharge from quarantine

Secondary objectives:

- To assess RSV viral dynamics by RT-PCR in upper respiratory samples
    - Peak
    - Duration
    - Area Under the Curve
- To assess RSV-induced symptoms by self-reported symptom scores
    - Peak
    - Duration

    - Area Under the Curve
- To assess antibody levels in blood before and after RSV challenge infection

Exploratory objectives:

- To assess antibody levels in nasal lining fluid before and after RSV challenge infection
- To assess RSV-specific T cell frequencies in blood before, during and after RSV challenge infection
- To assess T cell frequencies in nasal mucosa before, during and after RSV challenge infection
- To assess soluble mediator levels in blood before, during and after RSV challenge infection by multiplex protein assay
- To assess differential gene expression in nasal cells before, during and after RSV challenge infection by RNA sequencing
- To assess age-related changes in nasal epithelial cells before and after RSV infection using air-liquid interface cultures

3. STUDY DESIGN

This is a human challenge study involving healthy, non-smoking older adults aged 65–75 years. We aim to enrol 20 participants in this study.

Prior to infection, blood and respiratory samples will be collected. Participants will then be inoculated with 10^4 plaque-forming units of RSV A Memphis 37 intranasally.

Due to the older age range and potentially increased risk of more severe disease, participants will be quarantined in the Imperial Clinical Research Facility (ICRF) for up to 10 days post-inoculation, during which they will be closely monitored by clinical review and self-completed symptom diaries.

Clinical outcomes will be determined by symptomatology and viral detection using quantitative PCR. Based on recent elderly RSV challenge data, we anticipate a 70% attack rate, with a range of mild-moderate symptoms in the infected individuals. Symptoms typically commence 3 days post-inoculation and worsen to peak around days 7-8 before rapidly resolving without treatment. Viral shedding is expected to correlate closely with symptoms.

STUDY OUTCOME MEASURES

Primary:

- Solicited and unsolicited adverse events (AEs and SAEs) from virus inoculation (Day 0) to Day 28 post-inoculation
- Infection rate calculated by the number of infected individuals over the number of uninfected individuals expressed as a percentage, with infection defined as 2 or more quantifiable greater than lower limit of quantification (viral load ≥LLOQ) by RT-PCR from nasal wash, reported on 2 or more consecutive timepoints, starting from Day 2 post-inoculation and up to discharge from quarantine

Secondary:

- Nasal viral load by RT-PCR
- Antibody levels in blood by serum neutralisation assay and ELISA

Exploratory

- Antibody levels in nasal lining fluid before by ELISA

- RSV-specific T cell frequencies in blood by ELISpot assay
- T cell frequencies in nasal swab samples by flow cytometry, ELISpot or other cellular assay
- Soluble mediator levels in nasal lining fluid by multiplex protein assay
- Differential gene expression in nasal cells by RNA sequencing
- Nasal mucosal epithelial integrity and function in air-liquid interface cultures

4. PARTICIPANT ENTRY

SCREENING PHASE

The screening process will consist of the following stages: registering interest in the study; pre-screening questionnaire (online or by phone) and a full screening visit. Screening will occur between days -90 to -2 prior to date of virus inoculation.

Advertising

Advertising of the study will be done through a number of channels, such as:

- Website (e.g. study specific or sponsor's/site's website or webpages)
- Social media adverts and posts (e.g. Facebook, Twitter, LinkedIn and Instagram)
- GP invitation letters and GP text messaging services (local GP practices can be approached via the local CRN to contact potentially eligible participants registered with them and direct them to the study information on a website. The sponsor/site will not have access to their personal information and the GP practices will have the appropriate PIC agreements in place)
- Direct mailouts and emails through any volunteer databases or mailing lists where members of these databases/lists have previously agreed/consented to be contacted about taking part in research. Examples include the NIHR Be Part of Research database or Imperial College/NHS research databases, such as the ICRF Healthy Volunteer Database
- Radio (including Spotify) or newspaper adverts
- Posters and leaflets around the college and NHS campuses, or external locations such as: local community centres, charity centres, places of worship and/or travel hubs such as train and bus stations.
- University or NHS newsletters
- Referral
- Organic search (e.g. via Google or other search engines)

Registration and Pre-screening questionnaire

There will be information about the study on the Imperial College human challenge webpages and the PIS will be available to download and read from these webpages.

For volunteers interested in taking part, they will be asked to register their interest by completing an online pre-screening questionnaire and submitting their details to the study team. Alternatively, the participants can express interest directly using the study team's email and/or phone number. All advertising and media will direct volunteers to the webpage and will have the study team's email and/or phone number.

If participants express their interest in the study, they will be asked some initial pre-screening questions to assess their eligibility before being invited to a screening visit. The pre-screening questionnaire can be completed on the study webpage and the responses will be sent to the study or available for the study team to download. If the participant would prefer, the questions can be asked over email or telephone with the study team.

The following “yes or no” questions will be asked in the online pre-screening questionnaire:

1. Are you aged between 65-75years?
2. Do you currently smoke and/or use e-cigarettes?
3. Have you previously smoked or use e-cigarettes?
4. Do you have any current health problems? Please think about any health conditions you have that you see a healthcare provider for or take any medication for.
5. Have you had any health problems in the past? Please think about any significant medical conditions and/or hospital admissions and/or surgeries you have had, or any medications you have received for a condition in the past.
6. Are you currently taking any regular medications (including inhalers)?
7. Do you have any known or suspected allergies?
8. Do you have close contact with children or people who are deemed clinically vulnerable? For examples of clinical vulnerability, this could be people with a respiratory or cardiovascular condition/disease or problems with their immune system.
   By close contact we mean sharing a household with, caring for, or daily face to face contact.
9. Have you been vaccinated against COVID-19?
10. Have you had a cold or any cold-like symptoms, such as a cough, in the last 6 weeks?
11. Are there any dates you would be unavailable to attend study visits or the residential stay (e.g. planned holidays)? (Please specify the dates)

- Contact details (full name, date of birth, email address, phone number, home address and NHS number)

Depending on the responses given in the online pre-screening questionnaire, the study nurse and/or doctor will review and determine the participant’s eligibility to be invited for a screening visit. It is expected that the study team will need to follow up with the participant over email or phone to clarify some of the answers to the questions and gather more information to assess their eligibility to take part. This includes further details on medical history, medications, COVID-19 vaccination status etc.
If, on the basis of the pre-screening questionnaire response and further discussion with the study team, the participant is deemed eligible for screening, they will be invited to attend the Imperial Clinical Research Facility (ICRF) at Hammersmith Hospital, White City for a formal screening visit. If they have been deemed ineligible to take part, they will be informed of this.

Screening Visit

Participants will be sent an electronic version of the Participant Information Sheet (PIS) to read when they first express their interest in the study and again at least 24 hours prior to the screening visit, at both timepoints they will be encouraged to read the PIS ahead of the visit.
At the screening visit, the Study Doctor will explain the study and experimental procedures in detail and provide the participant a hard copy of the PIS. The participant will be encouraged to ask any questions and be shown the devices used for sample collection (swabs, nasal wash etc) as well as the unit where the residential stay takes place. When the participant has had time to consider their participation in this study, ask any questions they may have, and only when they have agreed to take part will they be asked to read, sign and date a consent form in the presence of the Study Doctor who will also sign the consent form.

There may be optional statements on the ICF. The participants will have these optional statements and procedures explained to them and these will be detailed in the PIS. The participant can then decide whether to agree to undergo these optional procedures and if they chose not to, this would not affect their ability to take part in the study.

Informed consent will be obtained prior to any study related procedures. The original signed ICF will be kept for the ISF, a copy a copy given to the participant and a copy put into their participant/medical notes.

Following informed consent, the following assessments will be completed at the screening visit:

- Full medical and medication history including questions about past and present health
- Quality of life questionnaires including the GAD-7 Anxiety Test questionnaire and PHQ-9 Depression Test questionnaire
- Questions about current weekly alcohol and/or smoking consumption and any history of use of drugs of abuse
- Examination for signs of illness or disease (a medical examination).
- Height and weight measurements to calculate BMI
- Pulse rate, blood pressure, temperature, saturations and breathing rate checked (Vital Signs).Electrocardiogram (ECG)
- Urine samples for drugs of abuse & cotinine
- Safety blood tests: full blood count, renal function, liver function tests, clotting, C-reactive protein (CRP), random glucose, lymphocyte subsets, immunoglobulins and HIV, HBV and HCV serology.
- Blood sample to save plasma and whole blood cells for HLA typing and other research assays if the participant passes screening
- Peripheral samples for research assays if the participant passes screening including: nasosorption, nasal lavage, nasal brushing, nasal scrape, oral swab
- Spirometry (Lung Function Test)
- Chest X-Ray

The participant's medical history will either be reviewed during screening (only after informed consent) using the hospital's electronic patient record system (such as Cerner) or using the ACCURX system as described in the PIS. If it is not possible to review their records using this system, or the record is not complete, the participant's medical history will be requested from their GP and reviewed to assess suitability. Participants may be invited for repeat assessment if required at the PI's discretion. If they are still eligible to take part, and when the participant has had enough time to consider their participation in this study, ask questions and if they are still willing to take part in the study, they will be invited to the residential stay.

The results from the screening visit, together with the GP summary, will be reviewed by the Study Doctor and if necessary, discussed with the PI to ensure that the participant meets all of the inclusion criteria, and none of the exclusion criteria, before inviting the individual to be enrolled into the study. If the results or any findings from the screening visit deem the participant ineligible, the study team will contact the individual and provide a full explanation. If required, and with the participant's permission, the study doctor will also inform the participant's GP of any incidental findings so that they may be appropriately followed up.

If a participant falls out of the 90-day screening window prior to challenge, they will be invited for repeat screening procedures including: blood and urine tests, vital signs, ECG, lung function test and physical examination. Their medical and medication history will also be reassessed. They will not be required to undergo a repeat chest X-ray unless it has been longer than 6 months since their previous X-ray or unless deemed a requirement by the Study Doctor or PI.

INCLUSION CRITERIA

- Healthy persons aged 65 to 75 years, able to give informed consent
- Non-smokers or ex-smokers with a pack year history of 10 or less
- Spirometry within the normal range for age and height (+/- 15%)
- FEV1/FVC >70% without bronchodilator
- Vaccination against SARS-CoV-2 at the minimum of 4 weeks prior to screening

EXCLUSION CRITERIA

- Chronic respiratory disease (asthma, COPD, rhinitis, sinusitis) in adulthood
- Inhaled bronchodilator or steroid use within the last 12 months
- Habitual use of any medication or other product (prescription or over the counter) for symptoms of rhinitis or nasal congestion within the last 3 months
- Acute upper respiratory infection (URI or sinusitis) in the past 6 weeks
- Participants with allergic symptoms present at baseline
- Clinically relevant abnormality on chest X-ray
- Those in close domestic contact (i.e. sharing a household with, caring for, or daily face to face contact) with children under 3 years, clinically vulnerable and/or immunosuppressed persons, or those with chronic respiratory disease
- Participants with known or suspected immune deficiency
- Receipt of systemic glucocorticoids (in a dose ≥ 5 mg prednisone daily or equivalent) within one month, or any other cytotoxic or immunosuppressive drug within 6 months prior to challenge
- Known IgA deficiency, immotile cilia syndrome, or Kartagener's syndrome
- History of frequent nose bleeds
- Any medical condition, prescribed or over-the-counter drug, or any other reason deemed by a study doctor to make the participant unsuitable for the study
- Recent or current use of recreational drugs, confirmed by a positive urine drug screen
- History of difficult blood draw, syncope or poor tolerance of sampling procedures
- Receipt of an RSV vaccine at any time

WITHDRAWAL CRITERIA

Any participant can withdraw from the study at any time if they wish to. Participants can also be removed from the study if an study doctor feels this is necessary or appropriate. Participants will be closely monitored throughout the residential stay, and followed up at subsequent study visits, by the study doctor and study team. This is defined further in the section on 'Serious Adverse Events'.

If a participant loses capacity during the study, the participant will be withdrawn from the study. Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected, or any other research procedures carried out on or in relation to the participant.

5. CHALLENGE AND FOLLOW-UP

The study is divided into screening, residential and follow-up periods. Following the screening visit, if deemed eligible, the participant will be invited to the residential part of the study. Participants will be admitted to the quarantine unit on Day -1, one day before the planned inoculation date.

The length of the residential period (8-10 days post inoculation) has been chosen to eliminate the possibility of participants in the study transmitting the virus to anyone not involved in the study (i.e. family, household contacts, and the wider community). It also allows the study team to closely monitor the participants during the infectious period. During the residential period, all study procedures will take place in the quarantine unit.

Residential period (Day -1 to Day 8-10)

Participants will be invited to the residential part of the study within 90 days of their screening visit, providing they are eligible to take part. The quarantine unit is at the Imperial Clinical Research Facility (ICRF) at

Hammersmith Hospital; participants will be admitted on the day before planned virus inoculation, known as Day -1.

A nasal sample will be taken at admission to exclude coincident infection and further baseline blood and nose samples will be taken from all participants. Also on Day -1, a blood sample for CMV serology may be taken as well as other assessments and samples listed in the Schedule of Events.

The next day, Day 0, subject to a satisfactory assessment by the Study Doctor (brief interview and medical examination), the participant will be inoculated with RSV using a dose of 10^4 PFU. After inoculation, participants will be observed for a minimum of 30 minutes in the ICRF to ensure no adverse reactions have occurred. They will then reside in the residential unit for the next 8-10 nights for monitoring and sample collection to evaluate immune responses and test the hypotheses laid out in this protocol. The sampling will include blood samples, daily throat swabs, nasal lavage, nasal SAM, nasal curretage and lung function tests.

Participants will stay overnight and within the quarantine unit for a period of 8-10 nights in total, from the day of viral challenge. Participants who remain uninfected can leave the quarantine unit from Day 8 post inoculation, onward but must return to the ICRF for their Day 9 and Day 10 sampling.

Participants who become infected with RSV must stay in the quarantine unit until Day 10.

Follow-up period (out-patient, Day 11 to Day 180)

To investigate the later stage of infection, participants will return for out-patient visits on Day 14, Day 28 and Day 180 post-infection for assessment and sampling. During the 6 months after challenge, participants will be asked to report all episodes of upper respiratory tract symptoms.

Participants will be given a contact card with the contact details for the Study Doctor and Research Nurses and be asked to contact the team should they experience any serious adverse events, such as hospitalisations, during the 6-month follow up period.

The participants will complete the study after their final Day 180 visit. When the study is completed the participants will not be routinely followed-up by the study team. Participants will return to the care of their GP following completion of the study and this will be highlighted to the participant at the final visit.

Those who have experienced an SAE will be followed up until resolution of the SAE or until it is deemed by the PI or clinician in charge of the participant's clinical care that no further follow-up is required.

6. STUDY PROCEDURES

Study procedures and their timing, including follow-up period are summarised in the SoE (Appendix 1). All screening evaluations must be completed and reviewed at Day -1 to confirm potential participants meet all eligibility criteria. A screening log will be maintained to record details of all participants screened and to document eligibility or record the reasons for screening failure, as applicable.

For all study assessments, the value obtained nearest to inoculation will be used as the baseline measure for assessments. Where applicable, unless otherwise stated, normal ranges will be filed in the Trial Master File (TMF).

Medical and Medication History

Medical and medication histories including any allergies will be recorded at screening, including, but not limited to, detailed histories on allergies (e.g. rhinitis, dermatitis, food, aspirin/non-steroidal anti-inflammatory drugs [NSAIDs] and asthma). Medical history will either be reviewed during screening (after informed consent) using the hospital's electronic patient record system (such as Cerner) or using the ACCURX system

as described in the PIS. If it is not possible to review their records using this system, or the record is not complete, the volunteer's medical history will be requested from their GP following screening and prior to challenge and reviewed for eligibility. Concomitant medications will be reviewed at each study visit.

Demographics

Demographic data will be recorded at screening. This will include:

- Age
- Sex/Gender
- Ethnicity/Race

Physical examination

Physical examination, including ENT, respiratory and cardiac assessment will be performed by the Study Doctor at screening and on admission to the residential stay on either Day -1 or Day 0, prior to inoculation, discharge, and at follow up appointments on Days 14 and 28.

Vital Signs

Participants will have their vital signs measured at the screening visit and each follow up visit. During the residential stay, the vital signs will be monitored twice daily at a similar time (+/- 1 hour) each day. The frequency of observations may increase in line with the National Early Warning Score (2) Scale 1 (NEWS 2) system implemented within ICHNT. The measurements will be recorded on a NEWS2 chart during the residential stay.

On Day 0, additional vital signs may be taken following the inoculation procedure. Vital signs will be taken in the morning prior to inoculation and then vital signs will be taken around 30minutes post-inoculation and again around 6-hours post inoculation. Depending on the time of day, they may be taken again before bedtime.

During vital signs assessments, participants will be rested in a quiet setting without distractions (e.g., television, mobile phones, computers). Participants will be asked to sit on a supportive chair or in a bed.
Vital signs will be measured in accordance with local SOP: "Measuring Physiological Observations incorporating NEWS 2 recording and SBAR escalation in adult patients.".

Vital signs assessments will be recorded as follows:

- Heart rate (HR) will be recorded in beats per minute (bpm)
- Respiratory rate (RR): respirations will be counted and recorded as breaths per minute (bpm)
- Blood Pressure (systolic BP and diastolic BP) will be measured in millimetres of mercury (mmHg); measurements will be made supine or with the participant sitting in a chair. Where possible, the same arm will be used for all measurements.
- Peripheral oxygen saturation (SpO2%) will be assessed using pulse oximetry.
- Temperature (tympanic, oral or axillary)

The NEWS 2 is the default tool for all adult patients admitted to ICHT, see Table 1. The tool allocates a score to each of the above parameters (plus alertness) in relation to normal and abnormal values. All physiological values have upper and lower limits that are pre-set nationally and all staff carrying out and documenting the measurements are fully trained.

The NEWS 2 is a track and trigger tool. It may not always be sensitive to all patients who are becoming acutely unwell and is not a substitute for clinical assessment and judgement. Concern that a patient may be deteriorating without a raised NEWS 2 score should still prompt escalation and review by a trained clinician.

The normal values for HR, RR, blood pressure, SP02% and temperature are listed below in white section of the NEWS chart.

Physiological parameter	Score 3	2	1	0	1	2	3
Respiration rate (per minute)	≤8		9–11	12–20		21–24	≥25
SpO_2 Scale 1 (%)	≤91	92–93	94–95	≥96			
SpO_2 Scale 2 (%)	≤83	84–85	86–87	88–92 ≥93 on air	93–94 on oxygen	95–96 on oxygen	≥97 on oxygen
Air or oxygen?		Oxygen		Air			
Systolic blood pressure (mmHg)	≤90	91–100	101–110	111–219			≥220
Pulse (per minute)	≤40		41–50	51–90	91–110	111–130	≥131
Consciousness				Alert			CVPU
Temperature (°C)	≤35.0		35.1–36.0	36.1–38.0	38.1–39.0	≥39.1	

Table 1 NEWS 2

The SPO2 (NEWS 2 Scale 2) is for use in patients who have a confirmed diagnosis of Hypercapnic Respiratory Failure (usually COPD) and will therefore not be used within this study.

In the event of a participant having an out of normal range result, the assessment may be repeated after at least 2 minutes of rest to exclude a technical fault and confirm the original reading.

If a result is out of the normal range and meets the criteria for an AE, the severity of the AE will be guided by the NEWS 2 scoring system Table 6 Severity Grading Criteria for physical observations.

In the unlikely event that a participant's NEWS2 aggregate score increases and meets the criteria for more frequent monitoring the national escalation guidance, detailed below in Table 2, will be followed, as per local NHS pathways.

NEW score	Clinical risk	Response
Aggregate score 0–4	Low	Ward-based response
Red score Score of 3 in any individual parameter	Low–medium	Urgent ward-based response*
Aggregate score 5–6	Medium	Key threshold for urgent response*
Aggregate score 7 or more	High	Urgent or emergency response**

*Response by a clinician or team with competence in the assessment and treatment of acutely ill patients and in recognising when the escalation of care to a critical care team is appropriate.
**The response team must also include staff with critical care skills, including airway management.

Table 2 NEWS 2 Escalation Guidance

Height, Weight and Body Mass Index (BMI)

Height and weight measurements will be recorded in compliance with local standard procedures.

BMI will be calculated as: BMI (kg/m2) = $\frac{\text{Weight (kg)}}{\text{Height (m)2}}$

Patient Health Questionnaire (PHQ-9) and Generalised Anxiety Disorder (GAD-7) Questionnaire

Participants will be asked to complete the PHQ-9 and GAD-7 questionnaires at screening, admission, discharge and optionally at the Day 14 and Day 28 follow up visits.

Chest X-Ray

All participants, who sign the informed consent and who are deemed eligible based on the information gathered at the screening visit, will have a single chest X-Ray performed during the screening visit. This will be carried out in the Hammersmith Hospital radiology department, according to local NHS SOPs.

12-Lead ECG

Twelve-lead ECGs will be obtained to evaluate the electrical activity of the heart, in accordance with local SOPs. ECGs will be reviewed by an appropriately qualified study doctor. ECGs taken post screening will be compared to the previous and baseline (screening) ECGs and will be documented as normal or abnormalities specified, and signed by the reviewing study doctor.

Any changes from baseline during the study will be assessed for their clinical significance. Clinically significant changes will be reported as aEs. The PI or delegate will assess non-clinically significant changes to determine whether they should be recorded. Study specific normal ranges are provided in below in Table 2.

ECG Parameters	Lower limit	Higher limit	Units
HR	**50**	**100**	**bpm**
QRS	**60**	**120**	**ms**
PR interval	**120**	**220**	**ms**
QT	**320**	**450**	**ms**
QTc	Normal for females is < 470		ms
	Normal for males is < 450		
QTcF	320	450	ms
QTcB	320	450	ms

Table 3 Normal Ranges for ECGs

Spirometry

A complete lung function test will be performed at the screening visit, with the participant's height, weight, age and smoking history determining their predicted values. A print-out report will form part of the eligibility check, detailing the individual's lung function in both relaxed and forced spirometry.

During the residential stay and at follow-up visits, a hand-held spirometry device will be used to measure the FEV_1,FVC and PEF. The measurement collected at the Screening visit will provide a baseline reading used for comparison for all subsequent measurements, in line with the local Spirometry SOP.

Predicted Values for Lung Function Measurements: the predicted or reference values for lung function measurements are those recommended by the Report Working Party for the European Community for Coal and Steel. Also incorporated are the recommendations of the British Thoracic Society and the Association of Respiratory Technicians and Physiologists.

Measuring FEV_1 and FVC by Spirometry

Screening Lung Function Test:
Spirometry is the most commonly performed lung function test; it provides us with basic information about a patient's airway function and vital capacity. The test itself measures exhaled volume and/or flow against time from a maximum intake of breath–- how much, and how quickly, patients can breathe out with data collected from both the relaxed and forced breath

- Posture must be consistent during the study, with participants sitting on a chair which has arms, feet on the floor and with no breathing limitations such as tight clothing.
- The participant should breathe in fully to maximum capacity. A good tight seal by the lips round the mouthpiece is essential, a nose clip will be used to minimise loss of breath via the nose.
- For the relaxed breath, the participant will exhale at a regular, smooth and consistent out breath until they reach residual volume. Three good breaths should be recorded with two of the three VC readings within 0.1L of each other, to ensure measurement is reliable and valid.
- For the forced breath the participant is instructed to forcibly exhale into the spirometer, blowing as hard as possible and continue to residual volume. Three good breaths should be recorded with two of the three FEV1 readings within 0.1L of each other, to ensure measurement is reliable and valid.
- A maximum of 8 forced breaths can be attempted, before a 30-minute rest if the test requires repeating further.
- The best value of 3 attempts will be recorded in the CRF, however the doctor will consider the full screening report for eligibility.

Spirometry parameters	Lower limit	Higher limit	Units
FEV1	Normal if ≥ 80% of the predicted value		litres
FEV1/FVC	Normal if ≥70% (≥ 0.7) of the base value		litres

Table 4 Normal Ranges for Spirometry

Peak Nasal Inspiratory Flow
A Peak Nasal Inspiratory Flow (PNIF) meter will be used to assess the patency of the nose.

Participants will be asked to exhale fully, then hold the PNIF device securely to their face to form an airtight seal around the nose and then inhale forcibly through the nose. The PNIF test will be repeated three times, with the highest result recorded in litres per minute (L/min).

Urine tests
Urinalysis will be performed for drugs of abuse and cotinine using commercially available kits that provide an instant result, which will be documented in the source data. The drugs of abuse screen will include (but is not limited to) amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines.

Virus Inoculation
The local inoculation SOP and inoculation checklist will be adhered to by all members of the inoculation team.

On the day of inoculation (Day 0), aliquots of challenge virus (10^4 plaque-forming units of RSV A Memphis 37) will be removed from storage in the -80 ^{o}C freezer and thawed. Inoculation will take place within the residential unit in the ICRF and where the participant will remain for the duration of their stay. A clinically trained staff member, with support from an assistant, will adhere to local Infection, Prevention and Control (IPC) guidelines regarding to donning and doffing of suitable PPE, including a facemask, eye protection, fluid repellent gown and show covers. During the inoculation and for all subsequent participant contact (excluding the show covers) during the residential stay, staff will adhere to the PPE requirements.

The participant will be supine (face and torso facing up) during the inoculation process, with their neck extended by either placing a pillow beneath their shoulders or tilting their head at the edge of the bed. The participant will remain supine with their neck in a comfortable position for 30 minutes post inoculation. The inoculator will inoculate the participants with diluted, at the pre-defined dose of 10^4 plaque-forming units of RSV A Memphis 37 inoculum using intra-nasal drops delivered equally into alternate nostrils with a pipette. This will be delivered slowly with a 30 second interval between each droplet to ensure maximum contact time between with the nasal and pharyngeal mucosa.

Participants will be asked not to talk or swallow during the procedure and to remain supine for 30 minutes post inoculation and to not drink a hot drink or shower for 2 hours post inoculation. They will also be asked to avoid blowing their nose for the rest of that day. Following inoculation, advice regarding hand hygiene will be given and participants will be provided with alcohol hand gel to reduce spread of virus within the shared environment.

BLOOD SAMPLING

Screening blood samples will be collected at the screening visit for a full blood count, renal function, liver function tests, random glucose, clotting, CRP, immunoglobulins, lymphocyte subsets, and HIV, HBV and HCV serology. These will be processed in the Haematology, Chemical Pathology, Immunology and Virology Laboratories of Imperial College Healthcare NHS Trust.

A blood sample for plasma will also be collected at the screening visit, and may be processed for HLA typing for those participants who have provided optional consent for genetic analysis. A blood sample for CMV serology may also be collected on Day -1.

Blood for peripheral blood mononuclear cells (PBMCs) will also be taken at Days -1, 1, 2, 3, 7, 10, 14, 28 and 180. On these occasions, 10-60mls of blood will be taken (see Appendix 1). In addition, blood for gene expression profiling, serum and plasma will be taken (see Appendix 1). Estimated bloods volumes at each visit are given in the Schedule of Events (see Appendix 1).

DNA processing will be taken from the residual or additional blood samples provided by the participants should the participants consent to this.

NASAL SAMPLING PROCEDURES

Nasal procedures will be performed in the order below for sample integrity and consistency. Further details on all the sampling procedures are given in the SOP Human Sampling Procedures for RSV Challenge Study.

Nasosorption (SAM)

The Synthetic Absorptive Matrix (SAM) will always be the first nasal sampling procedure to take place at each sampling session and is used to collect soluble mediators and antibodies from the nose. Participants will be asked to not blow their nose prior to the sample collection.
SAM strips are composed of a synthetic fibrous material and up to 2 will be used at each timepoint (one after the other) for 2 minutes to obtain repeated samples of neat nasal ELF.
The SAM is placed inside the desired nostril and the participant will be asked to either wear a nose clip or hold their nose closed for the 2 minutes while the sample is collected. This is a painless minimally invasive procedure. Following sampling, the SAM will be placed in a 1mL microfuge spin filter tube containing 250 µL of elution buffer (PBS/1% bovine serum albumin/0.05% sodium azide/0.05% Triton®) and placed immediately on wet ice before transfer to the laboratory.

Nasal Lavage

The nasal lavage may also be referred to at the nasal wash and will always be collected after the SAM and before the nasal curettage.

The nasal lavage (wash) is performed using the following technique:

- 5mL of 0.9% saline is introduced into one nostril using a syringe attached to a nasal olive with the participant sitting with the head tilted forward
- The saline is then washed in and out of the nose approximately 10 times by alternately withdrawing and advancing the plunger of the syringe while the participant maintains a tight seal between the nasal olive and the nostril; the aim is to recover ~80% of the saline from the nose
- The same procedure is repeated in the other nostril
- The sample is placed immediately on wet ice before transfer to the laboratory

Lavage fluid will later be analysed to quantify the degree of RSV shedding and determine antibody levels in nasal lining fluid.

Multiplex PCR will be performed on pre-inoculation nasal lavage or nasal swab to exclude the presence of other respiratory viruses prior to inoculation.

Multiplex PCR will be performed on the nasal lavage or a nasal swab collected on Day 6 to confirm RSV infection prior to discharge.

Multiplex PCR will be performed on the pre- and post-inoculation nasal lavage samples collected during the study for the primary endpoint viral load data.

Supernatants will be frozen and stored at -80°C.

Nasal Brushing

A small nasal brush will be used to obtain nasal epithelial cells from the nose for primary cell culture.
A nasal brushing will be performed as follows:

- Inspection of the nostril using a nasal speculum with a head lamp to assess for normal nasal anatomy prior to the procedure.
- A nasal brushing will be taken by inserting a cytology brush into the nostril and between the inferior nasal turbinate and the lateral wall of the nasopharynx and twisted 6 times.
- The procedure may be repeated on the same side to ensure adequate cell collection
- The brush will be placed in a 15ml falcon tube containing 2mls of media and transported to the laboratory for cell count and propagation.

Complications include slight bleeding immediately following the procedure and controlled by simple finger pressure and nasal discomfort which should not require any analgesia.
Where this sample is collected at the same timepoint as a Nasal Scrape, it will not be collected from the same nostril as the Nasal Scrape, in order to rotate sample site and minimise discomfort.

Nasal scrape using Rhinopro®

Rhinopro® curettes will be used to obtain a sample of nasal epithelial cells, goblet cells and mast cells. This may be referred to as a Nasal Scrape. It will not contain deeper layers of the mucosa. The Nasal Scape will be collected from the opposite side to the SAM. The following technique is used:

- The participant should be sat comfortably, ideally with their head resting against the back of a chair or pillow, with their chin tilted up towards the sample collector.
- A speculum will be used in the nose to keep the cavity open and employ good lighting
- Two Rhinopro® devices will be used to collect two samples from the same nostril
- Under direct visual inspection, the scooped end of the Rhinopro® will rest against the surface of the mid-inferior portion of the inferior turbinate. Note: avoiding the anterior bulb.
- The Rhinopro® should be approximately 3cm into the nostril; the floor of the nostril can be used to rest on.
- The cupped tip will be pressed on mucosal surface while it is withdrawn out of the nostril approximately 3mm to obtain a pin head of tissue.

- Note that this area has limited sensitivity and the participant should not find this procedure painful, although a nasolacrimal reaction can occur.
- The sample obtained should be placed immediately into a tube containing RNA Cell Protect® (Qiagen) or Trizol and frozen at -80°C for storage prior to analysis.

Nasopharyngeal swab

A nasopharyngeal (NP) FLOQ (flocked) swab will be used for analysis of T cells using flow cytometry, ELISpot or other cellular assays.

The patient will be asked to clear any mucus from their nasal passages and close their eyes to minimise discomfort prior to this sample being collected. The participant should be sitting comfortably, ideally with their head resting against the back of a chair or pillow, with their chin tilted up towards the sample collector

The swab will be gently inserted along the nasal septum, above the floor of the nasal passage to the nasopharynx until slight resistance is felt., and then rotated in this position in both directions, a total of 6 rotations and slowly removed.

The swab will be cut, using sterile scissors, ~2cm up from the swab tip and placed into a pre-cooled sterile cryogenic vial containing 90% heat inactivated fetal bovine serum and 10% of dimethyl sulfoxide (DMSO) and placed on ice. The cryovial will be moved either into a temperature-controlled cooling device (Mr Frosty) or directly onto dry ice for transport (as required) then to -80C storage as soon as reasonably possible.

Where a nasopharyngeal swab for cells for RNA is taken at the same time as a nasal scrape, this will be from the same nostril, with nostril side alternated each time to allow recovery.

Mid-turbinate FLOQ swab

FLOQ swabs will be used to collect cells from the nose (mid turbinate) for analysis of T cells using flow cytometry, ELISpot or other cellular assays.

These will be collected from the same nostril as the NP swab/scrape allowing the other nostril time to recover from the minimally invasive sample collection.

Throat swab

A sterile dry cotton-headed swab will be used to obtain samples from the pharynx for bacterial 16S gene analysis.

This is performed with the participants sitting with adequate lighting to visualise the pharynx, and a tongue depressor if required. The swab will be removed from the container carefully to ensure the tip is not contaminated, and the dorsal aspect of the pharynx and soft palate swabbed, using a rotation method for a total of 10 movements, avoiding the tongue. Some participants may experience a strong gag reflex.

The swab will return to the dry tube and immediately placed on ice until reasonably possible to transfer to the laboratory and frozen at -80°C later analysis.

Stool swab

Participants will be asked to collect the stool swab samples themselves and will be provided with a pre-labelled sterile dry cotton-headed swab. The participants will be provided with verbal and written instructions on how to collect the sample from the toilet paper after opening their bowels. as detailed below. The sample will be collected for bacterial 16S gene analysis. The study team will collect the sealed sample tube once per day, if a participant does not open their bowels and collect a sample within the preceding 24 hours, the sample time point will be missed. The sample will remain at room temperature until collection by the study team before transfer to the laboratory for storage at -80°C.

Duck Beak Mask Wearing Sample

Volunteers may be asked to wear a single use, duck beak face masks fitted with a polyvinyl alcohol (PVA, Orbi-Tech, Leichlingen, Germany) sampling matrix insert, capable of capturing virus. They will do this on

study Day -1 and then daily during quarantine from Study Day 1 plus, at the discretion of the PI/CI, on day 28, for up to 60 minutes per mask.

No hazards have been identified by the manufacturer of the PVA material and these face masks have been validated for the detection of M. tuberculosis in infected participants70. They have been successfully demonstrated to detect SARS-CoV-2 in exhaled breath. Up to five PVA strips will be harvested from each exposed mask and analysed for virus and virus-related signals.

SYMPTOM DIARIES

A self-completed diary card of upper respiratory tract clinical symptoms will be done twice daily from Day -1 to Day 14.

Upper Respiratory Tract Symptoms

A total 'upper respiratory clinical symptom score' (validated by Jackson *et al.* for common cold illnesses[44]) will be derived using a four-point scale (0-3 for absent, 1 mild, 2 moderate and 3 severe) for each of the following respiratory symptoms: sneezing, runny nose, stuffy/blocked nose, and sore throat according to established methods, giving a maximum clinical severity score of 12.
An example is shown below:

Symptom	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
Sneezing														
Runny nose														
Stuffy/blocked nose														
Sore throat														
Total Upper Respiratory score:														

0 = absent, 1 = mild, 2 = moderate, 3 = severe

Lower Respiratory Tract and Systemic Symptoms

An extended symptom diary based on influenza challenge scoring systems will be used to investigate the usefulness of recording lower respiratory and systemic symptoms in RSV challenge of older adults.

A total 'systemic symptom score' will be derived using the same four-point scale (0-3 for absent, mild, moderate and severe) for each of the following respiratory symptoms: fever, headache, malaise/fatigue and chills, giving a maximum clinical severity score of 12.

A total 'lower respiratory tract symptom score' will be derived using the same four-point scale (0-3 for absent, mild, moderate and severe) for each of the following respiratory symptoms: difficulty breathing, hoarseness, chest discomfort, wheezy chest and cough, giving a maximum clinical severity score of 15.

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 7-day period
- Nasal discharge is present on three or more days over the ten-day period post viral inoculation
- A subjective impression of a cold. 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

7. ADVERSE EVENTS

DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study participant.

Comment: An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, clinical observation, or disease temporally associated with the challenge agent or rescue therapy (if applicable), whether or not it considered related to either.

Serious Adverse Event (SAE): any untoward medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – *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*
- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement will be exercised in deciding whether an AE is serious in other situations. A study nurse/doctor can document the presenting details of an AE on the AE form, and a Study Doctor must review, decide on and complete the causality assessment and relationship of AE to the challenge virus/non-IMP on the AE CRF. Important aEs that are not immediately life threatening or do not result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

RISKS AND EXPECTED ADVERSE EVENTS

Potential adverse events related to RSV infection

We would expect infected participants to experience typical symptoms of a common cold (including, but not limited to: fever, headache, malaise, rhinorrhoea, nasal congestion, sneezing, sore throat, and cough). These would not be deemed adverse events, unless in the opinion of the study doctor. Participants will record any expected symptoms that they experience on the twice daily symptom diary between days –1 and day 14 which will be reviewed by the study team. Any symptoms that occur outside of the symptom diary capture time will be assessed whether it meets the AE/SAE criteria, even if deemed expected.

A fever greater than 38ºC for more than three consecutive days or withdrawal from the study due to intolerable symptoms in more than two participants will lead to a suspension of the study. The safety monitoring committee will be convened to determine any systematic cause for unexpectedly severe symptoms.

Any illness resulting in:

- **Sustained elevated heart rate >120bpm AND**
- **Sustained low blood pressure SBP<100**
- **Sustained elevated respiratory rate >30/min AND**
- **Sustained low blood oxygen SaO2<94%**
- **Evidence of pneumonia on clinical examination**

will lead to referral for assessment in Accident and Emergency. Hospitalisation of any participant will lead to immediate suspension of the trial. The safety monitoring committee will be convened to assess the clinical evidence to determine whether the study may proceed.

Potential adverse events related to study procedures

All study procedures involve no more than minimal risk to participants. Similar procedures have been used for many years without severe adverse effects and the risks are explained to participants at time of consent.

Blood draws: risks include discomfort as the needle goes through the skin and/or bruising. Infection, excess bleeding, clotting, or fainting are also possible, although unlikely. While these have been identified as potential risks, should a participant experience an event in relation to the blood draw, these will be documented and reported as an AE.

Nasosorption (SAM) strips, nasal curettage (scrape) and swabs (nose/throat/nasopharyngeal) may tickle, make the eyes water or be slightly uncomfortable during sample collection. They should not be painful or cause lasting discomfort.

While these have been identified as potential risks, should a participant experience an event in relation to any of these study procedures, these will be documented and reported as an AE.

Potential adverse effects of chest X-ray

Volunteers participating in this study will receive one chest X-ray, which is entirely for research purposes. The estimated dose will be 0.02 mSv (national Diagnostic Reference Level), which is approximately equivalent to 2.5 days natural background radiation and carries risk of inducing a cancer of approximately 1:1000,000 based on risk factors for a healthy adult. This is classified as a trivial risk level (ICRP 62).

RECORDING OF ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

All AEs and SAEs will be assessed from the time of written informed consent until study completion/final study contact or until the resolution of the AE. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

Any abnormal results identified from the time of consent up until inoculation, including laboratory results, clinical measurements and the physical examination, will be reviewed by the PI or delegated study doctor who will assess the result for clinical significance. If deemed clinically significant, the test may be repeated and consent gained from the participant for any further investigations required, whether that be organised by the study team or the GP. Abnormal, clinically significant findings discovered before inoculation will be deemed as a pre-inoculation AE. If not deemed clinically significant, they will not be recorded as an AE.

Investigators are not obligated to actively seek AE or SAE after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the Investigator must promptly notify the sponsor.

All adverse events should be recorded, with the following exceptions:

- Participants may experience typical symptoms of a common cold (including, but not limited to: fever, headache, malaise, rhinorrhea, nasal congestion, sneezing, sore throat, and cough). These would not be deemed adverse events, unless in the opinion of the study doctor. Participants will record any experienced symptoms on their symptom diary which will be reviewed by the study team.
- Abnormal vital signs at screening that are deemed not clinically significant by a study doctor.
- Abnormal individual or sporadic vital signs that are deemed not clinically significant, as per Table 6.
- The presence of a fever during the residential stay will not be recorded as an AE unless it persists for three consecutive days.

Assessment of AE and/or SAE

AEs will be fully recorded in the source documents as they are reported, whether spontaneously volunteered by a participant or in response to questioning about wellbeing at telephone or face-to-face study visits. Enquiries about AEs should cover the period between the previous and current visit. All ongoing AEs and/or SAEs will be reviewed at each visit time point. Depending on the nature of the event the reporting procedures below should be followed.

Care will be taken not to introduce bias when detecting aEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

The following are examples of open ended, non-leading questions that may be used to obtain this information:

- How are you feeling?
- Have you had any medical problems since your last visit/assessment?
- Have you taken any new medicines, other than those given to you in this study, since your last visit/assessment?

A Study Nurse can be delegated to document an AE on the AE CRF. However, a Study Doctor must review, decide on and complete the causality and relationship of AE to the challenge virus.
If the event consists of a cluster of signs and symptoms, a diagnosis should be recorded (e.g. gastroenteritis) rather than each sign and symptom.

The delegated staff member (may be a Study Nurse) capturing the AE must record:

- AE number
- Description of events (if the event consists of a cluster of signs and symptoms, a diagnosis should be recorded, including frequency if persisting AE)
- Onset date
- Severity (or grade)
- Concomitant medication
- Clinical outcome

The PI or delegated study doctor will record all other relevant information regarding an AE/SAE in the source documents and evaluate the following in relation to the AE/SAE:

- Relationship to challenge virus
- Frequency of AE (** see below)
- Seriousness/expectedness
- Date and time of resolution (or ‘continuing’ if unresolved)
- Action taken
- Clinical outcome
- Relationship or causality (Intervention treatment/ Challenge Virus/ study procedures/ concomitant medication/other).(*see below)

Relationship or causality of AE/SAE:

(*) For every AE, an assessment of the relationship of the event to the administration of the challenge virus will be undertaken by a study doctor. The study doctor will use clinical judgment to determine the relationship.

An interpretation of the causal relationship of the challenge virus to the AE in question will be made, based on the type of event; the relationship of the event to the time of challenge virus administration; and the known biology of the challenge virus.

Alternative causes of the AE, such as the natural history of pre-existing medical conditions, concomitant therapy, other risk factors and the temporal relationship of the event to challenge virus will be considered and investigated.

Causality assessment will take place during the study and at the final safety analysis, except for SAEs, which should be assigned by the reporting investigator immediately.

A “reasonable possibility” of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.

Classification	Definition
Not assessable	The AE relationship cannot be assessed.
Not related	The AE is related to an aetiology other than the challenge virus (the alternative aetiology must be documented in the participant’s medical record).
Unlikely to be related	The AE is unlikely to be related to the challenge virus and likely to be related to factors other than challenge virus
Possibly related	There is an association between the AE and the administration of the challenge virus, and there is a plausible mechanism for the AE to be related to the challenge virus, but there may also be alternative aetiology, such as characteristics of the participant’s clinical status or underlying disease.
Probably related	A reasonable temporal sequence of the AE and the challenge virus administration exists and, based upon the known pharmacological action of the drug, known or previously reported adverse reactions to the drug or class of drugs, or judgment based on the Investigator’s clinical experience, the association of the AE with the challenge virus seems likely.
Definitely related	A definite causal relationship exists between the AE and the administration of the challenge virus, and other conditions do not appear to explain the AE.

Table 5 Classification for AE Relationship

Unless an AE is ‘definitely related’ to the challenge virus, a causal relationship to one of the following should be considered, and full details provided on the AE reporting form as appropriate.

- Study procedures
- Concomitant medication
- Other

Frequency of AE

The frequency of the AE should be categorised as one of the following:

- Single
- Intermittent ($)
- Continuous

($) The study doctor is obligated to assess the relationship between challenge virus and each occurrence of each AE/SAE.

Severity assessment of an AE/SAE

The term ’severe’ is often used to describe the intensity (severity) of a specific event. This is not the same as ‘serious’ which is based on participant/event outcome or action criteria. The PI and delegated study doctors will use the grading scale for aEs as a reference when collecting, reporting and clarifying database queries of aEs and SAEs.

The severity table below will be used by study doctors when grading aEs that do not appear in the structured grading scales detailed later in this section.

An event is defined as ‘serious’ when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe. It is important to distinguish between serious and severe aEs. An AE of severe intensity needs not necessarily be considered serious. For example, a migraine

headache that incapacitates a participant for many hours may be severe, whereas a stroke that results in a limited degree of disability may be considered mild but should be reported as a SAE.

Grade	Definition
Grade 1	**Mild** level of discomfort, and does not interfere with regular activities
Grade 2	**Moderate** level of discomfort that intermittently interferes with regular activities
Grade 3	**Severe:** Significant level of discomfort and prevents regular activities

Table 6 AE Severity Grading

Assessment of AE/SAE

Assessment
Challenge Virus Symptoms A study doctor will assess, and review Challenge Virus related symptoms that are self-reported and/or recorded in participants' Symptom Diary Cards. Symptoms greater than Grade 0 will be expected and presumed to represent virus infection consequent to Viral Challenge and will not be additionally captured as aEs unless they meet the definition of an AE and are deemed to be clinically significant (in the opinion of the study doctor) to be classed as aEs. Following Viral Challenge all unexpected (in the opinion of the Study doctor) symptoms post inoculation will be captured as aEs, along with all other occurrences that meet the criteria for an AE.
Physical Examination Any clinically significant change in physical examination findings during the study will be documented as an AE.
Vital Signs Deterioration in a vital sign (compared to baseline) should only be reported as an AE if it is deemed clinically significant. For instance, a slightly elevated BP or slightly raised RR will not be recorded as an AE unless it has been deemed clinically significant by a study doctor. If deterioration in a vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated vital sign will be considered as additional information.
Temperature Following Viral Challenge, pyrexia will be expected and presumed to represent virus infection consequent to Viral Challenge and will not be additionally captured as an AE unless it meets the definition of an AE, and is deemed to be clinically significant (in the opinion of the study doctor) to be classed as an AE. Following Viral Challenge all unexpected (in the opinion of the study doctor) pyrexia post inoculation will be captured as an AE, along with all other occurrences that meet the criteria for an AE.
Spirometry A 15% drop in a spirometry value, compared to baseline measurement with handheld spirometer, and confirmed by a repeat on the same day, may be judged a Grade 1 (mild) AE. However, due to variability in participants' ability to perform these tests with adequate technique, the Investigator will use his/her clinical judgement to assess whether abnormal spirometry readings are consistent with a true drop and whether an AE should be raised. The study doctor will use his/her clinical judgement to assign severity grades above Grade 1, based on evaluation of clinical signs and symptoms. If a spirometry reading on repeat assessment has returned to normal an AE will not be raised.
Laboratory Values An abnormal laboratory value discovered at screening as part of the screening safety bloods, will be recorded as pre-inoculation AE. If it is deemed clinically significant, the participant may be invited back for repeat safety bloods to see if they return to normal range, otherwise, the participant will be deemed

ineligible to take part and, with consent, the study team will contact the participant and write to their GP describing the abnormality.

There are no follow up safety bloods included in this study, but the study doctor may wish to request safety bloods if they have any specific clinical concerns for the participant during the residential period or at any of the follow up visits. The clinical concern will be the adverse event, and any corresponding laboratory abnormalities will be considered additional information to the clinical symptom being recorded as an AE.

After the initial AE/SAE report, the study doctor is required to proactively follow each participant at subsequent visits/contacts. Additional Information/clarification may be required to ensure accurate completion of safety reports. The follow up reports should include a detailed Study doctor's clinical assessment. All aEs/SAEs will be followed until resolution, stabilisation, the event is otherwise explained, or the participant is lost to follow-up.

If an AE is not resolved at the end of the study, the AE should be followed until it has resolved, or a decision has been made by the Sponsor that no further follow-up is required.

Recording and reporting of abnormal laboratory results at screening

Abnormal laboratory results reported at screening will be reviewed by the study doctor and clinical significance assessed. Eligibility for enrolment in the study will be assessed by the study doctor. If not deemed clinically significant, the result will not be recorded as an AE unless it is later repeated, under the discretion of the study clinician and is found to have worsened or increased in grading according to the local CHIRP01 Toxicity Grading Scale.

If the result of 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 volunteer will be informed, and appropriate medical care arranged as appropriate and with the permission of the volunteer.

Decisions to exclude the volunteer from enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Study doctor.

Recording and reporting of abnormal vital signs

All vital signs will be reviewed by the study team at point of collection and assessed against the normal ranges. Abnormal vital sign measurements will be assessed for clinical significance by the study team.

If an abnormal vital sign is not deemed clinically significant at both screening or later time points, it is not classed as an AE.

If an abnormal vital sign is deemed clinically significant at either screening or prior to inoculation, the PI/CI will assess eligibility for virus challenge and may decide to exclude the participant from the study.

Vital signs that are deemed clinically significant by the study team will be graded as detailed (Table 7) below, based on the NEWS 2 scoring system.

Deterioration in a vital sign (compared to baseline) should only be reported as an AE if it is deemed clinically significant. For instance, a slightly elevated BP or slightly raised RR will not be recorded as an AE unless it has been deemed clinically significant by a study doctor.

If deterioration in a vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated vital sign will be considered as additional information.

Vital Sign	Grade 3	Grade 2	Grade 1	Normal Range	Grade 1	Grade 2	Grade 3
RR (bpm)*	≤ 8			12-20		21-24	≥ 25
SPO2%*	≤ 91	92-93	94-95	≥96			
Systolic BP*	≤90	91-100	101-110	111-219			≥ 220
Diastolic BP	>100	96-100	91-95	60-90			
HR*	≤40		41-50	51-90	91-110	111-130	≥131
Temperature	≤35.0		35.1-36.0	36.1-38.0	38.1-39.0	≥ 39.1	

Table 7 Vital Signs AE Severity Grading Table based on NEWS2 Scoring

* Taken after 5 minutes at rest

** When resting heart rate is between 50 – 100 beats per minute. Use clinical judgement when characterising bradycardia among some healthy participant populations, for example, conditioned athletes.

REPORTING PROCEDURES

The PI is responsible for ensuring that all SAEs are identified, evaluated, recorded and reported in a timely manner as per the Sponsor's SOPs, and also for ensuring that the medical management (including follow up) of SAEs and, where appropriate, pregnancy symptoms/complications is provided by site staff.

A Safety Monitoring Committee (SMC) will be established to advise the trial team on study progression in the event of a safety concern. The SMC will be informed of all unexpected aEs at least monthly during the clinical study period and convene during the study if there are any serious adverse events, protocol deviations, or other safety issues.

The SMC will be appointed and meet once (in person or by teleconference) prior to commencement of the study. The committee will consist of at least three members independent of the study team experienced in clinical trials and experimental medicine, with at least one member familiar with human challenge studies and one member experienced in respiratory infections. If any pausing rules or other safety issues arise, they will be convened to assess the clinical data and make a judgment on whether the study can proceed.

Serious aEs

Prompt notification by the Investigator (PI) to the CI and Sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of are met. An SAE form should be completed and emailed to the Chief Investigator within 24 hours of the Investigator becoming aware of the event. However, hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

An updated SAE report form should be forwarded to the CI and Sponsor within 24 hours of receipt of the new/updated information as relevant. Information relating to the participant's subsequent medical progress must be submitted to the CI and Sponsor as available, until the SAE has resolved or, in the case of permanent impairment, until it stabilises, and the overall clinical outcome has been ascertained.

The Investigator will also provide additional information, including a copy of the following documents (where applicable):

- Copies of test results, as available
- Concomitant medications
- Hospital discharge summary (as soon as it is available to the PI)
- Autopsy report (as soon as it is available to the PI)

The investigator at the site is responsible for ensuring that a member of the Sponsor study team is made aware of any SAE reports that have been transmitted.

Contact details for reporting SAEs
Please send SAE forms to: RGIT@imperial.ac.uk and the CI, c.chiu@imperial.ac.uk

All SAEs should be reported to the London Fulham Research Ethics Committee where in the opinion of the Chief Investigator, the event was:

- 'related', ie resulted from the administration of any of the research procedures; and
- 'unexpected', ie an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all related and unexpected SAEs. Annual safety/progress reports and final Study report will be generated and submitted to relevant ethics committee(s). This will be the responsibility of the study team and will include details of aEs/SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

There may be situations in which an SAE has occurred, and the Investigator has minimal information to include in the initial report to the Sponsor and CI. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data.

The causality assessment is one of the criteria used when determining regulatory reporting requirements – AE/SAE related to the challenge virus will be reported to the REC. The Investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.

In addition, any AE resulting in permanent study discontinuation for a participant, even if not serious and regardless of expectedness or causality, must be reported by email to the CI and Sponsor within 7 calendar days of the investigator or any other site personnel's knowledge of the event.

SAEs will be documented and reported in accordance with the Sponsor's SOPs.

Action and outcome in relation to AE/SAE

The Study doctor should ensure that adequate medical care is provided to participants for any aEs, including clinically significant laboratory values related to the study intervention. In addition, the Study doctor will describe whether any treatment was given for the AE.

The Study doctor will classify the action taken with regard to the AE. The action taken should be classified according to the following categories and full details provided as appropriate:

- None
- Non-drug therapy given
- Concomitant medication taken
- Study intervention dose not changed
- Study intervention dose adjusted
- Study intervention administration temporarily interrupted
- Study intervention administration permanently discontinued
- N/A Study intervention not administered
- Participant withdrawn
- Participant hospitalised

- Other

An AE should be followed until the Study doctor has determined and recorded the outcome or an alternative explanation. The outcome should be classified according to the categories shown in Table 7.

Classification	Definition
Resolved	Resolution of the AE with no residual signs or symptoms
Resolved with sequelae	Resolution of the AE with residual signs or symptoms
Persisting	Either incomplete improvement or no improvement of the AE, such that it remains on-going
Worsening	The AE has worsened since it was initially recorded.
Fatal	Outcome of the AE was death. 'Fatal' should be used when death was at least possibly related to the AE.
Not assessable	Outcome of the AE is not assessable or not known (e.g. the participant is lost to follow-up).

Table 8 Classification of Adverse Event Outcome

Follow up of AEs/SAEs

All clinically significant AEs and SAEs must be followed-up by the study doctor, or where appropriate, be referred to the participant's GP or other healthcare professional for follow-up until they are:

- Resolved (return to normal or baseline values), or
- Stabilised, or
- Judged by the PI/Study doctor to be no longer clinically significant, or
- An alternative explanation has been provided.

Additional measurements and/or evaluations may be necessary to investigate the nature and/or causality of an AE or SAE. This may include additional laboratory tests, diagnostic procedures, or consultation with other healthcare professionals. If the participant dies, any post-mortem findings (including histopathology) will be provided to the Sponsor if possible.

Post study AEs and SAEs

New AEs/SAEs will not recorded once the participation in the study has been completed.
Ongoing AEs/SAEs will be followed up until resolution by the study doctor, or where appropriate, be referred to the participant's GP or other healthcare professional with consent to follow up, or until they have been deemed clinically stable.

8. STATISTICS AND DATA ANALYSIS

This is a prospective cohort study of older adult volunteers challenged with RSV. Case-control analyses will also be performed using previously-collected data from young adults challenged in the same way (including transcriptomic data from an earlier study).

The CI has performed the statistical review for this study. Based on previous data from the human RSV challenge model, a sample size of 31 in each arm will be needed to detect a ≥2-fold difference in nasal IgA titres between groups with 80% power using a 2-sided unpaired t-test at 5% significance level (where the mean of log2(antibody titre) is 9.87 and standard deviation is 9.86). For comparisons of antibody responses, sample size will therefore be increased with samples from an additional 28 participants already challenged

in the safety and tolerability pilot study, giving a total sample size of 48. Based on our previous studies, a sample size of 11 in each arm will be sufficient to detect a ≥2-fold lower frequency in epitope-specific CD8+ T cells between older and younger groups, with 80% power using a 2-sided unpaired t-test and 5% significance level, where the mean and standard deviation are 1.67% and 0.974% of CD8+ lymphocytes respectively. The study will therefore be sufficiently powered to detect differences in T cell frequencies.

In our previous collaborative study with Merck, nasal cell samples from 20 RSV-infected young adults were analysed by RNAseq. Comparing day 7 post-infection with pre-infection, 1236 DEGs (adjusted $p<0.05$) were identified and permitted pathways enrichment analysis. Additionally, microarray analysis of bronchial brushings and biopsies from 7 RSV-infected individuals revealed 600-1000 DEGs. Bootstrapping analysis of preliminary data from these lower airway samples showed progressive decrease in DEG detection in bronchial tissues when <7 individuals were included. In this study, we expect an infection rate of 14 out of 20 challenged older individuals. We therefore anticipate that the study will be sufficiently powered to achieve the aim of adequate DEG identification to allow pathways enrichment and WGCNA.

Quantitative assessments of symptom scores, lung function, virus load, leukocyte numbers and inflammatory markers will be compared within subjects to determine differences between baseline and during infection, using linear mixed models or repeated measures ANOVA to identify significant changes over time. Intra-subject differences will be analysed using ANOVA and 2-tailed paired Student's t-tests or Wilcoxon signed rank test as appropriate. Correlations between inflammatory cell, illness severity, viral load and leukocyte counts will be examined using Spearman's rank correlations to investigate possible causal relationships.

The following analysis sets are defined for this study:
• Full Analysis Set (FAS) and Safety Analysis Set are defined as all subjects that are inoculated with the RSV challenge virus. The FAS will be considered as the primary analysis set for all primary, secondary and exploratory endpoints. The Safety Analysis Set is identical to FAS and will be used for all safety endpoints.
• Per Protocol (PP) Analysis Set is defined as all FAS subjects who have no major protocol deviations and who complete the quarantine period up to the final day of quarantine (study Day 8 or 10). The PP Analysis Set will be considered as the secondary analysis set for pre-specified primary, secondary and exploratory endpoints.

Membership of subjects in each analysis set will be determined at a planned data review meeting (DRM), prior to any analysis and database lock. A 'Laboratory confirmed infected' subgroup will be identified and will be presented for certain pre-specified analyses.

Primary, secondary and exploratory endpoints will be summarised using descriptive statistics. Continuous variables will be summarised using number of observations, mean (and/or geometric mean, where applicable), standard deviation, standard error, median, lower quartile, upper quartile, minimum and maximum values. Categorical variables will be summarised using proportions (counts and percentages). A 95% confidence interval (CI) may be presented for certain pre-specified endpoints.

Any missing data will be accounted for, and their possible impact on the study analysis will be described within the Statistical Analysis Plan (SAP).

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

9. REGULATORY ISSUES

ETHICS APPROVAL

The study has obtained approval from the London Fulham Research Ethics Committee (REC) and Health Research Authority (HRA). The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

CONSENT

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered, and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study, the doctor remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases, the participants remain within the study for the purposes of follow-up and data analysis.
All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

CONFIDENTIALITY

The Chief Investigator and all of the research team will preserve the confidentiality of participants taking part in the study and comply with the General Data Protection Regulation (GDPR) by the Data Protection Act (2018).

The processing of personal data of participants will be minimised by making use of a unique participant study number only on all study documents with the exception of informed consent forms and participant ID logs. All documents will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants' personal data. A separate confidential file containing identifiable information will be stored in a secured location in accordance with the current data protection legislation.

Pseudonymised data may be shared with collaborators in the UK and elsewhere, including industry collaborators such as Merck.

INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

SPONSOR

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the Imperial College Healthcare NHS Trust taking part in this study.

FUNDING

This study is funded by Merck through the Merck Investigator Studies Program.
The investigators will not receive any additional payment above their normal salaries.
Participants will be paid between £2,200 to £2,500 for the time and inconvenience of taking part in the study.

Screening Visit	£50
Quarantine Stay	£200 per full day in residence

Day 9 and Day 10 as an outpatient (if applicable)	£50 per visit
Day 14	£50
Day 28	£50
Day 180	£50
Minimum and maximum reimbursement:	**£2,200 - £2,500**

Participants can have their travel costs refunded up to £50 per visit with receipts.

Travel to and from screening	Up to £50
Travel for admission	Up to £50
Travel for discharge	Up to £50
Travel to and from Day 9 (if applicable)	Up to £50
Travel to and from Day 10 (if applicable)	Up to £50
Travel to and from Day 14	Up to £50
Travel to and from Day 28	Up to £50
Travel to and from Day 180	Up to £50
Maximum reimbursement for travel:	**£400**

AUDITS
The study may be subject to audit by Imperial College London/ Imperial College Healthcare NHS Trust under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Frame Work for Health and Social Care Research.

SAMPLE STORAGE AND USAGE

Samples of tissue, cells and fluids will be stored at the Commonwealth Building site at Imperial College London. Samples will be pseudonymised. These may be used for further assays or in other ethically approved studies, as detailed in the PIS and ICF. Samples and data may be shared with UK and international collaborators in studies that have been approved by local ethics committee and subject to a valid Materials Transfer Agreement.

10. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through Polly Fox, Clinical Project Manager, and Lydia Taylor, Senior Clinical Research Nurse, with oversight from Professor Christopher Chiu, Chief Investigator.

11. PUBLICATION POLICY

Our expectation is that after analysis the data from this study will be widely distributed in the medical and scientific community. Facilitated with presentations at local, national, and international meetings, we hope to publish widely in the medical literature. In addition, we have an excellent media department at Imperial College and publicise research that has public interest when it is published. We will also publish the results or links to relevant publications on the study website. No identifying participant information will be published.

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Appendix 1. Schedule of Events

Study Day	Screening	D -1	D0	D1	D2	D3	D4	D5	D6	D7	D8 (g)	D9 (g)	D10 (g)	D14	D28	D180
Time window (days)	**≤ -90**													**±2**	**±7**	**±14**
Written Informed Consent	X															
Eligibility assessment	X	X														
Medical & medication history	X															
Demographics	X															
Physical Examination (b)	X	X									X			X	X	
Vital Signs–- HR, RR, SBP, DBP, SpO2, Temp (a)	X	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD*	BD*	BD*	X	X	X
Height, weight and BMI	X															
PHQ-9 and GAD-7 Questionnaires (b)	X	X									X			(X)	(X)	
Chest X-Ray	X															
12-lead ECG (c)	X	X				X				X			X			
Spirometry +/- peak inspiratory flow (d)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine drugs of abuse & nicotine screen	X	X														
Virus inoculation			X													
Screening safety bloods (mls)	40															
Blood - HLA Sample (mls) $	4															
Blood - CMV Serology (mls)		(5)														
Blood - Serum (mls)		10													10	10
Blood - PBMCs (mls)		50		10	10	10				60			60	20	60	60
Blood – Plasma (mls) (d)	4		4	4	4	4	4*	4*	4*	4	4*	4*	4	4	4	4
Blood – PaxGene RNA (mls) (d)			5	5	5	5	5*	5*	5*	5	5*	5*	5	5	5	5
Cumulative Blood Volumes	48	108	117	137	157	177	187	197	207	277	287	297	367	397	477	557
Nasosorption (SAM) (d)	X	BD	X2	BD	BD	BD	BD	BD	BD	BD	BD*	BD*	BD*	X2	X2	X2
Respiratory PCR		X							X							
Nasal Lavage (Research)	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Nasal brushing	(X)	X														X

Study Day	Screening	D -1	D0	D1	D2	D3	D4	D5	D6	D7	D8 (g)	D9 (g)	D10 (g)	D14	D28	D180
Time window (days)	**≤ -90**													**±2**	**±7**	**±14**
Nasal scrape (Rhinopro®) and/or NP swab	X			X	X	X				X			X	X	X	X
Mid-turbinate FLOQ swab for airway cells	(X)	X		X	X	X	(X)	(X)	(X)	X	(X)	(X)	X	X	X	X
Throat swab	X	X		X	X	X	X	X	X	X	X	X	X	X		
Stool swab (e)		X	X	X	X	X	X	X	X	X	X	X	X			
Duck Beak Mask Sample (h)		X		X	X	X	X	X	X	X	(X)	(X)	(X)		(X)	
Symptom diary (f)		BD														
Collect symptom diaries														X		
AE/SAE Recording	X															
Concomitant Medications		X	X													

Appendix 2. Key for the Schedule of Events

X	Once
X2	Up to two SAM strips will be taken at the same timepoint.
(X)	Parenthesis indicates the assessment is optional, or at the PI's discretion
*	Optional blood drawer
$	The HLA typing sample will be collected at screening from all participants, but will be reserved and tested at a later date once a participant's eligibility has been confirmed. If a participant is not eligible for enrolment, this sample will be destroyed.
BD	Twice Daily
BD*	Taken twice daily if still in residential, taken once daily if this is an outpatient visit
a	During quarantine, vital signs will be taken at a similar time each day. On Day 0, additional vital signs may be taken following the inoculation procedure. Routine vital signs will be taken in the morning prior to inoculation, and then vital signs will be taken around 30minutes post-inoculation and then around 6hours post inoculation. Depending on the time of day, they may be taken again before bedtime. Vital signs may be additionally taken at any time during quarantine if the NEWS2 score indicates that the frequency of vital signs should be increased due to a clinical deterioration in vital signs.
b	A physical exam and the PHQ-9 and GAD-7 questionnaire will be completed on day of discharge from the residential stay. For uninfected participants this may be from Day 8 and for infected individuals it will be Day 10.
c	The 12-Lead ECG on Day 10 will only be performed for infected volunteers leaving quarantine on Day 10
d	The Plasma, Paxgene and Nasosorption samples collected on Day 0 will be collected prior to inoculation.
e	If a participant does not open their bowels on a given day, the stool swab sample will not be collected and this will not be considered a protocol deviation. It will also only be collected on Day 9 and 10 if the participant is still resident. We would not ask participants to perform stool swabs once discharged from quarantine.
f	The symptom diary will be done twice daily from Day -1 to Day 13. There will only be one completed symptom diary on the Day 14 visit. The participants will continue to fill out the symptom diary twice daily at home between the day of discharge and the day 14 visit.

g	Participants who are uninfected may be eligible for discharge from the quarantine unit on day 8 at discretion of the PI. They will be required to attend for daily visits on day 9 and day 10 for the procedures stated in the SoE. Procedures will be performed at a maximum frequency of OD, at the time of the day 9-10 visit. Participants who are infected will remain in the quarantine unit until at least Day 10 and continue with procedures as stated in the SoE.
h	**Optional sampling at the Investigator's discretion:** Participant will be asked to wear one or two single-use facemasks for up to 60 minutes per mask, once a day to capture exhaled virus. If the participant remains uninfected at Day 7, the sampling may be withheld at further time points.