Imperial College London

The impact of age on adaptive immunity in adults infected with respiratory syncytial virus

RESEARCH PROTOCOL

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Study Management Group

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Study Site

The study will take place at Hammersmith and St Mary's Hospitals, London – part of the Imperial College Healthcare NHS Trust.

Study Coordination Centre

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Clinical Queries

Clinical queries should be directed to Prof Christopher Chiu who will answer or direct the query to the appropriate person.

Sponsor

Imperial College London is the research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Research Governance and Integrity (Ruth Nicholson) at:

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Funder

The Medical Research Council (MRC) has provided funding for this study, in conjunction with Glaxo Smith Kline (GSK). This protocol describes the above 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 framework for health and social care research (Version 3.3, 01/11/17). It will be conducted in compliance with the protocol, the Data

Protection Act and other regulatory requirements as appropriate including Good Clinical Practice (GCP).

STUDY SUMMARY

TITLE Adaptive immunity in older adults infected with respiratory syncytial virus

DESIGN Human viral challenge study in healthy older volunteers

AIMS 1. To test the hypothesis that immune (especially T cell) responses to RSV are quantitatively and qualitatively impaired with aging

2. To identify the mechanisms underlying reduced antigen-specific cell-mediated immunity (CMI) to RSV by comparing the phenotypic and transcriptional changes in T-cells following experimental challenge with RSV in healthy older adults with young adult volunteers.

POPULATION Healthy persons aged 18 to 40 years and 60 to 75 years. Up to 42 subjects in total

ELIGIBILITY Healthy persons aged 18 to 40 years and 60 to 75 years that fit the inclusion and exclusion criteria

DURATION 5 years

GLOSSARY OF ABBREVIATIONS

AE	Adverse Event
BAL	Bronchoalveolar Lavage
BTS	British Thoracic Society
CI	Chief Investigator
CMI	Cell Mediated Immunity
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case Report Form
CRP	C-Reactive Protein
DC	Dendritic Cell
ECG	Electrocardiogram
ELF	Epithelial Lining Fluid
ENT	Ears/Nose/Throat
FEV_1	Forced Expiratory Volume in One Second
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GMP	Good Manufacturing Practice
GSK	Glaxo Smith Kline
HBV/HCV	Hepatitis B/C Virus
HIV	Human Immunodeficiency Virus
HRA	Health Research Authority
ICRF	Imperial Clinical Research Facility
ICRRU	Imperial Clinical Respiratory Research Unit
IMP	Investigational Medicinal Product

MRC	Medical Research Council
NHLI	National Heart and Lung Institute
NHS	National Health Service
NRES	National Research Ethics Service
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
PFU	Plaque-Forming Unit
PIS	Participant Information Sheet
PPE	Personal Protective Equipment
PRB	Protocol Review Board
R&D	Research & Development
REC	Research Ethics Committee
RSV	Respiratory Syncytial Virus
SAE	Serious Adverse Event
SAM	Synthetic Absorptive Matrix
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
URI	Upper Respiratory Infection
WHO	World Health Organisation

KEYWORDS

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

INTRODUCTION

1.1. Background to research

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. This is now improving as more detailed epidemiological studies are published that highlight 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. While RSV is the leading cause of severe respiratory illness in young children, it is also a major problem in adults. Incidence increases in old age, with RSV 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[2].

Despite decades of research, there is still no specific treatment or active vaccine against RSV. 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[3], even healthy adults can be repeatedly infected with an identical RSV at as little as 2 month intervals[4].

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 this incomplete immunity remain unclear and factors that determine whether an individual will develop symptomatic infection following exposure to a respiratory pathogen remain ill defined. Traditionally, serum antibody has been used as a correlate of protection against many infections. However, in RSV, the protection associated with antibody is partial at best. 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)[5]. 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[6]. Current vaccine candidates primarily aim to induce levels of antibody higher than those generated during natural infection but it may be unrealistic to expect vaccine-induced antibody on its own to mediate complete 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[7]. 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[8]. 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[9]. Conversely, stimulation of epitope-specific CD8+ T cells using peptide immunisation of HLA-A*0201 transgenic mice ameliorates disease and improves viral control[10]. Immunisation using peptide-loaded dendritic cells (DCs) from the closely related pneumonia virus of mice confers partial protection against subsequent RSV challenge[11]. 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[12]. 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[13]. 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[14]. Thus it is likely that CMI 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[15]. 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[16]. This usually translates to reduced vaccine efficacy[17]. However, the effect of aging on immune factors related to protection from symptomatic RSV disease has been relatively under-studied. Anti-RSV antibody levels 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[18]. 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[19–21]. None of these studies have correlated these observed reductions with increased risk of infection.

The importance of airway mucosal immunity 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 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. Our recent work has demonstrated the role of nasal IgA in protection from RSV infection. In healthy young adults, 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. More detailed analysis of mucosal immune factors such as these in older adults will further define their contribution to increased susceptibility.

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[22]. 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[23]. Our recent work using experimental human infection with RSV has demonstrated the predominance of CD8+ T_{RM} cells in the lower airway. Following RSV infection, antigen-specific CD8+ T_{RM} cells are 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. Since T_{RM} cells exist in tissues with little trafficking through peripheral blood, their role in immune protection against respiratory viral infections can only be assessed *ex vivo* during infection by bronchoscopy. Analysis of these cells in the controlled conditions of an experimental human infection trial will permit their correlation with risk and severity of infection.

Based on extensive preclinical and clinical research, we hypothesise that severe RSV disease (particularly in the setting of older age and pre-existing airways disease such as chronic obstructive pulmonary disease (COPD)) represents a dysregulated and over-exuberant inflammatory response to infection. Steroids have limited efficacy, and identification of druggable inflammatory pathways in severe RSV disease would be of great potential value. A number of promising clinical assets are ready for investigation, however, our ability to test novel therapeutics is limited by the currently available models of viral disease and clinical endpoints tied to disease processes. Observational studies of natural infection are constrained by sampling, timing and access. We therefore propose to extend the experimental human RSV infection studies established at Imperial College to investigate the pulmonary response to RSV infection in older adults, with the development of novel clinical endpoints which can then be used to evaluate the possible impact of treatment with validated novel anti-inflammatory agents in human RSV disease.

Safety of experimental human infections with RSV Since 2010, we have been conducting experimental human challenge studies in volunteers aged between 18 and 55. To date, 61 individuals have been inoculated safely, with around 55% 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.

Challenge studies overcome the many difficulties associated with studying a naturally infected patient group where diagnosis is often delayed; the timing and dose of inoculating virus is extremely variable;

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 will allow 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.

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 suggested 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. However, it is known that older people of advanced years are at higher risk, so we have set the upper age limit at 75 years. With these safeguards, we believe the risk of severe disease in our healthy older (55-75 year old) group is likely to be low. In addition, since our study design includes a confinement period in a residential research facility (see below) signs of disease severity will be monitored very closely.

Dr. Patrick Mallia and Professor Sebastian Johnston in our department have already 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. There have never been any cases of pneumonia in volunteers for experimental respiratory infection studies at Imperial College irrespective of the virus used. Although we recognise that RSV is generally believed to cause a more severe disease in high-risk patients than rhinovirus, we believe that in a carefully screened healthy non-smoking group of older volunteers there will be no severe disease.

This study will for the first time systematically investigate the immune responses in an elderly cohort challenged with a well-defined RSV inoculum. With a global aging population and continuing difficulties in generating vaccines that can reliably induce protective immunity in the elderly, these data will indicate the targets at which development of vaccines against RSV and other infections should be directed.

1.2. Research Hypotheses

1. RSV challenge remains safe and tolerable in older adults

2. Humoral and cell mediate immune responses in the airway represent direct correlates of protection against RSV

3. Aging leads to impaired RSV-specific cell mediated immunity and, to a lesser extent, humoral immunity

4. Impaired antigen-specific adaptive immunity in elderly adults leads to increased susceptibility to symptomatic RSV disease

STUDY OBJECTIVES

1.3. Primary Objective:

- Safety and tolerability of experimental challenge with RSV Memphis 37

1.4. Secondary Objective:

- To measure RSV-specific antibody, B cell and T cells in the blood and airway before, during and after infection with RSV in an elderly cohort

1.5. Exploratory Objectives:

- To compare the quantitative and qualitative features of adaptive immune responses against RSV in the elderly with young adults

STUDY IMPLEMENTATION

1.6. Pilot safety study

A pilot safety study will be performed to determine safety and tolerability of deliberate RSV infection in older adults.

This will be performed according to the sub-protocol “Accelerating RSV vaccine development in elderly (60-75 year old) adults by extending the controlled human infection model: a pilot safety study” (see Appendix 1-3). To ensure that the clinical team can closely monitor participant safety and to remain within the capacity of the quarantine unit, study participants will be divided into groups. The first 10 participants will be challenged in sequential cohorts as follows, with a younger sentinel group followed by older groups with pausing rules to ensure RSV disease remains within an expected mild-moderate range. Safety will be assessed at the end of quarantine (day 10 post-inoculation) for each group at which time, if no pausing criteria are met, the next group will be allowed to proceed as follows:

1. Group 1 (n=2, aged 60-67)
2. Group 2 (n=3, aged 60-67)
3. Group 3 (n=2, aged 68-75)
4. Group 4 (n=3, aged 68-75)

If any of the following pausing criteria are met, further viral challenges will be suspended until the Safety Monitoring Committee has met and approved re-commencement.

1. Occurrence of any death occurring after RSV challenge
2. Occurrence of any SAE
3. Occurrence of any unexpectedly severe disease as described in Section 1.20.1
4. Occurrence of any severe (non-serious) unexpected AE considered at least possibly related to the viral challenge

At the end of each quarantine period, the next group will be scheduled for quarantine if none of the following pausing rules is met.

At the end of the quarantine period (Day 10 post-inoculation) of Group 4, a safety review of the first 10 participants will be carried out to assess the extent of expected and unexpected adverse events, viral shedding and symptom scores. If at least 4 subjects are infected (as defined by PCR detection of virus on at least 2 consecutive days) and none of the infected subjects exhibit any safety concerns (that would trigger a pausing rule), the investigators will discuss the feasibility of intensifying the sampling regime, for example with the addition of bronchoscopy. If less than 4 subjects are infected in the first 10 challenge participants, the pilot study will continue with no intensification of sampling (i.e. no bronchoscopy). A further interim analysis will be performed after the Day 28 post-infection time-point of the first 10 participants. The remaining participants will be challenged in groups as follows:

5. Group 5 (n=5, aged 60-67)
6. Group 6 (n=5, aged 68-75)

The last interim analysis will be performed after the Day 28 post-infection time-point of Group 6 for assessment of attack rate, symptom scores and viral load. A minimum of 4 infected subjects without safety concerns in the first cohort supports a probability of at least 80% for the absence of safety concerns in the next 5 infected subjects from the second cohort. Prior to the first cohort, we assume a probability of 50% or more for the absence of safety concerns in the first Group of 4 or 5 infected subjects, based on our experience in younger subjects. The calculations assume, prior to the first cohort, a 95% credible interval for the probability of safety concerns covering a range between 0% and 97% and centred around 27.5%. Prior to the second cohort, assuming at least 4 infected subjects and no safety concern, the 95% credible interval would cover a range between 0% and 34% and centred around 5.5%. The probability of an absence of safety concerns in a Group 4 or 5 subjects is calculated using a beta-binomial distribution with parameters 0.275 and 0.725 prior to the first cohort, and 0.275 and 4.725 prior to the second cohort. The probability of no event in 4 or 5 subjects from those distributions are equal to ~50% and ~80% respectively prior to the first and prior to the second cohort.

1.7. Main study

If experimental RSV infection in this age group is shown to be safe and well-tolerated in the pilot sub-study, we will move onto the main part of the study with further recruitment and implementation of the study as per protocol.

PARTICIPANT ENTRY

1.8. Recruitment

Subjects will be recruited by advertisement in local newspapers, around College sites, the Surrey Clinical Research facility database, and online: the Imperial Trust website, Gumtree, Student Union websites, ICRF website, and on social media such as the Imperial CRF Twitter page. Additionally, respondents to adverts for prior research projects in the department will be contacted and invited to take part in our study (they have previously given their consent to be contacted). If interested, they will be invited for screening. The total number includes up to 20 in the pilot safety study. We aim to enrol up to 12 young non-smoking (18-40 year olds) and up to 30 older non-smoking (60-75 year olds) in the main study.

1.9. Pre-registration evaluations

1.9.1. Screening Visit

The screening visit will involve each participant attending the Imperial Clinical Research Facility (ICRF) at Hammersmith Hospital, White City or Imperial Clinical Respiratory Research Unit (ICRRU) at St Mary's Hospital, Paddington for a brief interview and medical examination to find out if they are suitable for the study. Potential subjects will be given a participant information sheet (PIS) detailing the study and experimental procedures. When the subject has had enough 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. Consent will be obtained prior to any history-taking, examination or tests are carried out. A copy will be kept in the research file, a copy given to the patient and a copy put into their medical notes.

A medical history will then be taken and clinical examination, ECG, lung function tests, chest X-ray and blood tests performed by the study doctor. Blood tests will include general screening for underlying illness, particularly full blood count, urea and electrolytes, liver function tests, coagulation, C-reactive protein, lymphocyte subsets, immunoglobulins, and HIV, HBV and HCV serology. A urine drug screen for illicit drugs will also be performed.

These will all be done in the ICRF at Hammersmith Hospital or ICRRU at St Mary's Hospital. If the evaluation and the results of these tests show no evidence of infection or any other problems with the participants' health and matches the inclusion/exclusion criteria then they will continue to the main part of the study.

1.10. Inclusion criteria

- Healthy persons aged 18 to 40 years or 60 to 75 years, able to give informed consent
- Non smokers or ex-smokers with smokers with less than or equal to 5 pack years smoking history.
- 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

1.11. 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
- Subjects 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 elderly adults (>65 years), immunosuppressed persons, or those with chronic respiratory disease
- Subjects 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 significant medical condition or prescribed drug deemed by the study doctor to make the participant unsuitable for the study
- Women of childbearing potential must have a negative BhCG urine pregnancy test*
- Positive urine drug screen

*Women of childbearing potential will have a pregnancy test performed prior to virus inoculation to exclude pregnancy and be required to use contraception throughout the study.

1.12. Withdrawal criteria

Any subjects can withdraw from the study at any time if they wish to. Subjects can also be removed from the study if an investigator feels this is necessary or appropriate. Subjects will be closely monitored throughout by the study doctor. This is defined further in the section on 'Serious Adverse Events'.

If a participant loses capacity during the study, the participant would 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.

Table 1: Study procedures

Procedures	DAY (relative to viral inoculation)																			
	Screen	**-14**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**28**	**166**	**180**
Time window (days)	≤ -90	±7															±1	±7	±7[&]	±14
Consent	X																			
Physical Examination	X	X	X									X					X	X		X
Chest X-Ray	X																			
12 lead ECG	X			X		X		X		X		X								
Lung Function Tests	X	X	X	X	X	X	X	X	X	X	X	X	X				X**	X		X
Safety bloods	X																		X	
Screening urine tests	X																			
Blood - serum			10															10		10
Blood - PBMCs (mls)		40	50	10***	10***	10***				60*			60				20	60		60
Blood – plasma (mls)	4		4	4	4	4	(4)	()	(4)	4	(4)	(4)	4				4	4		4
Blood – RNA (mls)		6	6	6	6	6	(6)	(6)	(6)	6	(6)	(6)	6				6	6		6
Oral swab			X	X	X	X	X	X	X	X	X	X	X				X	X		X
Throat swab X 2	X	X	X	X	X	X	X	X	X	X	X	X	X				X			
Stool swab X 2			X	X	X	X	X	X	X	X	X	X	X							
Nasal lavage (daily)	X	X	X	X	X	X	X	X	X	X	X	X	X	(X)	(X)	(X)	X	X		X
Nasosorption (SAM)	X	X	X2	X	X	X	X	X	X	X	X	X	X				X	X2		X
Nasal scrape (Rhinopro®)	X	X		X	X	X				X			X				X	X		X
Nasal brushing		X																		X
Bronchoscopy(*):		X								X*								X		X
Virus inoculation			X																	
Symptom diary check			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Collect symptom diaries																	X			

(*) Bronchoscopy includes: Bronchosorption using SAM (4)
Bronchial biopsy (8), Bronchial brush (4) and Bronchoalveolar lavage (BAL),
(number): the blood collection on days 4, 5, 6, 8 and 9 are optional and will be collected or collection withheld at the investigator's discretion (plasma and RNA).

*Second bronchoscopy will take place on Day 7 post-inoculation at the earliest and at Day 9 post-inoculation at the latest. PBMCs will be collected on the same day as the bronchoscopy.
** Optional if normal spirometry throughout quarantine period
*** Optional 10ml PBMC samples may be taken at these time points
& The Day 166 safety blood visit can occur at anytime between 21 and 7 days prior to the final bronchoscopy visit. This visit must be conducted before the final bronchoscopy visit in order to ensure the safety bloods have been collected and reviewed prior to the bronchoscopy.
Note: In addition, SARS-CoV-2 samples may be taken as per local guidelines.

1.13. Study visits for all subjects

The study is divided into out-patient and confinement phases. Subjects will stay overnight for a period of 8 - 10 nights in total, from the day of viral challenge, to the 8 - 10th day after viral challenge. This period of confinement has been chosen to eliminate the possibility of subjects in the study transmitting the virus to anyone not involved in the study (i.e. family, household contacts, and the wider community). Also, confinement will be used to enable closer monitoring and to enhance safety for study subjects. During the confinement period, all study procedures will take place in the confinement facility (except bronchoscopy – see sections 1.13.2 and 7) (Figure 1 & Tables 1. There will be follow-up visits after discharge (1.13.3).

1.13.1. Baseline visit (out-patient, Day -14)

On the first visit (baseline – one to two weeks before infection with the virus) the study doctor will assess the subject through a brief interview and medical examination. We will perform lung function test, blood tests (serology and PBMCs), a throat swab, take washings from the participant's nose (nasal lavage), a nasal scrape, nasal brushing a nasal SAM and bronchoscopy. At this visit the participant will also be given a symptom diary card and asked to complete it (before any nasal procedures), detailing any respiratory symptoms they experience in their upper and lower airways. They will be asked to complete the symptom diary daily for one week following the first bronchoscopy and then daily for two weeks after infection with the virus.

1.13.2. Confinement period (Day 0 to Day 10)

On the next visit (viral challenge), 1-2 weeks later, participants who have satisfactory blood and nasal sample results and assessment by the study doctor (brief interview and medical examination) at the baseline visit, will be asked to attend the ICRF/ICCRU. A single nasal lavage sample will be taken to exclude coincident infection and further blood and nose samples will be taken from all participants. Following this, subject to a satisfactory assessment by the study doctor (brief interview and medical examination) on the day, we will infect participants with RSV using a dose of 10^4 PFU. After infection, participants will be provided with facemasks (if appropriate) and observed for a period of one hour on the ICRF/ICCRU to ensure no adverse reactions have occurred. They will then enter a residential research facility (confinement facility), where they will reside for the next 8-10 nights so we can monitor the development of cold symptoms and collect samples to evaluate immune responses and test the hypotheses. These will include intermittent blood samples, daily throat swabs, nasal lavage, nasal SAM, nasal scrapes, lung function tests, as well as bronchoscopies around Day 7-9, Day 28 and Day 180 post-infection. Participants will be seen daily by the study team.

On the 8th day of residence, assuming all significant symptoms have resolved and at the discretion of the principal investigator, participants will leave the confinement facility and asked to return daily for assessment and sampling. If symptoms continue or the study doctors deems it necessary, the volunteer will be asked to remain until the 9th or 10th day of residence at which time they will be discharged, subject to a satisfactory assessment by the study doctor. If a subject is discharged before the 10th day or wishes to withdraw after virus inoculation but before the 10th day, we will strongly advise them to confine themselves in their own homes, and to strictly avoid any contact with young children, the elderly or other high-risk individuals for the remainder of the period during which viral shedding may occur.

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

To investigate the later stage of infection, participants may be asked to attend out-patient visits on Days 11, 12 and 13 for additional nasal wash samples if feasible. All participants will also return on Day 14 post-infection for assessment and sampling, and for bronchoscopy on Day 28 and Day 180. During the 6 months after challenge, subjects will be asked to report all episodes of upper respiratory tract symptoms. If an episode of natural infection occurs, the subject will be asked to re-attend for blood and upper respiratory tract samples to be collected.

Prior to the final bronchoscopy visit at Day 180, the participants will attend the study site for a safety blood visit. This is to check there are no safety concerns prior to their final bronchoscopy.

STUDY PROCEDURES

A variety of procedures are carried out during the study period. The frequency and timing of these procedures are shown in Table 1 above. For the screening, and Days -14, 14, 28 and 180 visits, procedures will take place in the procedures room on ICRF/ICCRU; for Days 0 to 10 subjects will reside in the confinement facility in the ICRF and procedures will take place here (with the exception of virus inoculation and bronchoscopy).

1.14. Virus Inoculation

On the day of inoculation (Day 0), RSV stock will be defrosted from its storage in the -80°C freezer. Subjects will be inoculated using intra-nasal drops on a single occasion with diluted inoculum at a given dose divided equally between the two nostrils. This will be done slowly with sufficient interval between each inoculation (at least 30 seconds) to ensure maximum contact time between with the nasal and pharyngeal mucosa. Subjects will be asked not to swallow during the procedure to ensure maximal pharyngeal contact. The inoculation procedure will be performed in isolation in the ICRF/ICCRU. The subject will be supine and inoculated by intranasal drops, using a pipette. Following inoculation, advice regarding hand hygiene will be given and subjects will be provided with alcohol hand gel and facemasks to reduce spread of virus in the environment.

1.15. Throat swab, oral swab, stool swab

For the throat swab a sterile dry cotton-headed swab is used to obtain samples from the pharynx for bacterial 16S gene analysis. This is performed with the subject sitting. Adequate lighting will be ensured and a tongue depressor used 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, avoiding the tongue. Some subjects may experience a strong gag reflex. Two samples will be obtained; one used swab will be placed into a dry ice container and frozen at -80°C prior for later analysis and another into bacteriology culture medium.
FLOQ swabs will be used to collect respiratory samples from the nose (mid turbinate) and the throat for exploratory virology research to determine viral shedding.

The oral swab collects oral fluid and is run across the gums & inside of the cheek by the volunteer for 1-2 minutes, then kept on ice prior to processing.

Sterile dry cotton-headed swabs will be used to obtain stool samples for bacterial 16S gene analysis. These will be collected by the subject from the toilet paper after opening their bowels.
Procedure:

- Remove the swab from the collection tube by holding it firmly by the cap. Do not touch the cotton part with your bare hands.
- Collect a small amount of fecal material by rubbing the cotton tip of the swab on a faecal sample: a piece of used bathroom tissue is the best material possible. A small amount is enough: it should cover half of the cotton tip. Do not try to collect too much biomass.
- Replace the swab in the collection tube and close it by pushing firmly on the cap.
- Store the swab at -80°C within 48h. If it is not possible to store at -80°C, store the sample at 4°C until transfer into a cryogenic environment.

1.16. Nasal sampling procedures

All nasal procedures will be performed in the order below **prior** to the bronchoscopy to avoid contamination by the local anaesthetic and the effect of the bronchoscope on the nose.

1.16.1. Nasosorption

Up to two strips of SAM will be used (2 per nostril, one after the other) for 2 minutes to obtain repeated samples of neat nasal ELF. This is a painless minimally invasive procedure that will not require any local anaesthetic. Following sampling, SAM will be placed in a 1mL microfuge spin filter tube containing 250µL of elution buffer (PBS/1% bovine serum albumin/0.05% azide/0.05% Triton®). Further details are given in the SOP Human Sampling Procedures for RSV Challenge Study.

The SAM will be transported on ice to the laboratory.

1.16.2. Nasal Lavage

Nasal lavage 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 subject 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 subject 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 fluid is then aliquoted into sterile microfuge tubes and centrifuged for analysis of cells

Lavage fluid will later be analysed to quantify the degree of RSV shedding. Multiplex PCR will be performed on the pre-inoculation lavage and post-inoculation lavage collected during the study to exclude the presence of other respiratory viruses. Supernatants will be frozen and stored at -80°C. Further details are given in the SOP Human Sampling Procedures for RSV & Flu Challenge Study.

1.16.3. Nasal scrape using Rhinopro®

Rhinopro® curettes will be used to obtain a sample of nasal epithelial cells from each nostril. This is a painless procedure and will not require local anaesthetic. The following technique is used:

- The subject should be sat comfortably, ideally with their head fixed, looking forward, while their chin rests on a support (if available)
- Tear bag and remove the flexible plastic Rhinopro® without contaminating the scoop end
- Place a speculum in the nose to keep the cavity open and employ good lighting
- Under direct visual inspection, insert the cupped probe onto the surface of the mid-inferior portion of the inferior turbinate. Note: Avoid the anterior bulb.
- The Rhinopro® should be 3cm up the nose; the floor of the nostril can be used to rest on
- Have the cup of the Rhinopro® at the correct angle
- Gently press the cupped tip on mucosal surface and move out and in of nostril 3mm up to 3 times
- Note that this area has limited sensitivity and the subject should not find this procedure painful, although a nasolacrimal reaction usually occurs
- The cell harvest is epithelial cells, goblet cells and mast cells. It does not contain deeper layers of the mucosa. 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.

1.16.4. Nasal Brushing

A nasal brush is used to obtain nasal epithelial cells from the nose for primary cell culture. A nasal brushing is performed as follows. Inspection is performed 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 through the nostril and between the inferior nasal turbinate and the lateral wall of the nasopharynx and then removed with a twisting motion of the wrist. The brush will be placed in a 15ml falcon tube containing 2mls of media and transported to the laboratory for cell count and seeding. Complications include slight bleeding immediately following the procedure and controlled by simple finger pressure and nasal discomfort which should not require any analgesia.

1.17. Bronchoscopy

Bronchoscopies will be performed in either the ICRF at Hammersmith Hospital, or the Endoscopy suite at St Mary's or Hammersmith Hospitals, in accordance with BTS guidelines. During the confinement period, private transport from the confinement facility to the Endoscopy suite will be mandatory. Subjects will be instructed to wear facemasks during this visit, and staff will use appropriate personal protective equipment (PPE). Bronchoscopies will be performed at baseline (Day -14), during the in-patient stay (between Day 7-9), and at Day 28 and 180 follow-ups.

- Subjects will sign a consent form prior to being screened for the study and will **sign a separate consent form for each bronchoscopy**. The procedure will be explained during the assessment stage and **subjects will be given written information specifically regarding bronchoscopies in addition to the Participant Information Sheet**
- Subjects will fast for four hours prior to the procedure.

- Resuscitation equipment (for intubation, ECG monitoring and defibrillation) and necessary drugs (salbutamol, theophylline, adrenaline, hydrocortisone) will be available in the bronchoscopy room.

- Premedication will be given including:

  - Sedation – Midazolam (2-10mg) and/or Fentanyl (25-100µg) as necessary.
  - Topical anaesthesia – Lignocaine solution (1-4%). The total dose will not exceed 400mg.

- Supplemental oxygen at a rate of 2Lmin^{-1} is given via a nasal cannula and adjusted as appropriate, and oxygen saturations and heart rate are monitored with a pulse oximeter continuously. Intravenous access will be mandatory in all cases

- The subject will be monitored during the bronchoscopy by a separate nurse and a second suitably qualified physician will be present to act as an advocate

- The following samples are collected in this order: (i) Bronchosorption using Synthetic Absorptive Matrix (SAM) (ii) Bronchial Brushings (iii) Bronchial Biopsy (iv) Bronchoalveolar lavage (BAL)

- All adverse events – pain, bleeding, hypoxia etc. will be recorded and reported according to Section 8 of this protocol.

- Subjects will be observed for a minimum of 2 hours and will remain nil by mouth after the procedure recovered.

- Transportation will be arranged as subjects should not drive on the day of the procedure.

- All subjects will have a contact telephone number on discharge to ring in case of any issues.

1.17.1. Bronchosorption:

Bronchosorption™ FX·i is a non-sterile, single-use device consisting of a synthetic absorptive matrix (SAM™) strip enclosed within a catheter for bronchial sampling. Bronchosorption™ FX·i is designed to operate through a flexible video bronchoscope with a maximum working length of 815mm. The SAM™ strip is 1.0mm wide, designed to work with a minimum instrument channel diameter of 2.0mm.' Hunt Developments UK (Ltd).

- The device will be passed down the bronchoscope.
- The probe will be deployed for up to 120 seconds in segmental and larger bronchi to allow the SAM to absorb local epithelial lining fluid (ELF).
- The probe will then be re-sheathed and removed via the operating port of the bronchoscope.
- This will be repeated up to 4 times with a new device.

  The SAM will be transferred to polypropylene tubes for transport on ice to the laboratory and stored at -80°C until analysis.

1.17.2. Bronchial Biopsies:

Up to eight bronchial biopsies will be taken from the segmental and sub-segmental bronchi of the right lower and middle lobe (RLL, ML). This is performed using Keymed 2mm biopsy channel cupped and fenestrated biopsy forceps [FB-19C-1 (1111065)]; four biopsies will be placed in 4% paraformaldehyde and stored in paraffin blocks and two placed immediately into RNAlater® (Qiagen) stabilisation solution and refrigerated for 24 hours prior to freezing at -80°C.

1.17.3. Bronchial Brushings:

Four bronchial brushings will be taken from the left lower lobe (LLL) sub-segmental bronchi with a standard cytology brush. The brush is washed in a tube containing RNA Cell Protect® (Qiagen) to preserve cells prior to freezing at -80°C. A new brush is used each time the brushes are washed and discarded.

1.17.4. Bronchoalveolar Lavage (BAL):

- BAL is performed by instillation of sterile physiological (0.9%) saline at room temperature into the left upper lobe (LUL) bronchus in 30-60ml aliquots to a total of 180-240ml.
- Aiming for 80% volume recovery and aspirating after each instillation.
- The BAL fluid is collected into a plastic chamber and transferred to polypropylene tubes for transport on ice to the laboratory.
- BAL processing - Keep BAL collected at bronchoscopy on ice at all times.

1.18. Blood sampling

Screening visit blood will be taken for full blood count, renal function, liver function tests, glucose, clotting, and 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. Serology will be performed at screening and Day 28 by IgG microneutralisation and ELISA assays.

The total amount of blood taken at screening would amount to 42.5mL. Blood for peripheral blood mononuclear cells (PBMCs) will also be taken at Day -14, 0, 3, 7, 10, 14, 28 and 180. On these occasions, 20-60mls of blood will be taken (see Table 1). In addition, blood for gene expression profiling, serum and plasma will be taken. Blood for gene expression profiling will be taken once or twice day. A maximum of 80 mls of blood will be taken on any single day for a total of 455 mls over the 28 day challenge period (see Table 1).

DNA processing will be taken from the residual or additional blood samples provided by the subjects.

1.19. Physical examination

Physical examination, including ENT, respiratory and cardiac assessment will be performed by the study doctor at screening and Days -14, 0 (prior to inoculation), discharge, and 14 and 28 days post inoculation.

1.20. Chest X-Ray

All patients eligible to participate in the study will have a single chest X-Ray performed prior to their first bronchoscopy. This is as per our local research bronchoscopy guidelines. The radiation dose associated with a single chest X-Ray is minimal and the risk of adverse effects is negligible.

1.21. Lung Function Tests

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

Performing the measurement:

- Posture must be consistent during a study, either standing or sitting, with no breathing limitation
- The subject should breathe in fully. A good tight seal by the lips round the mouthpiece is essential. The subject should then exhale forcibly into the spirometer, blowing as hard as possible and continue to residual volume
- The best value of 3 attempts will be recorded.

At the screening visit and at other visits as described in Table 1, measurements of FEV1 and FVC will be made as outlined above.

1.22. Clinical symptom scores

A self-completed diary card of upper respiratory tract clinical symptoms will be made at baseline 14 days prior to inoculation (prior to nasal washing and/or bronchoscopy), on Day 0, and daily for 14 days after inoculation.

Additionally, the symptom diary will be completed daily for 7 days after the 1st bronchoscopy (i.e. Day -14 to Day -7). This is to allow the effect of bronchoscopy on symptoms to be measured and adjusted.

Individual symptom scores will be accumulated over the ten-day period after inoculation and the baseline recording (including any effect measured from the 1st bronchoscopy where appropriate) subtracted from the post inoculation recordings. Thus, for a patient who has a score of zero on day 0 prior to inoculation, the maximum cumulative score for the following 10 days is 240.

Upper Respiratory Tract Symptoms

A total 'upper respiratory clinical symptom score' will be derived using a four-point scale (0-3 for absent, mild, moderate and severe) for each of the following eight respiratory symptoms: sneezing, nasal discharge, nasal obstruction, and sore throat according to established methods, giving a maximum clinical severity score of 12. This is an established method for studies of common cold illnesses.[44] Symptoms will be recorded at the same time of day and before any procedures such as bronchoscopy or nasal lavage are performed.

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

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 subjects 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

Lower Respiratory Tract and Systemic Symptoms

An extended symptom diary (Appendix 1) 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.

ADVERSE EVENTS

1.23. Definitions

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

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

- **results in death**
- **Is life-threatening** – refers to an event in which the subject 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**
- **results in persistent or significant disability or incapacity**
- **is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

1.24. Expected adverse events

1.24.1. Potential adverse events related to RSV infection

We would expect subjects 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. However, fever greater than 38ºC for more than three consecutive days or withdrawal from the study due to intolerable symptoms in more than two subjects in any arm 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 subject 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.

1.24.2. Potential adverse events of bronchoscopy

Bronchoscopy is a safe procedure, with serious complications occurring in less than 1 in 1000 procedures. It is frequently performed on patients with lung diseases such as asthma, and even in critically-ill and frail elderly patients. The bronchoscopy may cause cough for a few hours, and there may be some discomfort during the procedure, but this is reduced with local anaesthetic and sedation.. The sedation makes participants drowsy, but this usually wears off after a few hours. Participants should not drink alcohol after receiving sedation. Minor bleeding caused by irritation of the airways from the telescope may occur (less than 1 in 100), but this does not require treatment. Serious bleeding requiring treatment is very rare (less than 1 in 1000). The fluid used to wash the airways (lavage) can cause some irritation, which manifests as a brief fever in 10-30% of cases. This is self-limiting and can be treated with paracetamol. On rare occasions the airways can narrow – if this occurs medication can be given to open the airways. Occasionally after taking the tissue sample (biopsy) people can cough up small amounts of blood but this will stop without further treatment. Very rarely (less than 1 in 1000), the lung can collapse (pneumothorax) after bronchial biopsy; we would arrange for all necessary treatment in the unlikely event should this occur.

Bronchosorption is likely to have less adverse effects than bronchoalveolar lavage, brushing and biopsy (the standard bronchoscopy tools) as it should not cause bleeding, infection or a reactive pyrexia. The only additional adverse event relating specifically to bronchosorption is dislodgement of the SAM from the forceps. If this does occur the SAM can be retrieved using standard endobronchial forceps and snares available routinely in the bronchoscopy suite.

1.23.3 Potential adverse effects of chest X-ray

Volunteers participating in this study will receive one chest Xray, which is entirely for research purposes. The estimated dose will be 0.014mSv (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).

1.25. Reporting procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

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.

1.25.1. Non serious AEs

All such events, whether expected or not, should be recorded. These will be discussed monthly by the safety monitoring committee (see Section 12)

1.25.2. Serious AEs

An SAE form should be completed and faxed to the Chief Investigator and the Sponsor within 24 hours. The safety monitoring committee (see Section 12) will also be informed and a meeting convened as soon as possible.

All SAEs should be reported to the West London REC 2 Research Ethics Committee where in the opinion of the Chief Investigator, the event was:

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

Reports of related and unexpected SAEs should be submitted to ethics, the sponsor and the R&D office within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies.

Contact details for reporting SAEs
RGIT@imperial.ac.uk
Fax: 020 7262 8913 for the attention of Professor Peter Openshaw and/or Professor Christopher Chiu
c.chiu@imperial.ac.uk

Please send SAE forms to: Section of Infectious Disease, 8th Floor Commonwealth Building, Imperial College London, Hammersmith Campus, Du Cane Road, London W12 0NN
Tel: 020 8383 2301(Mon to Fri 09.00 – 17.00)

ASSESSMENT AND FOLLOW-UP

Study participants will be seen frequently during the study period, and at least daily for 10 days following infection. They have details to contact the study doctor and research nurses and will be offered daily telephone contact. In this way participants will be assessed regularly by the investigating team and any adverse events detected rapidly; subjects meeting the criteria for a serious adverse event will be offered prompt treatment as appropriate.

Subjects will have completed the study when they have had final convalescence investigations, expected to be 180 days after initial inoculation with the RSV virus. The overall study will be completed when sufficient numbers of subjects have been recruited. The end of the study is defined as the last visit of the last participant.

When the study is completed they will not be routinely followed-up. If a participant loses capacity during the study, then the participant would 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. Subjects will return to the care of their GP following completion of the study. Those who have experienced an SAE will be followed up until resolution of the SAE or until it is deemed by the CI or clinician in charge of the participant's clinical care that no further follow-up is required.

The study is a low-risk non-CTIMP, carried out by an experienced CI and study team. The study has appropriate risk assessments in place and will be checked through the PRB system at ICRF. Local monitoring will be carried out by study staff to ensure that CRFs are being completed, with no key data missing.

STATISTICS AND DATA ANALYSIS

Based on pilot data from Cherukuri et al., we calculate that a sample size of 8 in each arm would be sufficient to find a difference of 267 SFU/10^6 between RSV-specific interferon-γ T cell counts by ELISpot in blood, with 80% power using a 2-sided unpaired t-test with 5% significance level (where the variability in each group is 130). Previous data from de Bree et al. studied epitope-specific T cells detected by tetramer that was normally distributed with standard deviation 0.0338. If the true difference in the experimental and control means is 0.0528 as their data indicated, we would need to study 8 experimental subjects and 8 control subjects to be able to reject the null hypothesis with a type I error probability of 0.05. However, tetramer technology (which we are using to mark RSV-specific T cells) depends on matching of certain cell surface markers known as Human Leukocyte Antigen (HLA). Only a proportion of the population have each type of HLA, so not all participants will be suitable for tracking with all tetramers. We therefore aim to recruit 32 volunteers to each group in order to obtain at least 8 individuals with each HLA type required for tetramer labelling. T cell numbers will be compared between young and elderly cohorts using an unpaired t-test (if normality of the T cell distribution, or its transformation, is satisfactory as assessed by histogram with q-qplot and Shapiro-Wilks test) or a Mann-Whitney test (if normality is not satisfactory).

We will perform gene expression analysis on each of 6 patients prior to inoculation and at 3 post-baseline time points. Our main analysis will identify differentially expressed genes between young and elderly adults at each post-baseline time point. The sample size, calculated using the size package from bioconductor which provides pilot data of gene expression variability from the U95 package, predicts that 6 individuals in each group will give us 80% power to detect a potential fold change of 3 in up to 67% of the genes with a Bonferroni multiple testing correction with a genome-wide 5% significance level.

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. 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.

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 according to Imperial College London policy.

REGULATORY ISSUES

1.26. Ethics approval

The Chief Investigator has obtained approval from the Fulham Research Ethics Committee (REC) and Health Research Authority (HRA) for this study. The study will be submitted for Site Specific Assessment (SSA) at Imperial College Healthcare NHS Trust. The Chief Investigator will require a copy of the R&D approval letter before accepting participants into the study. 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, Declaration of Helsinki 1964 and later revisions.

1.27. Consent

Consent to enter the study will be sought from each participant only after a full explanation has been given, an information leaflet offered, time allowed for consideration, and any questions participants may have answered. Signed participant consent will be obtained prior to any screening tests being carried out. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician 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.

1.28. 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.

1.29. Indemnity

Imperial College, London as sponsor of this study holds negligent and non-negligent harm insurance policies which apply to this study. These have been arranged through the Joint Research Office.

1.30. Sponsor

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

1.31. Funding

This study will initially be funded by a MRC Clinician Scientist Fellowship. The MRC are currently acting as sole funders and this agreement is in place. This project will then act as the basis for a MRC Senior Clinical Fellowship application, which will continue funding for this work. The investigators will not receive any additional payment above their normal salaries. Participants in the study will have their travel costs refunded. They will also be given a donation of up to £3000 to compensate for the time and inconvenience of taking part in the study.

1.32. Audits and inspections

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

1.29. Sample storage and usage

Samples of tissue, cells and fluids will be stored at the Commonwealth Building and/or National Heart and Lung Institute sites at Imperial College London. Samples will be fully anonymised. These may be used for further assays or in other ethically approved studies. 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.

STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through Professor Christopher Chiu, Clinical Research Fellow, with support from Professor Peter Openshaw. In addition, a safety monitoring committee will convene monthly during the study to discuss all adverse events, protocol deviations, and other safety issues.

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. No identifying participant information will be published.

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