Nasal Immune Challenge Study

Protocol

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Signatures:	Dr Akhilesh Jha 04th July 2022

Confidentiality Statement

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1. AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1 (non-substantial)	1.1	18.05.22	Akhilesh Jha	Altering wording of post-nasal space biopsy to "a minimum of 6 weeks prior to nasal challenge (pages 15, 17 and appendix B) and added in section 15.4 to clarify epithelial cells will be cultured and stored within Cambridge.
2 (non-substantial)	1.2	04.07.22	Akhilesh Jha	Confirmed that only SARS-CoV-2 lateral flow (rather than PCR) is required prior to nasal sampling (as per University Department of Medicine Biological Safety Committee Review). Changed wording on pages 13, 16 and 35

2. SYNOPSIS

Title	Nasal Immune Challenge Study
Internal reference no	A095696
Design	Open label design using nasal challenge with a topical spray using saline or a Toll-like receptor (TLR)7/8 agonist and viral analogue (R848)
Participants	Healthy non-allergic volunteers Volunteers with allergic rhinitis with/without asthma
Sample Size	6 volunteers for sample collection to enable optimisation of protocols 24 for challenge (12 healthy and 12 allergic rhinitis with/without asthma)
Duration	2 years
Aims	1. To compare changes in gene expression at single-cell resolution in nasal mucosal samples and blood before and 24 hours after nasal TLR7/8 agonist (R848) challenge 2. To compare differences in nasal mucosal and blood single-cell gene expression between healthy and allergic participants 24 hours after R848 nasal challenge. 3. To assess systemic physiological measures and clinical tolerability of repeat R848 nasal challenge two weeks apart in healthy participants and those with allergic disease. 4. To measure change in levels of cytokines and chemokines in nasal mucosal lining fluid after repeat R848 nasal challenge. 5. To measure changes in peripheral immune (blood) responses to ex-vivo stimulation with lipopolysaccharide (LPS) before and after nasal challenge with R848.
Outcome Measures	1. Increase in CXCL10 nasal mucosal gene expression at 24 hours after nasal challenge with R848 compared to baseline. 2. Difference in CXCL10 nasal mucosal gene expression at 24 hours after R848 challenge between healthy subjects and those with allergic rhinitis. 3. Change in CXCL10 nasal mucosal protein in nasal mucosal lining fluid over 24 hours expressed as area under curve (AUC) between first and second nasal challenge with R848. 4. Change in participant's temperature between first and second nasal challenge with R848 (as a marker of clinical tolerability).
Eligibility	Adult males and females aged 18 years and above will need to meet inclusion and exclusion criteria. Participants in this study will be recruited from Cambridge University Hospital, University of Cambridge, volunteer databases and advertising in the Cambridge area.

3. ABBREVIATIONS

AE	Adverse event
AR	Allergic Rhinitis
ARwA	Allergic Rhinitis with Asthma
CUHFT	Cambridge University Hospital Foundation Trust
CI	Chief Investigator
CRA	Clinical Research Associate (Monitor)
CCRC	Cambridge Clinical Research Centre
CRF	Case Report Form
CRO	Contract Research Organisation
CT	Clinical Trials
EC	Ethics Committee (see REC)
GCP	Good Clinical Practice
GP	General Practitioner
ICF	Informed Consent Form
LPS	Lipopolysaccharide
NHS	National Health Service
NRES	National Research Ethics Service
PI	Principal Investigator
PIL/S	Participant/ Patient Information Leaflet/Sheet
PNIF	Peak Nasal Inspiratory Flow
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
R848	Resiquimod
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
TNSS	Total Nasal Symptom Score

4. BACKGROUND AND RATIONALE

Background

Acute respiratory viral infections cause significant morbidity, especially in vulnerable individuals and is a topic of immense significance during the current COVID-19 global pandemic. Respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) involve inflammation of the airways and viruses are a major cause of exacerbations (1). Whilst new biological drugs targeting allergic and eosinophilic immune pathways have been developed for allergic asthma, therapeutic options for treating asthma exacerbations remain limited and rely on decades-old blunt tools such as oral corticosteroids. Development of new therapies and vaccines to combat viral respiratory tract infections is slow, partly because of the limited understanding of innate immune responses at the respiratory mucosal site of disease (2). Detailed characterization of such responses can facilitate biomarker definition for respiratory diseases, providing mechanistic insights and a platform for the testing of novel therapeutics.

Our understanding of the role of innate immunity to infection has recently undergone a paradigm shift as it is now recognized that innate cells (and not just adaptive immune cells) have the capacity to undergo “training” after infection via metabolic changes and epigenetic reprogramming (3). Pathogens can therefore modulate chromatin structure and the transcriptional programme of innate immune cells, which can result in altered but non-specific responses upon reinfection either with the same or heterologous stimuli. These epigenetic mechanisms may be particularly relevant in understanding how viral triggers (e.g. influenza) can lead to increased susceptibility to bacterial infections and cause pneumonia (e.g. due to *streptococcus spp.*) (4).

Most studies have focused on peripheral immune cells such as macrophages, but the concept of innate immune training has been extended to long-lived non-immune skin epithelial stem cells, which have the capacity for “inflammatory memory” (5). Allergic inflammatory memory has also recently been established in respiratory progenitor cells (6). Murine studies have highlighted the potential for structural cells (such as epithelial cells) to have the potential to mount immune responses (7). However, mechanisms of innate immune “memory” in human respiratory mucosal epithelial and immune cells in response to microbes and pathogen associated molecular patterns (PAMPs) are not well understood despite the respiratory tract being under a constant barrage of infectious stimuli.

Toll-like receptors (TLRs) play a critical role in the initial detection of microbes and PAMPs. TLR7/8 specifically detects RNA viruses such as influenza, rhinovirus, and coronavirus. Airway epithelial and immune cells express TLR7/8 and are therefore vital in mounting the innate immune response to viral infections, as well as helping shape adaptive immunity (8–10). Resiquimod (R848) is a single-stranded RNA viral analogue that binds to TLR7/8. When given systemically to treat human hepatitis C infection, it causes cytokine release and a flu-like syndrome (11). However, R848 has been successfully employed topically as a skin cream for actinic keratosis (12). TLR7 agonists have been developed for therapeutic use by repeated dose intranasal administration to target allergic inflammation in healthy volunteers and those with allergic rhinitis (13, 14).

Rationale for current study

Intranasal R848 administration has now been established in a human challenge model in healthy volunteers and in those with allergic rhinitis (AR) and allergic rhinitis with asthma (ARwA) by the chief investigator (15). In total, 44 volunteers have received intranasal R848 at a range of doses; 9 healthy volunteers received 10µg per nostril (n=9) with one person receiving 100µg per nostril (n=1); subsequently 35 volunteers received between 1-2µg per nostril based on weight (0.02µg/kg, healthy n=12, AR n=12, ARwA n=11). This dose was well

tolerated by all volunteers with minimal nasal symptoms and no significant change in airflow obstruction (FEV1) in volunteers with asthma. Intranasal R848 was well tolerated systemically with no change in physiological measures or serum inflammatory mediators compared to saline challenge. The study demonstrated that individuals with AR and ARwA have increased nasal mucosal interferon and chemokine responses compared to healthy volunteers. This is coupled with the observation that there is a small and transient reduction in peripheral eosinophil counts at 4 hours and in lymphocyte counts at 24 hours, a finding limited to volunteers with AR and ARwA. This suggests that leukocyte trafficking may be occurring from the peripheral blood to the nasal mucosa. These findings highlight that dysregulated mucosal innate immune responses are likely to be important in determining the clinical outcome of viral triggers.

R848 nasal challenge (up to 2μg per nostril) is therefore a practical, tolerable, and non-invasive method to stimulate and investigate TLR-driven respiratory mucosal innate immune responses in both health and disease.

Defining the cellular basis for TLR-driven innate immunity will help provide mechanistic insights into how these responses differ between individuals, its dysregulation in disease (such as during an asthma exacerbation) and susceptibility to secondary infections. Understanding the epigenetic mechanisms which regulate interferon and chemokine production may help identify the molecular drivers of excessive or dampened inflammation, helping to refine potential therapeutic targets. Given the current COVID-19 pandemic, the clinical translational model utilised in this study allows for a practical and straightforward method to assess the host factors which determine respiratory mucosal innate immunity.

The goal of this project is to utilize an established tolerable human nasal TLR agonist challenge model combined with single-cell transcriptomic and epigenetic techniques to precisely characterize innate immune responses and mechanisms of innate immune training in airway epithelial and immune cells. Samples will be collected from the nasal mucosa and blood before and after saline and R848 challenge. Cell cultures from airway epithelium and blood will also be established to assess the functional impact of R848 on cellular response to repeat stimulation with viral and bacterial ligands. Study populations will include healthy participants and those with allergic rhinitis with/without asthma in order to investigate how *in-vivo* innate immunity is altered in these populations. Some participants will also undergo repeat nasal challenge to assess mechanisms of innate immune memory in the respiratory tract.

5. OBJECTIVES

5.1 Primary Objectives

To compare changes in gene expression at single-cell resolution in nasal mucosal samples and blood before and 24 hours after nasal TLR7/8 agonist (R848) challenge

5.2 Secondary Objectives

To compare differences in nasal mucosal and blood single-cell gene expression between healthy and allergic participants 24 hours after R848 nasal challenge.

To assess systemic physiological measures and clinical tolerability of repeat R848 nasal challenge two weeks apart in healthy participants and those with allergic disease.

To measure change in levels of cytokines and chemokines in nasal mucosal lining fluid after repeat R848 nasal challenge.

To measure changes in peripheral immune (blood) responses to ex-vivo stimulation with lipopolysaccharide (LPS) before and after nasal challenge with R848.

5.3 Exploratory Objectives

To establish cell culture models from primary nasal epithelium and immune cells to understand the functional impact of R848 on cellular responses to repeat stimulation with viral and bacterial ligands.

6. STUDY DESIGN

6.1 Summary of Trial Design

The first stage of the study will include up to 6 participants (healthy or allergic disease) to undergo nasal mucosal tissue and blood collection without any nasal challenge. These samples will be used to optimise the protocols required for downstream processing, e.g. for single cell and epigenetic analysis.

The main component of the study is an open label design employing nasal challenge with a topical spray using saline or a TLR7/8 agonist R848. The aim is to perform deep immunophenotyping of nasal mucosal tissue and peripheral blood before and after nasal challenge with R848 and compare responses between non-allergic and allergic individuals. Some participants will undergo repeat challenge two weeks later with assessment of cytokine, chemokine, and gene expression profiles to determine which cells contribute to innate immune memory of previous exposures and how this may be altered in disease.

After an initial screening visit to determine eligibility, participants constituting equal numbers of non-allergic and allergic individuals will undergo nasal challenge with R848 (n=24). They will have sampling performed for up to 24 hours and can choose to go home or stay overnight in the Cambridge Clinical Research Centre (CCRC). This will be the end of the study for half of the participants.

The remaining half will be invited to subsequently undergo repeat saline (n=2) or R848 (n=10) challenge approximately two weeks later to determine clinical tolerability, assess for exaggerated or dampened nasal mucosal immune responses and investigate mechanisms of innate immune memory, and how they may differ between the groups.

The maximum duration for participants in the study are as follows (Appendix A):
Single challenge study (n=12): one day
Double challenge study (n=12): two days spread two weeks apart

6.2 Primary Outcome Measure

1. Increase in CXCL10 nasal mucosal gene expression at 24 hours after nasal challenge with R848 compared to baseline

Secondary Outcome Measures

1. Difference in CXCL10 nasal mucosal gene expression at 24 hours after R848 challenge between healthy subjects and those with allergic rhinitis

2. Change in participant's temperature between first and second nasal challenge with R848 (as a marker of clinical tolerability)

3. Difference in CXCL10 nasal mucosal protein in nasal mucosal lining fluid over 24 hours expressed as area under curve (AUC) between first and second nasal challenge with R848.

Exploratory Outcome Measures

1. Difference in CXCL10 after ex-vivo stimulation of blood samples with LPS before and 24 hours after nasal challenge with R848

2. Single-cell gene expression and epigenetic profiles in mucosal tissue and blood before and after nasal challenge with R848

3. Comparison of single-cell and epigenetic profiles between healthy and allergic disease groups

7. TRIAL PARTICIPANTS

7.1 Overall Description of Trial Participants

Adults will be considered for inclusion in this study. They will either be healthy individuals with no history of allergy, or they will have a history of allergic rhinitis (hay fever) with/without physician diagnosis of asthma.

For the sample optimisation phase of the study (n=6), volunteers will only be required to provide nasal mucosal tissue (using curettage and or brush) and a blood sample and will therefore not undergo a detailed medical screening visit.

For participants undergoing nasal challenge, they will be recruited within the parameters of the following criteria:

7.2 Inclusion Criteria

- Participant is willing and able to give informed consent for participation in the study.
- Male or female aged 18 years and above
- Able (in the Investigators opinion) and willing to comply with all study requirements.
- Willing to allow his or her General Practitioner and consultant, if appropriate, to be notified of participation in the study.
- Female participants of child-bearing potential and male participants whose partner is of child-bearing potential must be willing to ensure that they or their partner use effective contraception during the study
- Participant has clinically acceptable laboratory and ECG at enrolment.
- Negative lateral flow or polymerase chain reaction (PCR) test for SARS-CoV-2
- For healthy volunteers:
  - No clinical history of allergic rhinitis, asthma or eczema
  - Negative skin prick tests or specific IgE response to a panel of common aeroallergens: cat, dog, grass pollen, tree pollen, house dust mite, fungal spores
  - Normal blood eosinophil count (< 300 cells/μL)
  - Normal baseline forced expiratory volume (FEV1) i.e. ≥80%
- For volunteers with allergic rhinitis with or without asthma:
  - A clinical history of allergic rhinitis symptoms (sneezing, runny or itchy nose) in response to aeroallergens
  - At least one positive skin-prick test or specific IgE response to a panel of common aeroallergens: cat, dog, grass pollen, tree pollen, house dust mite, fungal spores
  - Pre-bronchodilator $_{FEV1}$ ≥50% predicted
  - Participants are permitted to have physician-diagnosed mild to moderate asthma which is not poorly controlled as evidenced by an Asthma Control Questionnaire (ACQ-5) score of ≤1.5.
  - If they have asthma, they are permitted to be on inhaled corticosteroid (ICS) and a long-acting beta agonist (LABA), but no other controller medication.
- Have had no other courses of medication including nasal and systemic corticosteroids, whether prescribed or over-the-counter, in the four weeks before first study dose other than mild analgesia, vitamins and mineral supplements or, for females, oral contraceptives.

7.3 Exclusion Criteria

- Recent infections in past 14 days before screening: especially upper respiratory tract illnesses (including colds and influenza), sore throats, sinusitis, infective conjunctivitis.
- Lower respiratory tract infection in past 28 days
- Nasal anatomical defects, precluding use of nasal sampling techniques
- The participant may not enter the study if any of the following apply:
  - Female participants who are pregnant, lactating or planning pregnancy during the study.
  - Respiratory diseases (other than hay fever or asthma where specified)
  - Significant medical history of hepatic, cardiovascular, gastrointestinal, renal, endocrine, infective, haematological, autoimmune, metabolic, rheumatological, neurological, dermatological or neoplastic conditions
  - Extreme obesity (BMI >40)
  - Depression and psychiatric disorders
- Any other significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the study, or may influence the result of the study, or the participant's ability to participate in the study.
- Participants who have participated in another research study involving an investigational product in the past 12 weeks
- Smoking in previous 6 months

8. STUDY PROCEDURES

Pictures of the study procedures are outlined in appendix A and an outline of study procedures in relation to study visits is outlined in appendix B.

Nasal lavage: 10ml of normal saline will be administered and collected gently through a disposable plastic olive-shaped device into both nostrils prior to each challenge. This acts to "clean" the nasal mucosa of nasal debris prior to challenge and the nasal washing will be discarded.

Nasosorption: Nasal mucosal lining fluid will be collected non-invasively using nasosorption initially regularly (0min, 15min, 30min, 1h, 2h) then at two-hourly intervals (4h, 6h, 8h, 24h) after each challenge to assess the kinetics of interferon, cytokine and chemokine production.

Nasal mucosal epithelium: Nasal mucosal epithelial cells will be collected at the screening visit, baseline (0h) and 24 hours after each challenge to assess transcriptional and epigenetic signatures and to culture epithelial cells in the lab. Therefore, a maximum of up to three sets of samples per volunteer will be taken from half of the group undergoing single challenge (n=12), whilst the remainder of the group undergoing double challenge will have a total of five sets of samples taken (n=12). Up to five sets of nasal curettage samples were taken per volunteer in a prior study using R848 with good tolerability and no withdrawal of participants from the study[15]. Topical anaesthetic spray may be offered prior to procedures.

- **Nasal brushings** are taken using a small cytology brush from the nasal inferior turbinate – up to two samples will be taken (one from each nostril).
- **Nasal curettage** involves the use of a small plastic curette to gently "scrape" a small amount of tissue from the nasal inferior turbinate – up to four samples will be taken (two from each nostril).

Post-nasal space biopsy will be an optional procedure in order to obtain a small sample of tissue (0.5cm) taken from the postnasal space using endoscope and forceps under topical local anaesthetic. It will be performed by a trained Ear, Nose and Throat (ENT) surgeon (Mr Coates) in a specific area of Addenbrookes hospital with dedicated facilities for the procedure. The purpose of this is to obtain a larger and deeper volume of tissue than the nasal curettage/brushings to facilitate detailed molecular phenotyping particularly of immune cell subsets, which are found underneath the epithelial layer. The procedure is generally well tolerated, and complications are rare. This procedure is more invasive than performing nasal curettage/brushings and will therefore only be performed twice in up to 12 people. These participants will only undergo a single nasal challenge and not the second repeat challenge. Volunteers who agree to have the procedure will have the first sample collected at the screening visit. There will then be a minimum of 6-weeks (to ensure wound healing) before performing nasal challenge with saline (up to n=2) or R848 (up to n=10), and a subsequent post-nasal space biopsy performed 24 hours after challenge.

Blood: Blood will be collected on the following occassions:
- Screening visit for routine safety and eligibility (9ml) and DNA analysis (8.5ml)
- Before (0h) and after (24h) each nasal challenge for single cell analysis and Truculture (13ml each time-point)
- Before (0h) and twice after (4h, 24h) nasal challenge for serum and full blood count (9ml each time point).

This means that half of the volunteers undergoing single challenge (n=12) will provide a total of approximately 70ml blood over three visits and the remaining half undergoing 2 challenges (n=12) will provide a total of approximately 125ml blood over five visits.

8.1 Informed Consent

The participant must personally sign and date the latest approved version of the informed consent form before any study specific procedures are performed.

Written and verbal versions of the participant information and Informed consent will be presented to the participants detailing no less than: the exact nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their GP or other independent partiefs to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the informed consent. The person who obtained the consent must be suitably qualified and experienced and have been authorised to do so by the Chief/Principal Investigator as detailed on the Delegation of Authority and Signature log for the study. The original signed form will be retained at the study site within the Trial Master File (TMF) or Investigator Site File (ISF). A copy of the signed Informed Consent will be given to participants and a copy retained in the participant medical notes.

8.2 Screening and Eligibility Assessment

Participants in this study will be recruited from Cambridge University Hospital (CUHFT), University of Cambridge, volunteer databases and advertising in the Cambridge area. Participants from CUHFT may be recruited from outpatients (in particular departments of allergy and asthma) or comprise of staff working there. Employees or students at the University of Cambridge who express an interest in taking part are also eligible. Advertisements for the study will be through organisational poster. Pre-screening of interested potential participants will involve telephone screening and if potentially eligible they will receive the participant information sheet and consent form by email at least 24 hours prior to the screening visit.

A comprehensive screening visit will be undertaken at the CCRC to evaluate the following:
Demographics: date of birth, gender, race, smoking and drinking habits
Medical History: Details of any history of disease or surgical interventions and a systems review to determine inclusion/exclusion criteria
Concomitant Medication: All over-the-counter or prescription medication, vitamins, and/or herbal supplements will be recorded on CRFs.
Physical Examination as well as physiological observations, height, weight and oral temperature will be recorded.
ECG Test: A 12-lead ECG will be taken for each participant. At least the following ECG parameters will be recorded: heart rate (HR), PR, QT and QRS intervals and QT_C. The report will be signed by the Investigator who will record in the CRF whether it is normal, abnormal but not clinically significant, or abnormal AND clinically significant. In the latter case the eligibility of the participants will be reviewed.
Laboratory Tests: Biochemistry, full blood count, liver function test, total IgE, skin prick allergy testing, pregnancy test (for women) will be performed.
COVID Testing: All participants will be required to be free of any potential symptoms of COVID (fever, cough, anosmia) for at least 14 days prior to enrolment and not have any history of long-term symptoms due to COVID. A negative SARS-CoV-2 lateral flow or PCR test will be required prior to nasal sampling procedures. Staff and students from the University of

Cambridge as well as staff from CUHFT who are undergoing routine surveillance screening for COVID-19 and who test negative for the disease will also be invited to participate in the study.

All laboratory results will be reviewed by the Investigator who will record in the CRF whether they are normal, abnormal but not clinically significant, or abnormal AND clinically significant. In the latter case the eligibility of the participants will be reviewed.

Nasal curettage and brushings: This will be collected from all volunteers

Post-nasal space biopsy: For up to half the group (n=12) who volunteer for post-nasal space biopsies to be collected, a negative COVID test is required prior to sampling. These participants will undergo nasal challenge experiments a minimum of 6-weeks after the biopsy to allow for adequate time for wound healing.

8.3 Subsequent Assessments

All study visits and procedures are outlined in appendix B – the following details pertain to participants undergoing nasal challenge:

Visit 1 This visit will take place as close to a negative COVID test result. They will undergo a brief medical assessment including baseline physiological observations. They will then undergo nasal lavage followed by nasal curettage/brushing and blood sampling.

Subsequently, nasal challenge with saline (n=4) or R848 (n=20) will then be performed and non-invasive nasosorption samples will be taken every 2 hours until 8 hours after challenge. Physiological observations, total nasal symptom scores (TNSS) and peak nasal inspiratory flow (PNIF) will also be assessed 2-hourly. Blood will be collected for white blood cell differential and serum 4 hours after nasal challenge.

Participants can then choose to go home or have the option of remaining at the CCRC overnight as it has the facilities to provide this support (e.g. if the patient has travelled from further away).

Visit 2 This will take place at 24h with collection of nasosorption, curettage/brushing and a blood sample along with assessment of physiological observations, TNSS and PNIF.

For up to half the group who volunteered for an initial post-nasal space biopsy (6 weeks earlier), a second post-nasal biopsy will be undertaken. For these participants this will be the end of the study.

Visit 3 For the remaining half of the participants (n=12), they will undergo a second nasal challenge with saline (n=2) or R848 (n=10) to investigate mechanisms of innate immune memory in epithelial cells. All assessments and procedures will be identical to visit 1.

Visit 4 All study assessments and procedures will the same as visit 2.

8.4 Definition of End of Trial

The end of trial is the date of the last visit of the last participant.

8.5 Discontinuation/Withdrawal of Participants from Study Treatment

Each participant has the right to withdraw from the study at any time. In addition, the investigator may discontinue a participant from the study at any time if the investigator considers it necessary for any reason including:

- Ineligibility (either arising during the study or retrospective having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or study requirements
- An adverse event which requires discontinuation of the study or results in inability to continue to comply with study procedures
- Consent withdrawn

The reason for withdrawal will be recorded in the CRF. If the participant is withdrawn due to an adverse event, the investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilised.

8.6 Source Data

Source documents are original documents, data, and records from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g., there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent, the participant will be referred to by the study participant number/code, not by name.

9. TREATMENT OF TRIAL PARTICIPANTS

9.1 Description of Study Treatment

Challenge Agent	Manufacturer	Catalogue Dose	Production Purity
Resiquimod (R848) VacciGrade TLR7/8 agonist Synthetic vaccine grade	Invivogen, California 3950 Sorrento Valley Boulevard, Suite 100, San Diego, CA 92121, USA www.invivogen.com	5 mg lyophilized R848 Reconstitute in endotoxin-free physiological water CAS 144875-48-9	Non-biological origin: does not contain any animal nor human components. Endotoxin <1.25 EU/mg

9.2 Storage of Study Equipment or Related apparatus

R848 will be stored in labelled storage vials in the laboratory freezer and defrosted on the day of usage. Saline will be stored at room temperature. All other clinical equipment will be stored either in the PI's laboratory or the CRF.

9.3 Compliance with Study Treatment

Participants will be administered saline or R848 via a nasal spray directly by the investigators and therefore there will be no issues in assessing compliance.

10. SAFETY REPORTING

10.1 Definitions

10.1.1 Adverse Event (AE)

An AE or adverse experience is any untoward medical occurrence in a participant, which does not necessarily have to have a causal relationship with the intervention. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study, whether or not considered related to the study.

10.1.2 Serious Adverse Event (SAE)

A serious adverse event or reaction is any untoward medical occurrence that at any dose:

- Results in death,
- Is life-threatening,

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe, including an event which:

- Requires inpatient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.
- Other important medical events*

*Other events that may not result in death, are not life threatening, or do not require hospitalisation, may be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

Planned admission to hospital for a pre-existing condition will not be considered an SAE.

10.1.3 Expected Adverse Events and Serious Adverse Events Exempt from Recording

The nasal sampling procedures outlined in this document have been performed on numerous occasions by the PI (>300 times by Akhilesh Jha, AJ) and Co-I (ENT surgeon Matthew Coates, MC) and are very well tolerated. Nasal curettage has also been performed in >1200 young people including babies as young as 5 days old and was well tolerated (16).

Expected Adverse Events from nasal procedures include the following:

- Nasal irritation and runny nose
- Minor epistaxis
- Watering of the eyes
- Sneezing
- Transient bitter taste if topical anaesthesia is used

Obtaining a post-nasal space biopsy is more invasive than performing nasal curettage/brushings but the use of topical local anaesthetic means that it is generally

well tolerated. Prolonged epistaxis, requiring nasal packing and/or admission to hospital, is rare following this procedure.

Nasal R848 challenge (higher dose 10µg/nostril n=9, and low dose 1-2µg/nostril n=35) is well tolerated with no reported SAEs (a mixture of healthy, AR and ARwA participants). Given that R848 is a substance that binds to the same pattern recognition receptors as influenza, some of these participants experienced transient flu-like symptoms such as:

- Runny nose
- Mild headache
- Lethargy
- Myalgia

Symptoms diminished over a 24 to 36-hour period and there were no changes in systemic physiological observations (temperature, pulse and blood pressure) at multiple time-points after R848 challenge[15]. **A table of symptoms from the previous nasal challenge study is provided in appendix C**.

The current study will assess the impact of two nasal challenges with R848 administered two weeks apart. The second nasal challenge is expected to induce similar flu-like symptoms to the first challenge. However, one of the main outcomes of the study is to assess the tolerability of the second challenge. Therefore, a small increase in the severity of flu-like symptoms (e.g. runny nose, headache, lethargy, myalgia, fever) may be expected.

10.1.4 Suspected Unexpected Serious Adverse Reactions (SUSAR)
A serious adverse reaction, the nature or severity of which is not consistent with the applicable product information

10.2 Reporting Procedures for All Adverse Events
All AEs occurring during the study observed by the investigator or reported by the participant, whether or not attributed to study, will be recorded on the CRF.
The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to study, other suspect device and action taken. Follow-up information should be provided as necessary.
AEs considered related to the study as judged by a medically qualified investigator or the sponsor will be followed until resolution or the event is considered stable. All related AEs that result in a participant's withdrawal from the study or are present at the end of the study, should be followed up until a satisfactory resolution occurs.
It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the participant's removal from treatment (see section 7.7). A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant must undergo an end of study assessment and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable. The relationship of AEs to the study will be assessed by a medically qualified investigator.

10.3 Reporting Procedures for Serious Adverse Events
All SAEs, except those expected ones defined in section 10.1.3 that do not require immediate reporting, must be reported to the Sponsor within one working day of discovery or notification of the event. The Sponsor will perform an initial check of the information and ensure that it is reviewed at the next R&D Management meeting. All SAE information must be recorded on an SAE form and sent to the Sponsor using

the appropriate reporting form and the contact details on there. Additional information received for a case (follow-up or corrections to the original case) needs to be detailed on a new SAE form which must be sent to the Sponsor using the appropriate reporting form and the contact details on there.

The Sponsor will report all SUSARs to the Research Ethics Committee concerned. Fatal or life-threatening SUSARs must be reported within 7 days and all other SUSARs within 15 days. The PI will inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.
In addition to the expedited reporting above, the PI shall submit once a year throughout the study or on request an Annual Report to the Ethics Committee which lists all SAEs / SUSARs that have occurred during the preceding 12 months.

11. STATISTICS

11.1 Description of Statistical Methods

This is an open label interventional trial to investigate basic mechanisms of innate immunity. The following data outputs will be generated:

- Single-cell transcriptomic signatures
- Epigenetic signatures
- Nasal and blood cytokine levels

This data is typically of a non-parametric distribution and the statistical plan will follow a similar template to the previous publication using R848 challenge (15). For the single-cell and epigenetic datasets, a series of pre-processing quality control checks will take place followed by cell and gene-level downstream analysis with the support of specialised bioinformaticians.

For protein analysis, data will be plotted for individual cytokines using raw data at an individual level. For the repeat R848 challenge, area under curve (AUC) values will be calculated for each cytokine and paired Wilcoxon signed-rank test will be used to compare differences between each challenge with the hypothesis that repeat R848 challenge causes increased responses on secondary challenge.

AUC values of cytokine production after R848 challenge will also be compared between healthy and allergic participants using unpaired Mann-Whitney test.

11.2 The Number of Participants

In the prior publication using R848 nasal challenge (15), 12 participants in each group (healthy, allergic rhinitis and asthma) were sufficient to see differences in bulk gene expression and immune mediators in the nasal mucosa. The present study is based on utilising a powerful single-cell transcriptomic approach which is able to distinguish differences in immune responses at an individual cellular level. It may therefore be possible to see differences between groups in fewer numbers of participants but will allow recruitment of up to 12 participants in each group.

11.3 The Level of Statistical Significance

A P-value of < 0.05 will be taken to indicate statistical significance.

11.4 Procedure for Accounting for Missing, Unused, and Spurious Data.

Missing clinical data points will be imputed as the mean of values from that volunteer.

12. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Direct access will be granted to authorised representatives from the sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

13. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures. The University of Cambridge operate a risk-based audit programme to which this study will be subject.

14. CODES OF PRACTICE AND REGULATIONS

14.1 Participant Confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participant's ID number on the CRF and an electronic database. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the Data Protection Act 2018 which requires data to be anonymised as soon as it is practical to do so.

14.2 Other Ethical Considerations

The study will not be initiated before the protocol, and all study relevant material such as the informed consent forms and participant information sheets have received a favourable opinion from the Research Ethics Committee (REC) and the HRA, and Cambridge University Hospitals NHS Foundation Trust has issued trust confirmation of capacity and capability. Any changes to protocol or relevant study documents will be approved by the Sponsor. Should an amendment be made that requires REC approval, as defined by REC as a substantial amendment, the changes will not be instituted until the amendment has been reviewed and received favourable opinion from the REC and the HRA, and Cambridge University Hospitals NHS Foundation Trust have issued confirmation of continuing capacity and capability. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately providing that the REC is notified as soon as possible, and an approval is requested. Minor amendments as defined by REC as a non-substantial amendment may be implemented upon receipt of HRA approval.

14.3 Informed Consent

It is the responsibility of the Chief Investigator and Principal Investigator, or a person designated by the CI or PI (if acceptable by local regulations), to obtain written informed consent from each patient participating in this study, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study using the PIS. The consent process will be documented in the patient's notes.

The process for obtaining participant informed consent will be under the REC guidance, and GCP and any other regulatory requirements that might be introduced. The CI/PI/delegate and the participant shall both sign and date the Informed Consent Form before the person can participate in the study.

The participant will keep a copy of the PIS and a signed and dated Consent Form. The original will be retained in the Trial Master File. A second copy, along with the PIS, will be filed in the participant's medical notes and a signed and dated note made in the notes of when the PIS was provided, and that informed consent was obtained for the study.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to the participant that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled.

14.4 Declaration of Helsinki

The Investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki (last amended October 2000, with additional footnotes added 2002 and 2004).

14.5 ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

15. DATA HANDLING AND RECORD KEEPING

15.1 Data Handling

All data will be handled within the conditions of the Data Protection Act 2018

15.2 Data Collection Forms

Enrolment into the study will be documented in each participants' medical notes. Data collection will comprise of a paper case report form, including participant characteristics, disease severity, medication lists, clinical examination, pulmonary and blood test results.

15.3 Data Quality and Security

The data will be securely stored in line with GCP standards and the data protection principles. Standard Operating Procedures (SOPs) will be followed to ensure quality control. Only staff authorised to work on this study will have access to participants' data. The Principal Investigator will facilitate access to study records for monitoring, audits, and regulatory inspections. Participant's consent to this will be sought at the time of enrolment into the study.

15.4 Sample handling

Nasal epithelial cells may also be collected, stored and cultured in a laboratory on the Cambridge Biomedical Campus or at other sites within the University of Cambridge.

16. FINANCING AND INSURANCE

16.1 Insurance and Liability

CUHFT, as a member of the NHS Clinical Negligence Scheme for Trusts, will accept full financial liability for harm caused to participants in the clinical trial caused through the negligence of its employees and honorary contract holders. There are no specific arrangements for compensation should a participant be harmed through participation in the trial, but no-one has acted negligently.

The University of Cambridge will arrange insurance for negligent harm caused because of protocol design and for non-negligent harm arising through participation in the clinical study.

16.2 Research Costs

The research is funded by Wellcome Trust and Cambridge Academy of Therapeutic Sciences via a Developing Concept Fund grant (Reference number RG93172/BRYANT/34636), Wellcome Trust Investigator Award to Professor Clatworthy (220268/Z/20/Z) and through the Evelyn Trust (21/05).

16.3 Service Support Costs

Not applicable

16.4 Study Sponsorship

This study is being jointly sponsored by the University of Cambridge and CUHFT.

17. PUBLICATION POLICY

Results of this study will be submitted for publication in a peer-reviewed journal. The manuscript will be prepared by the Principal Investigator in conjunction with the study team; authorship will be determined by agreement.

Anonymised data containing genetic information will be deposited on publicly accessible databases in order to share and archive the data for the benefit of the wider scientific community. For example, data will be deposited with The European Genome-phenome Archive (EGA) (https://ega-archive.org/) or/and the Human Cell Atlas (https://www.humancellatlas.org/).

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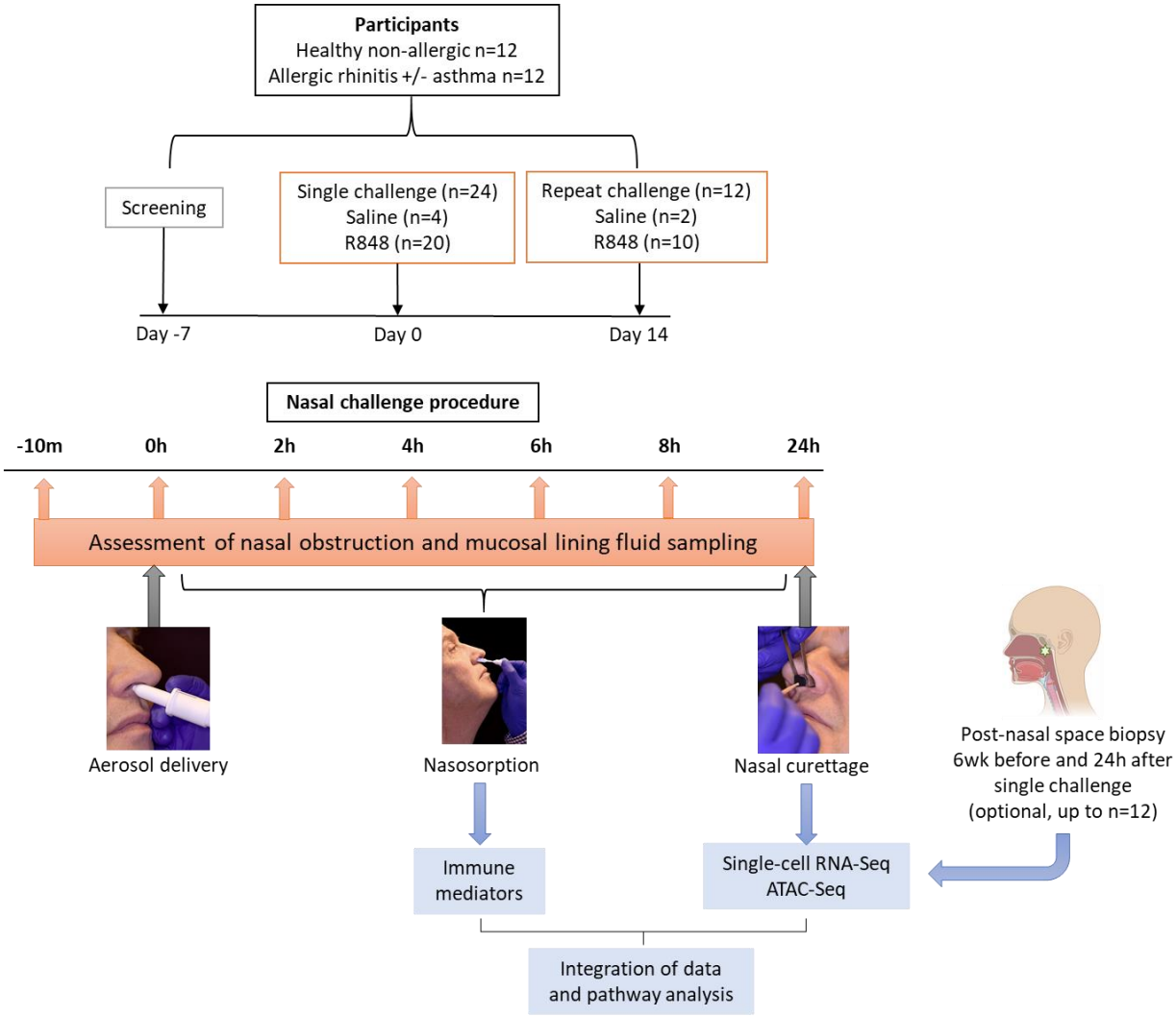
19. APPENDIX A: STUDY FLOWCHART



20. APPENDIX B: SCHEDULE OF PROCEDURES

Procedures	Visits				
	Screening	**Visit 1 (duration 10h)** 1st nasal challenge	**Visit 2 (at 24h)** 1st nasal challenge	**Visit 3 (duration 10h)** 2nd nasal challenge	**Visit 4 (at 24h)** 2nd nasal challenge
Informed consent	**X**				
Demographics, Medical History and Medications	**X**				
General physical examination	**X**				
ECG and Laboratory tests	**X**				
DNA blood test (up to 8.5mL)	**X**				
SARS-CoV-2 Lateral Flow or PCR nasal swab		**X** (negative test prior to nasal sampling)		**X** (negative test as close as possible to sampling)	
1st Nasal challenge with saline (n=4) or R848 (n=20)		**X**			
2nd Nasal challenge with saline (n=2) or R848 (n=10)				**X**	
Vital signs, clinical assessment and symptom scores		**X** (2 hourly)	**X**	**X** (2 hourly)	**X**
TNSS and PNIF		**X** (2 hourly)	**X**	**X** (2 hourly)	**X**
Nasal lavage		**X** (0h)		**X** (0h)	
Nasosorption (filter strips to absorb mucosal fluid)		**X** (15min, 30min, 1hr, 2hr, then 2 hourly)	**X**	**X** (15min, 30min, 1hr, 2hr, then 2 hourly)	**X**
Nasal curettage	**X**	**X**	**X**	**X**	**X**
Nasal brushing	**X**	**X**	**X**	**X**	**X**
Post-nasal space biopsy (a minimum of 6 weeks prior to challenge) and at visit 2	**X**		**X**		
Blood (9ml FBC, serum 0h, 4h, 24h), (13ml single cell analysis and Truculture at 0h and 24h)		**X** (0h and 4h)	**X**	**X** (0h and 4h)	**X**

TNSS Total Nasal Symptom Score; PNIF Peak Nasal Inspiratory Flow

21. APPENDIX C: TABLE OF SYMPTOMS FROM PREVIOUS CHALLENGE STUDY

The following symptoms were recorded from the previously established R848 (1-2µg/nostril) nasal challenge study performed in 35 healthy, AR and ARwA volunteers (reference 15). All were mild and none were serious adverse events.

Symptom	Number of cases (out of total 35 volunteers)
Nasal blockage/rhinitis	11
Lethargy	6
Headache	3
Myalgia	1
Sore throat	1