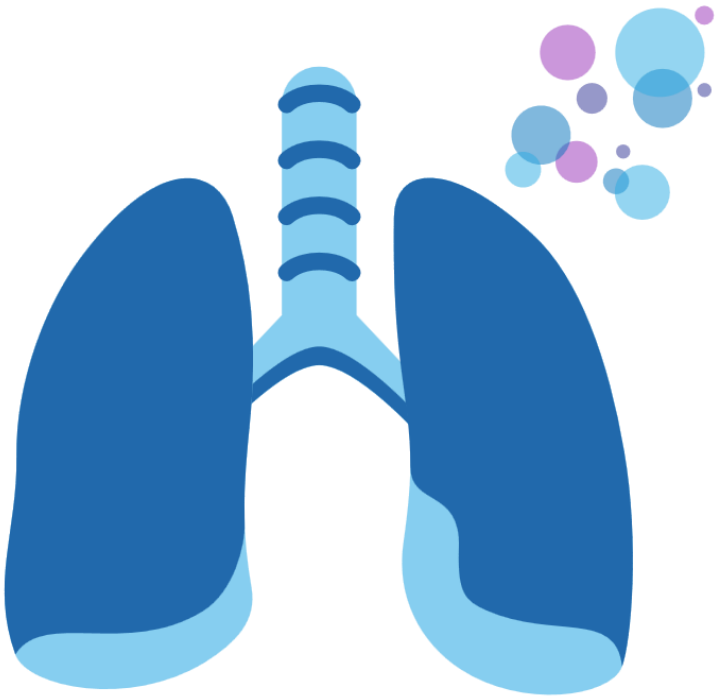
Study Protocol

Lung Immune Challenge Study

Controlled Exposure to Inhaled Resiquimod (R848)

to Study Mechanisms of Inflammation



Lung Immune Challenge Study

1. STUDY DETAILS

Sponsor Reference Number:	R&D A096886
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Signatures:	Dr Akhilesh Jha 14th March 2024

Confidentiality Statement

All information contained within this protocol is regarded as and must be kept confidential. No part of it may be disclosed by any Receiving Party to any Third Party, at any time, or in any form without the express written permission from the Principal Investigator and / or Sponsor.

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2. AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made

3. SYNOPSIS

Title	Lung Immune Challenge Study: Controlled Exposure to Inhaled Resiquimod (R848) to Study Mechanisms of Inflammation
Internal Reference No.	A096886
Design	Randomised single ascending dose inhaled exposure to Toll-like receptor (TLR)7/8 agonist R848
Participants	Participants without asthma Participants with asthma
Sample Size	36
Duration	3 years
Aims	1. To identify a suitable dose of inhaled R848 that induces innate immune responses in the lungs and blood 2. To identify a suitable dose of inhaled R848 that is clinically tolerable
Outcome Measures	Primary Outcome i. Change in CXCL10 in serum or induced sputum 24 hours after single ascending dose inhaled R848 Secondary Outcomes i. Change in FEV_1 after 1, 4, 8, 24 and 48 hours of single ascending dose inhaled R848 ii. Change in physiological observations (temperature, pulse rate and blood pressure) after 1, 4, 8, 24 and 48 hours of single ascending dose inhaled R848 iii. Change in peripheral eosinophil and lymphocyte counts after 4, 24 and 48 hours of single ascending dose inhaled R848 iv. Change in serum C-Reactive Protein (CRP) after 4, 24 and 48 hours of single ascending dose inhaled R848
Eligibility	Adult males and females between 18 and 60 years of age meeting inclusion and exclusion criteria.

4. ABBREVIATIONS

AE	Adverse event
AR	Allergic Rhinitis
ARwA	Allergic Rhinitis with Asthma
CUH	Cambridge University Hospitals
CCRC	Cambridge Clinical Research Centre
CRF	Case Report Form
CRP	C-Reactive Protein
CT	Clinical Trials
FEV_1	Forced Expiratory Volume in First Second
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
HLRI	Heart and Lung Research Institute
ICF	Informed Consent Form
LPS	Lipopolysaccharide
NHS	National Health Service
PBMC	Peripheral Blood Mononuclear Cell
PI	Principal Investigator
PIS	Participant Information Sheet
R&D	NHS Trust R&D Department
R848	Resiquimod
REC	Research Ethics Committee
REDCap	Research Electronic Data Capture
RPH	Royal Papworth Hospital
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File

5. BACKGROUND AND RATIONALE

5.1 Background

Acute respiratory viral infections cause significant morbidity, especially in vulnerable individuals and has been a topic of immense significance during the recent 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 chronic allergic asthma, therapeutic options for treating acute asthma attacks 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 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.

The innate immune system is crucial for effectively detecting and mounting responses to respiratory viruses. Toll-like receptors (TLRs) inside cells play a critical role in the initial detection of viral nucleic acid. 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 appropriate innate immune responses to viral infections (3-5).

Resiquimod (R848) is a synthetic compound that binds to TLR7/8. As R848 activates anti-viral responses, it has been given systemically to treat viral hepatitis C infection (6) and employed topically as a skin cream for actinic keratosis (7). Related 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 (8, 9). A TLR7 agonist has also been trialled by the nebulised route and found to activate anti-viral innate immune responses (as evidenced by a rise in the biomarker CXCL10) in the lungs and blood (10). R848 can therefore be used as a suitable translational approach for studying innate immune responses in humans.

5.2 Rationale

An intranasal R848 human challenge model has been established by the Principal Investigator (PI). In total, it has been administered intranasally to 54 volunteers at different doses and is well tolerated with no reported serious adverse events (SAE) (11, 12). Nasal R848 induces anti-viral innate immune responses (interferons and chemokines) in a similar manner to respiratory viruses. It therefore represents a very useful research tool for studying innate immune responses to viral infections in the respiratory tract without needing to use live viral challenge, which is significantly more technically and resource intensive.

Intranasal R848 has been given to healthy volunteers and those with allergic rhinitis (AR) or allergic rhinitis with asthma (ARwA). Individuals with AR and ARwA have increased nasal mucosal interferon and chemokine responses compared to healthy volunteers highlighting that dysregulated respiratory mucosal innate immune responses are likely to be important in determining the clinical outcome of viral triggers in individuals with allergy and asthma (12).

The nose however has a different microenvironment, structure and composition of cells compared to the lungs (13). Furthermore, in those with asthma, distinct changes occur in the lungs such as airway remodelling and airway smooth muscle hypertrophy (14). It is therefore crucial to study host innate immune responses directly at the site of disease.

Defining the cellular basis and molecular drivers of innate immune responses to respiratory

viral infections in the lungs will help provide mechanistic insights into how these responses might be dysregulated in diseases and help identify potential therapeutic targets.

The goal of this study is to develop a human lung immune challenge model using controlled exposure to inhaled R848. The aim is to build upon the already established nasal challenge model to identify a clinically tolerable dose for use in the lungs that induces anti-viral innate immune responses. It will first be trialled in healthy individuals and subsequently in volunteers with asthma.

Once established, inhaled R848 challenge is a clinical research approach that will enable:

- Study of the effect of different host states on respiratory innate immune responses
- Understanding of the molecular and immune drivers of asthma exacerbations
- A platform for testing the efficacy of anti-inflammatory compounds
- Significant practical advantages over live viral challenge models

6. OBJECTIVES

6.1 Primary Objectives

i. To identify an appropriate dose of inhaled R848 which induces innate immune responses in serum and lungs

6.2 Secondary Objectives

i. To assess clinical tolerability of inhaled R848 with regards to FEV_1

ii. To assess clinical tolerability of inhaled R848 with regards to physiological observations (temperature, pulse, blood pressure)

iii. To assess change in peripheral eosinophil and lymphocyte counts after inhaled R848

iv. To assess change in serum CRP after inhaled R848

7. STUDY DESIGN

7.1 Summary of Trial Design

Nasal administration of R848 has been shown to cause dose-dependent (10 to 100 µg/mL) induction of mucosal innate immune responses (11, 12). The current study will aim to nebulise R848 into the lower airways using a similar dosing regimen and using a nebulisation technique well established using inhaled lipopolysaccharide (LPS) challenge (15). The design of the study is broadly based on a previously published clinical trial of an inhaled TLR7 agonist (10).

Single ascending dose (0.1, 1, 10, 100 µg/mL) nebulisation of R848 will be performed in healthy volunteers (up to n = 24) and subsequently in volunteers with asthma (n = 6).

All volunteers will receive only a single dose and the starting dose cohort for nebulised challenge will be 100-fold lower than the dose administered in the nose to ensure clinical tolerability.

For each dose-cohort (n = 6), participants will be randomised to either R848 (n = 4) or saline (n = 2).

A summary of the study design is outlined in **Appendix A**.

The study will start with a small “sample only’ cohort of six participants without asthma who will be recruited for the collection of nasal brush, sputum, and blood samples to optimise sample processing protocols prior to the main study.

7.2 Primary Outcome Measures

i. Change in CXCL10 in serum or induced sputum 24 hours after single ascending dose inhaled R848

7.3 Secondary Outcome Measures

i. Change in FEV_1 after 1, 4, 8, 24 and 48 hours of single ascending dose inhaled R848

ii. Change in physiological observations (temperature, pulse rate and blood pressure) after 1, 4, 8, 24 and 48 hours of single ascending dose inhaled R848

iii. Change in peripheral eosinophil and lymphocyte counts after 4, 24 and 48 hours of single ascending dose inhaled R848

iv. Change in serum CRP after 4, 24 and 48 hours of single ascending dose inhaled R848

8. TRIAL PARTICIPANTS

8.1 Overall Description of Trial Participants

The study will start with a small "sample only' cohort of six participants without asthma who will be recruited for the collection of nasal brush, sputum, and blood samples to optimise sample processing protocols prior to the main study.

The interventional part of the study will be performed in participants without asthma (n = 24), and subsequently in participants with asthma (n = 6).

8.2 Inclusion Criteria

- Male or female aged between 18 and 60 years.
- Willing and able to give informed consent for 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.
- Clinically acceptable laboratory measurements and ECG at enrolment.
- Ability to expectorate sputum.
- Optional additional swab for SARS-CoV-2 testing will be collected from participants if required by local or/and national health and safety policies at the time of sampling.

For volunteers without asthma:

- No clinical history of asthma
- Normal baseline spirometry i.e. FEV_1/Forced Vital Capacity (FVC) ratio z-score greater than the lower limit of normal.

For volunteers with asthma:

- Physician-diagnosed mild to moderate asthma which is not poorly controlled as evidenced by an Asthma Control Questionnaire (ACQ-5) score of ≤1.5.
- They are permitted to be on inhaled corticosteroids (ICS), long-acting beta agonist (LABA) and long-acting muscarinic antagonists (LAMA).
- Pre-bronchodilator FEV_1 ≥70% predicted.
- Evidence of bronchial hyperreactivity as evidenced by either (i) Bronchodilator reversibility (increase FEV_1 ≥12% and 200 mL); (ii) Positive methacholine challenge (PC_{20} < 8mg/ml), or (iii) Positive challenge test as per current CUH policy.

8.3 Exclusion Criteria

- Upper respiratory tract infection in preceding 14 days.
- Lower respiratory tract infection in preceding 28 days.
- Female participants who are pregnant, lactating or planning pregnancy.
- Respiratory diseases (other than asthma where specified).
- Significant extrapulmonary medical conditions.
- Extreme obesity (BMI >40).
- 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.
- No newly prescribed courses of medication including corticosteroids in the four weeks before first study dose other than mild analgesia, vitamins, and supplements.
- Smoking tobacco or vaping products in previous 6 months.

- Smoking history of >5 pack years.

8.4 Treatment Assignment and Stratification

A web-based randomisation and treatment allocation system using REDCap (Research Electronic Data Capture) will be used. Participants will be stratified by sex to ensure equal allocation to active and saline groups.

For each dose cohort of 6 participants, individuals will be randomised (2:1) to either:

- Nebulised R848
- Nebulised Saline

The PI (or delegate) will prepare the solution for nebulisation in a location away from the participant. When it is administered to the participant, they will be unaware of the intervention to which they have been allocated.

9. STUDY PROCEDURES AND ASSESSMENTS

A schedule of study procedures in relation to study visits is outlined in **Appendix B**.

9.1 Study Procedures

9.1.1 Nasosorption

Nasal mucosal lining fluid will be collected non-invasively using nasosorption at baseline (0 hours) and 24 hours after intervention to assess whether immune changes in the lungs can also be detected in the nose.

9.1.2 Nasal Brushing

- A small cytology brush will be used to gently and non-invasively collect epithelial cells from the nasal inferior turbinate to enable epithelial cell culture in the laboratory.

9.1.3 Blood

Blood will be collected on the following occasions:

- *Screening (31.5 mL)*: For routine safety and eligibility (9 mL), DNA testing (2.5 mL) and blood for isolation of peripheral blood mononuclear cells (PBMCs) (20 mL).

- *Baseline (14 mL)*: At 0 hours for full blood count (FBC) (4 mL), CRP (5 mL) and serum collection (5 mL).

- *Post intervention (52 mL)*: At 15 minutes, 1 hour, 4 hours, 24 hours and 48 hours for serum collection (25 mL); At 4 hours, 24 hours, and 48 hours for full blood count (FBC) (12 mL) and CRP (15 mL) measurement.

Therefore, a total volume of approximately 100 mL blood will be collected from each volunteer throughout the whole trial including the screening and main study period.

9.1.4 Induced sputum

This will be performed using hypertonic saline at the screening visit and 24 hours after intervention using standardised techniques (16) over a 15-20 minute period to obtain sputum that can be analysed for CXCL10 levels. Salbutamol will be co-administered as a bronchodilator to reduce the chances of bronchoconstriction.

9.1.5 Spirometry

Volunteers will perform spirometry at the screening visit and throughout the study period.

9.1.6 Bronchodilator Reversibility or Methacholine Challenge or Exercise Testing

For volunteers with asthma, as part of their screening they will be asked to perform either bronchodilator reversibility, methacholine challenge or challenge testing as per latest CUH policy (e.g. exercise testing), to confirm bronchial hyperreactivity. If their baseline FEV_1 is ≤80%, they will be asked to perform spirometry before and after inhalation of salbutamol to check for reversibility by ≥12% and 200 mL. If their baseline FEV_1 is ≥80% they will instead perform a methacholine challenge or challenge test as per CUH policy (e.g. exercise test).

9.2 Informed Consent

A written or electronic version of the Participant Information Sheet (PIS) will be provided to participants detailing the nature of the study and any known side effects and risks involved in taking part. The participant will be 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 time to consider the information, and the opportunity to question the Investigator, to decide on participation in the study. Electronic Informed Consent (eConsent) will then be obtained by means of participant dated and investigator dated electronic signatures. The person obtaining consent will be suitably qualified and experienced and have been authorised to do so by the PI as detailed on the Delegation log for the study. A copy of the signed form will be retained in the Trial Master File (TMF). A written or electronic copy of the signed consent form will also be provided to participants.

9.3 Screening and Eligibility Assessment

Participants in this study will be recruited from Cambridge University Hospitals (CUH), Royal Papworth Hospital (RPH), Heart and Lung Research Institute (HLRI), University of Cambridge, volunteer databases, local advertising in the Cambridge area and wider East of England region, as well through online platforms. Participants from CUH 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 posters, newsletters, online bulletins, local newspapers, online platforms, and social media channels.

Pre-screening of interested participants will primarily involve interaction with a dedicated study website (www.lungchallenge.org.uk) or by email and telephone screening. They will be able to download the Participant Information Sheet (PIS) from the study website.

An online pre-screening questionnaire hosted using REDCap software will be utilised to ascertain basic eligibility for the study in an anonymised form. If participants are not eligible no data will be stored. If participants may be eligible, they will be asked for further demographic and medical details to help ascertain eligibility and their data will be stored using the University of Cambridge's Secure Data Hosting Service which has a Safe Haven for Personal Identifiable Data https://cscs.medschl.cam.ac.uk/server-services/secure-data-hosting-service/.

Subsequently, for potentially eligible participants, a screening visit will be undertaken at the Cambridge Clinical Research Centre (CCRC) to evaluate the following:

Demographics: date of birth, gender, race, smoking status.

Medical History: Details of any history of disease or surgical interventions and systems review to determine inclusion/exclusion criteria.

Concomitant Medication: All over the counter or prescription medication, vitamins, and/or herbal supplements will be recorded.

Physical Examination as well as physiological observations, height, weight, and temperature will be recorded.

ECG Test: A 12-lead ECG will be undertaken for each participant.

Laboratory Tests: Biochemistry, full blood count, liver function test, total IgE, PBMC isolation, skin prick allergy testing, and a pregnancy test (for women) will be performed.

Nasal Brushing: At the discretion of the Investigator, this will be done to collect nasal epithelial cells.

Induced Sputum: Volunteers will undergo induced sputum sampling. For volunteers with asthma this will take place at a second screening visit approximately 7 days (+/- 3 days) after

the reversibility testing is done at the screening visit. This is to limit any confounding effects of reversibility testing on cellular counts in the induced sputum.

All screening results will be reviewed by the Investigator to determine participant eligibility.

For participants in the "sample only" part of the study, their screening will briefly involve collection of data on demographics, medical history, medication usage, spirometry, and physiological observations to ensure they meet the inclusion and exclusion criteria. They will not require other screening tests. Sample collection will involve a nasal brushing, induced sputum, and blood sampling (20 mL – for isolation of peripheral blood mononuclear cells). This will be the end of the study for them.

9.4 Subsequent Assessments

For volunteers who are eligible for the study, the following study visits and procedures will take place and are outlined in **Appendix B**.

9.4.1 Visit 1 (Day 1)

This will take place over 24 hours including an overnight stay in the CCRC. Baseline assessments will include:

- Spirometry
- Physiological observations (temperature, pulse, and blood pressure)
- Nasosorption - to determine baseline nasal immune profile
- Blood tests (FBC, CRP, Serum)

Volunteers will have been randomised to receive either R848 or saline via a breath-actuated nebuliser (Aeroeclipse II). After administration, they will be monitored at regular intervals by the clinical research team to check for clinical tolerability and repeat blood tests. The following morning after 24 hours, volunteers will subsequently have repeat blood tests as well as a repeat induced sputum performed.

9.4.2 Visit 2 (Day 2)

This will take place at 48 hours to monitor physiological observations, spirometry and perform blood tests.

9.4.3 Visit 3 (Day 8)

This will take place at 7 days after challenge to monitor physiological observations, spirometry and perform blood tests. This will then be the end of the study for participating volunteers.

9.5 Definition of End of Trial

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

9.6 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.

9.7 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 and medicine history may be summarised), 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 and not by name.

10. TREATMENT OF TRIAL PARTICIPANTS

10.1 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

R848 has been administered *in-vivo* in pre-clinical models and humans in a wide variety of formulations and routes at significantly higher doses than the proposed study, with extensive available data on tolerability:

- R848 was well tolerated in pre-clinical models in mice and rats (17, 18).
- Oral R848 has been given to patients with chronic hepatitis C infection and was well-tolerated at doses of 0.01mg/kg, equating to 700 μg for a 70 kg human (6).
- Topical dermal R848 is safe and effective as a skin cream for the treatment of anogenital warts, actinic keratosis and skin cancer. Human studies have been carried out giving topical R848 to inflamed skin with actinic keratoses at doses up to 0.25%, which is equivalent to 2500 μg/mL (19). A related compound, the TLR7 agonist Imiquimod (marketed as Aldara), is approved for clinical use as a topical application for genital warts.
- Nasal R848 at doses between 10-100 μg/mL has been given by the PI to 54 participants ranging from those who are healthy to those with allergic rhinitis and asthma. This was effective at inducing mucosal innate immune responses whilst being clinical tolerable (11, 12).

For the present study, R848 will be reconstituted as per the manufacturer's instructions and diluted to the appropriate doses at CUH. A sample of the prepared dose vials will be sent for external quality control sterility testing to British Pharmacopeia standards before use in the clinical study.

Saline will be obtained from commercial medicine stock from within the hospital. It will be manufactured to GMP and considered sterile therefore no additional sterility testing will be required.

10.2 Dose Escalation

The following doses of nebulised R848 will be tested using a single dose escalation design: 0.1, 1, 10, 100 μg/mL. Six volunteers will be involved in each dose cohort with only a single dose given to each participant.

Each participant will be administered a dose of R848 or saline in a volume of approximately 1 mL (optimal volume to be confirmed after testing) using a breath-actuated nebuliser.

At the end of each dose cohort, CXCL10 levels in sputum and blood will be assessed and compared between saline and R848. If there are no statistically significant differences

between groups, and the dose is tolerable for volunteers, then the study will proceed to the next dose cohort.

Spirometry will be monitored regularly after inhaled challenge. Bronchodilator drugs will be available on standby for administration if volunteers have symptoms of breathlessness or tight chest associated with a significant drop in FEV_1 (≥20%) from baseline.

10.3 Storage of Study Equipment or Related apparatus

R848 will be stored in labelled storage vials in a -20°C freezer within CUH in PI's laboratory 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.

10.4 Compliance with Study Treatment

Participants will be administered R848 or saline via a nebuliser directly by the research team and therefore compliance will be directly assessed.

11. SAFETY REPORTING DEFINITIONS

11.1 Adverse Event (AE)

An AE or adverse experience is any untoward medical occurrence in a participant, which does not necessarily 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.

11.2 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.

11.3 Expected Adverse Events and Serious Adverse Events Exempt from Recording

Nasal R848 challenge administered at doses between 10 - 100 µg/mL in healthy participants and those with asthma has previously been well tolerated with no reported SAEs (11, 12).

R848 is a synthetic substance that when given to in the respiratory tract is designed to mimic the effects of a viral infection such as influenza. Therefore, expected AEs that participants may experience include transient flu-like symptoms such as:

- Cough
- Runny nose
- Mild headache
- Low grade fever
- Lethargy
- Myalgia

If a high-grade fever occurs that is more sustained or causes symptoms, then volunteers will be offered an antipyretic such as paracetamol.

Participants with asthma will be carefully monitored after R848 dosing. If their FEV1 drops by >20% then they will be administered nebulised salbutamol as a bronchodilator.

Nasal sampling using nasosorption and nasal cytology brush may cause:
- Nasal irritation and runny nose
- Minor epistaxis
- Watering of the eyes
- Sneezing

There are minimal if any side effects anticipated from the induced sputum procedure and salbutamol will be given as a bronchodilator at the same time of administration in healthy and asthma participants, but recognised side effects of the procedure include:
- Cough
- Tight chest

Expected AEs from blood sampling procedures may include:
- Mild discomfort during venepuncture
- Bruising at venepuncture site

11.4 Suspected Unexpected Serious Adverse Reactions (SUSAR)

A serious adverse reaction, the nature or severity of which is not consistent with the applicable product information

11.5 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 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. 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.

11.6 Reporting Procedures for Serious Adverse Events

All SAEs, except those expected ones defined in section 10.3 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. 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.

12. STATISTICS

12.1 Description of Statistical Methods

CXCL10 will be measured in sputum and serum 24 hours after each ascending dose of inhaled R848 (n = 4) and compared to inhaled saline (n = 2). CXCL10 levels will be compared between groups using unpaired t-tests.

Spirometry (FEV_1), physiological assessments (temperature, pulse, blood pressure) and leukocyte differential counts from FBC samples measured after challenge will be compared to baseline values using one-way analysis of variance (ANOVA).

12.2 The Number of Participants

Nasal R848 is a suitable method of inducing nasal innate immune responses (evidenced by a rise in interferon levels) in healthy individuals and those with asthma (11, 12). Therefore, it is anticipated that inhaled R848 will have a similar effect in inducing lung and peripheral innate immune responses even in a small number of participants.

The number of participants in each dose cohort of the dose finding phase of the study (n = 4 R848, n = 2 saline) is based on AstraZeneca's clinical trial of their inhaled TLR7 agonist (a compound with similar effects) (10), and was a suitable number of participants for assessing innate immune responses as evidenced by a rise in CXCL10 in sputum and serum.

12.3 The Level of Statistical Significance

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

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

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

13. 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.

14. 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 program to which this study will be subject.

15. CODES OF PRACTICE AND REGULATIONS

15.1 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 electronic databases. 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.

15.2 Other Ethical Considerations

The study will not be initiated before the protocol, and all study relevant material such as the PIS and ICF 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.

15.3 Informed Consent

It is the responsibility of the PI, or a person designated by the PI (if acceptable by local regulations), to obtain signed 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 process for obtaining participant informed consent will be under the REC guidance, and GCP and any other regulatory requirements that might be introduced. The PI or delegate and the participant shall both sign and date the ICF before the person can participate in the study.

The participant will receive a written or electronic copy of the PIS and a signed and dated ICF. The ICF will also be printed and retained.

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.

15.4 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 in October 2013).

15.5 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: ICH E6(R3).

16. DATA HANDLING AND RECORD KEEPING

16.1 Data Handling

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

16.2 Data Collection Forms

A record of a participant's involvement in the study will be made in their electronic healthcare record. Data collection will comprise of an electronic case report form using REDCap and will include participant characteristics, disease severity, medication lists, clinical examination, pulmonary and blood test results.

16.3 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 PI 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.

The Cambridge Integrated Data Environment (CAM:IDE) System will support digital management of data within the study. It will utilise the REDCap Safe Haven, which is suitable for personally identifiable data and anonymised research data and hosted on the Cambridge University Information Services (UIS) ISO 27001 accredited environment which is also NHS Toolkit Compliant.

16.4 Sample handling

Induced sputum and serum will be processed and analysed in laboratory facilities on the Cambridge Biomedical Campus.

Nasal epithelial cells will be collected, stored, and cultured in a laboratory on the Cambridge Biomedical Campus or within the University of Cambridge.

17. FINANCING AND INSURANCE

17.1 Insurance and Liability

CUH, 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.

17.2 Research Costs

The research is funded by the Medical Research Council (MRC; Reference Number MR/Y000935/1).

17.3 Service Support Costs

Not applicable

17.4 Study Sponsorship

This study is being jointly sponsored by the University of Cambridge and Cambridge University Hospitals NHS Foundation Trust.

18. PUBLICATION POLICY

Results of this study will be submitted for publication in a peer-reviewed journal. The manuscript will be prepared by the PI 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 may 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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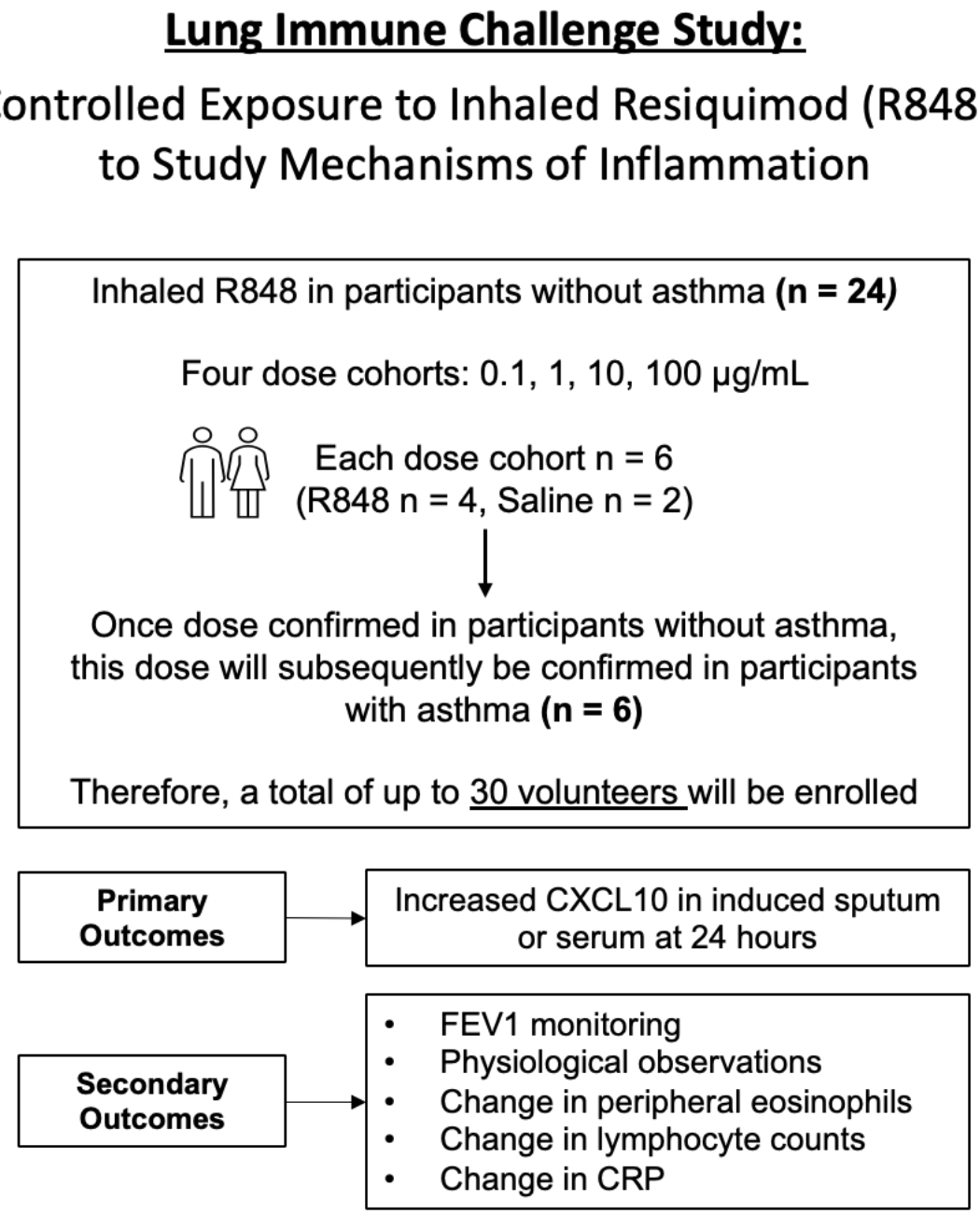
20. APPENDIX A: STUDY FLOWCHART



21. APPENDIX B: SCHEDULE OF PROCEDURES

Procedure	Screening Healthy (n = 24)	Screening Asthma (n = 6)	Day 1							Day 2	Day 8
			Visit 1							Visit 2	Visit 3
			0h	Challenge	15 min	1 h	4 h	8 h	24 h	48 h	Day 7
Informed consent	X	X									
Demographics, Medical History, Medications	X	X									
General physical examination	X	X									
ECG and baseline laboratory tests	X	X									
DNA blood test + PBMC isolation	X	X									
Nasal brush	X	X									
Skin prick testing for aeroallergens	X	X									
Bronchodilator reversibility, methacholine challenge or test as per CUH policy		X									
Induced sputum	X	**7 Days Post Reversibility**							X		
Nasosorption			X						X		
Spirometry (FEV_1) and Observations (temperature, pulse, blood pressure)	X	X	X		X	X	X	X	X	X	X
Serum			X		X	X	X	X	X	X	X
Full blood count + C-Reactive Protein			X				X		X	X	X
Nebulisation with R848 or Saline				X							