NON-EOSINOPHILIC BIOLOGICAL EFFECTS OF INTERLEUKIN 5 (IL-5) - ROLE OF IL-5 IN SUPPRESSING ANTI-VIRAL IMMUNITY IN BRONCHIAL EPITHELIAL CELLS AND DENDRITIC CELLS IN ASTHMA

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Clinical Queries

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Sponsor

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

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This protocol describes the non-eosinophilic biological effects of IL-5 study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

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

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GLOSSARY OF ABBREVIATIONS

IL-5	Interleukin 5
BECs	Bronchial epithelial cells
PBMCs	Peripheral blood mononuclear cells
PDCs	Peripheral dendritic cells
MAELABA	Mechanisms of Adverse Effects of Long-Acting Beta-Agonists in Asthma
IFN	Interferon
RV	Rhinovirus

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KEYWORDS

As listed in the glossary.

STUDY SUMMARY

TITLE Non-eosinophilic biological effects of interleukin 5 (IL-5) - role of IL-5 in suppressing anti-viral immunity in bronchial epithelial cells and dendritic cells in asthma

DESIGN Laboratory study

AIMS To take primary BECs and PBMCs/pDCs from n=5 people with asthma:

a. To determine their expression levels of *IL5RA* & *CSF2RB* mRNA and IL-5RA & CSF2RB protein by Western-blot and immunofluorescence as in[18], to determine whether they express receptor proteins at baseline
b. To incubate them with IL-5 protein (at 10-100ng/mL) or medium control, then after 24hrs, infect them with RV/medium control, as in[16] and then harvest cells, cell lysates and supernatants at 8, 24 & 48 hr time points post infection to determine whether IL-5 receptor expression levels are increased by infection/IL-5
c. To study IFN-β and IFN-λ and ISG (intracellular ISGs viperin, OAS, MxA, RIG-I and secreted chemokines CXCL9/10/11) induction by mRNA in lysates and protein assays (MSD) in supernatants. IFN-α will also be studied in PBMCs
d. As CSF2RB was reported to be expressed to a greater degree on BEC monolayers than on ciliated cells[18], we plan to study both submerged monolayer cultures and air-liquid interface differentiated ciliated cell cultures

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e. To use IL-3 and GMCSF, which bind the CSF2RB chain, but not the IL5RA chain, to determine whether signals are via one chain or the other
f. To measure virus load by PCR of viral RNA in cell lysates and virus release into supernatants by $TCID_{50}$ assay to determine whether IL-5 treatment augments virus replication

OUTCOME MEASURES These studies should determine whether IL-5 suppresses anti-viral immunity in BECs and in PBMCs/pDCs directly via IL-5 receptor signalling suppressing RV induction of IFNs. If the findings are positive this will provide a novel mechanism through which anti-IL-5 therapy is able to protect against exacerbations in severe eosinophilic asthma.
If positive, it would then justify further clinical studies to determine whether anti-IL-5 therapies suppress IL-5 concentrations in stable and exacerbated asthma *in vivo*, and whether they restore anti-viral immunity in BECs and PBMCs *in vivo* and thereby protect against exacerbations.

POPULATION People with asthma ≥18 years old

ELIGIBILITY If they meet the inclusion and exclusion criteria for the study Mechanisms of Adverse Effects of Long-Acting Beta-Agonists in Asthma (MAELABA)

DURATION 43 days

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1. INTRODUCTION

1.1. BACKGROUND

This study aims to investigate the role of IL-5 in suppressing anti-viral immune responses in bronchial epithelial cells (BECs) and in peripheral blood mononuclear cells (PBMCs) from 5 people with asthma, using BECs that are being collected at bronchoscopies for an ongoing study (the MAELABA study). An additional blood sample will also be collected at the same bronchoscopies (as the MAELABA participants are being cannulated for their bronchoscopies, this will require no additional venepuncture) to provide the PBMCs required for this study.

1.2. RATIONALE FOR CURRENT STUDY

Virus infections cause asthma exacerbations via IFN deficiency in asthma

Virus infections, particularly rhinoviruses (RVs), are the major cause of acute asthma exacerbations. We reported that type I (α/β) and III (λ) interferon (IFN) responses to virus infection are delayed and deficient in many people with asthma, using *ex vivo* infection of BECs and bronchoalveolar lavage (BAL) cells[1,2]. Others have shown the same in PBMCs and plasmacytoid dendritic cells (pDCs)[3-5]. Importantly, deficient IFN responses were predictive of subsequent virus-induced exacerbation severity in experimental RV infection studies in asthma[2,6] and in further experimental RV infection studies virus loads were increased in asthma compared to healthy controls[7,8]. Furthermore, anti-IgE therapy has been shown to restore deficient IFN responses in PBMCs[9] and pDCs[10] in asthma and to reduce exacerbations by >85% in those with the most robust restoration of their IFN responses[9]. Finally, although both studies missed their primary end points due to various design/execution issues, the clinical importance of IFN deficiency in asthma exacerbations is supported by two clinical trials showing clinical benefit (improved symptoms and lung function) with inhaled IFN-β in moderate asthma in subjects experiencing significant exacerbation of their asthma as a result of virus infections[11,12].

Mechanisms of anti-interleukin (IL)-5 therapy exacerbation reduction are poorly understood

Anti-IL-5 therapy markedly reduces asthma exacerbations in eosinophilic severe asthma[13]. However the mechanisms through which exacerbation reduction occurs in asthma are poorly understood. Co-culture of eosinophils with pDCs has been shown to inhibit RV-induction of IFN-α via soluble factor(s) in eosinophil supernatants[14]. Eosinophil-derived neurotoxin (EDN) and transforming growth factor (TGF)-β1 were both detectable in these eosinophil supernatants and both were shown to (rather weakly) suppress RV-induction of IFN-α from pDCs. Surprisingly other eosinophil products including major basic protein, eosinophil peroxidase, IL-4, IL-5 and IL-13 were not detectable in these eosinophil supernatants. A role for IL-5 in directly suppressing IFN induction in pDCs was not investigated further, however children with severe asthma being treated with mepolizumab (3 children) or benralizumab (1

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child) were collectively shown to have stronger IFN induction than 5 children with severe asthma not treated with anti-IL-5 therapy[14]. Co-culture of eosinophils with the BEAS-2B human BEC cell line has also been shown to inhibit RV-induction of IFN-β and IFN-λ and to increase RV replication in these cells, and TGF-β1 was again detectable in co-culture supernatants and was shown to suppress RV-induction of IFN-λ mRNA induction in BEAS-2B cells[15]. IL-5 was not investigated in these experiments and no studies using primary BECs have been reported. Thus anti-IL-5 therapy exacerbation reduction may in part result from reduced numbers of circulating and lung eosinophils leading to reduced levels of EDN and TGF-β1, resulting in less suppression of innate antiviral immune responses, however, this effect was not strong and a direct role for IL-5 in suppressing antiviral immunity has not been investigated.

IL-4 and IL-13 directly suppress IFN responses in BECs

It is well established that airway concentrations of the type (T) 2 cytokines IL-4, IL-5 and IL-13 are increased in asthma and we have shown that IL-4 and IL-13 directly suppress RV-induced IFN responses and increase RV replication in human BECs[16,17]. IL-5 has not been studied in this context.

BECs have functional IL-5 receptors and respond to IL-5

IL-5 has not been studied in this context principally because it was thought that the IL-5 receptor α-subunit (IL5RA) is expressed exclusively by eosinophils, basophils and some B-lymphocytes[18]. However recent studies report that IL5RA and to a lesser degree the IL-5 receptor ß-subunit (CSF2RB) were both expressed in ciliated BECs[18,19], and stimulation of BECs with IL-5 protein (10 ng/mL) has shown the receptor to be functional, with CSF2RB protein expression tending to increase after 15 minutes stimulation with IL-5 protein[18]. Function was also shown via increases in intracellular signaling pathway activation (phosphorylation of ERK and Akt) and via alterations in gene expression in response to exogenous IL-5 protein: after 24 hours of stimulation, 1403 genes displayed increased expression while 1851 genes had decreased expression (False discovery rate (FDR) < 0.01)[18]. Finally function was confirmed by inhibition of intracellular signaling pathway activation by a neutralizing anti-IL5RA antibody[18].

IL-5 levels are increased in asthma and are further increased during exacerbations

Virus infections are classically associated with T1 rather that T2 responses, however, we have shown that RV infection in asthma (but not healthy controls) induced nasal IL-4, IL-5 and IL-13 and bronchial IL-5 and eosinophilia[7]. We confirmed RV-induction of bronchial IL-5, and have also shown RV-induction of bronchial IL-13 and CRTH2 in asthma in a second separate study[20].

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IL-5 likely directly suppresses virus induced IFN responses in BECs

Given that the Th2 cytokines IL-4 and IL-13 directly supress virus induction of IFN-β and IFN-λ in BECs and thereby increase RV replication in BECs, and given that BECs express the IL-5 receptor and respond to IL-5, it is highly likely that IL-5 also directly suppresses IFN induction by RV infection in BECs.

IL-5 likely directly suppresses virus induced IFN responses in pDCs

BECs are the primary site of RV replication in the lung, but BECs do not produce detectable quantities of IFN-α in response to RV or influenza virus infection[21]. Robust restoration of PBMC IFN-α production in response to RV stimulation is associated with reduction of asthma exacerbation frequency of >85%[9]. pDCs are potent producers of IFN-α[5] and are likely to be the most important source of IFN-α in the human lung *in vivo*. pDCs express the high affinity IgE receptor, and ligation of this receptor profoundly suppresses virus induction of IFN-α[5]. I have been unable to find any reports indicating that pDCs express the IL-5 receptor or that pDCs respond to IL-5, however, eosinophils, basophils, B-lymphocytes and BECs all express the IL-5 receptor and respond to IL-5[18], and a recent report finds that blood and especially lung neutrophils consistently and abundantly expressed the IL-5 receptor and responded to IL-5, and further that expression was higher in the presence of respiratory viruses[22]. Thus it is highly likely that pDCs also express the IL-5 receptor and are able to respond to IL-5.

HYPOTHESES

- Increased IL-5 in stable eosinophilic asthma and asthma exacerbations suppresses anti-viral immunity in bronchial epithelial cells (BECs) and in peripheral blood mononuclear cells (PBMCs) directly via IL-5 receptor signalling suppressing RV induction of IFNs.
- Anti-IL-5 therapies suppress IL-5 concentrations in stable and exacerbated asthma *in vivo*, suppression of IL-5 *in vivo* restores deficient anti-viral immunity in BECs and PBMCs and thereby protects against asthma exacerbations.

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2. STUDY OBJECTIVES

To demonstrate *in vitro* that IL-5 suppresses anti-viral immunity in BECs and PBMCs/pDCs directly via IL-5 receptor signalling, thereby suppressing RV induction of IFNs.

3. STUDY DESIGN

- Sample collection for laboratory analysis
- 43 day study
- 5 subjects with mild asthma using as required salbutamol only

PBMCs/pDCs will be obtained from blood samples taken during cannulation for bronchoscopy for MAELABA. 100mls of blood will be taken solely for this study. There will be 4 bronchoscopies for MAELABA from which blood samples can be obtained until successful analysis of a sample. If cannulation is difficult, venepuncture will be used to acquire the blood samples.

BECs will be obtained from bronchial brushes taken during bronchoscopy for MAELABA. There are 4 bronchoscopies as part of the MAELABA study with ethical approval of 8 brushes being collected at each bronchoscopy. No more than 5 of the 8 brushes will be used for this study. Therefore a total of up to 20 brushes per participant can be collected for this study.

No further blood samples or bronchial brushes will be taken following completion of the study MAELABA.

3.1. STUDY OUTCOME MEASURES

Successful bronchial epithelial cell culture and PBMC isolation from 5 subjects for laboratory studies.

4. PARTICIPANT ENTRY

4.1. PRE-REGISTRATION EVALUATIONS

Patients will be recruited and screened as part of the MAELABA study. For this, participants will be recruited from hospital outpatient clinics, primary care / general practice (with the assistance of the NIHR Clinical Research Network), existing databases of asthma patients who have previously consented to being contacted about research and through advertisements (including through medical / charitable organisations). Social media platforms (including, but not limited to, Facebook) will be used to advertise the study. Those expressing an interest in participating in the MAELABA study will be asked to contact the study team. An initial discussion will take place over the telephone, during which the study team will explain what the MAELABA study involves as well as the additional samples that will be utilised for this study. During the discussion the study team will confirm if the potential

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participants satisfy some of the basic criteria for entry into the study and arrange an appointment to meet the study team in person at St Mary's Hospital. A copy of the Participant Information Sheet (PIS) for both studies will be sent to participants in advance of the in-person meeting, either by e-mail or post, so that they have time to read over the material prior to attending hospital.

At the initial in-person visit, an investigator will explain the studies to the participants and will discuss the PIS, inform them that participation is voluntary, give them time to consider whether they wish to participate and offer them the opportunity to ask any questions. If they wish to take more time to consider whether to participate, a follow up appointment will be made to discuss the study again. If they agree to take part in the study, they will be asked to sign a consent form for each study which will be countersigned by a member of the study team. Copies of the consent form will be provided to the patient and added to their research file.

Initial procedures undertaken at the screening visit for MAELABA will comprise: taking a medical history; performing a clinical examination; blood tests including full blood count and coagulation screen; clinic spirometry; chest x-ray; electrocardiogram (ECG); urine pregnancy test for female participants. If participants are successfully screened for MAELABA, they will be suitable for this study and no further screening will be required.

Once participants pass screening, they will have a single blood sample taken during cannulation for their bronchoscopy for the study MAELABA. 100mls of blood will be taken for this study which should take less than a minute, this is just under 6 tablespoons of blood. Further blood samples will be taken at further bronchoscopies only if the first sample is unsuitable for analysis. No further blood samples will be taken following the completion of the 4th bronchoscopy for MAELABA, this means participants will be included in this study for 43 days

4.2. WITHDRAWAL CRITERIA

Participants will be free to withdraw from the trial at any time without needing to provide a reason for doing so. Data pertaining to a participant that has withdrawn from the study will continue to be used in the study, unless the participant withdraws their consent for this.

Investigators will withdraw a participant from the study if:

- There is a clinical indication for withdrawal; this may include, but is not limited to, withdrawal of participants who require additional treatment such as extra inhaled therapies or corticosteroids to control their asthma symptoms during the course of the study (as the necessary treatment may mask the effect of the study intervention that they are receiving)
- The participant experiences a severe asthma exacerbation (As per American Thoracic Society (ATS) guidelines 32, the definition of a severe asthma exacerbation for clinical trials should include at least one of the following:

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(i) Use of systemic corticosteroids (tablets, suspension or injection), or an increase from a stable maintenance dose, for at least 3 days.
(ii) A hospitalisation or Emergency Department visit because of asthma, requiring systemic steroids

- The participant develops an intercurrent upper or lower respiratory tract infection at any time during the study
- The participant is unable to safely tolerate the interventions that are required as part of the study
- The participant demonstrates less than 80% adherence to either the salmeterol monotherapy protocol or salmeterol/fluticasone combination therapy protocol
- The participant becomes pregnant during the study
- The participant starts breastfeeding during the study
- The participant commences smoking during the study
- The participant commences vaping during the study
- The participant commences illicit drug use/abuse during the study
- The participant commences any medications that would impair their ability to safely continue with the study
- The participant commences any systemic or nasal topical steroids, inhaled
- corticosteroids (other than those that are part of the study protocol) or systemic immunosuppressants
- The participant withdraws consent for continuing with the study
- The participant experiences a serious or severe adverse event that prevents continued participation in the study
- The participant experiences significant deviation from the study protocol
- The Sponsor makes a reasonable and justified request for participant withdrawal
- The participant is lost to follow up
- If a participant experiences signs or symptoms suggestive of an asthma exacerbation at any time during the study, they will be asked to seek medical attention immediately and to notify the study team. Depending on the severity of the presentation, participants may seek medical attention via their GP, the Emergency Department or the study team. The need for inhaled short-acting beta-agonists, corticosteroids or antibiotics will be directed by the clinical signs and symptoms. The specific treatment plan for an exacerbation will therefore be a clinical decision based on the reviewing clinician's judgement.
- If a participant is withdrawn from the study, data obtained from the participant until that point in the study will be retained for analysis. The reasons for withdrawing any participants will be recorded and reported.

5. ADVERSE EVENTS

5.1. DEFINITIONS

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

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Serious Adverse Event (SAE): any untoward 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, or prolongation of existing inpatients' 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.

5.2. 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.

5.3.1 Non serious AEs

All such events, whether expected or not, should be recorded- it should be specified if only some non-serious AEs will be recorded, any reporting should be consistent with the purpose of the trial end points.

5.3.2 Serious AEs

An SAE form should be completed and emailed to the Chief Investigator within 24 hours. However, relapse and death due to pre-existing conditions, and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the Haydock Research Ethics Commitee where in the opinion of the Chief Investigator, the event was:

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

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all related and unexpected SAEs.

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

Contact details for reporting SAEs
RGIT@imperial.ac.uk
CI email (and contact details below)
Attention Professor Sebastian Johnston
Please send SAE forms to: s.johnston@imperial.ac.uk

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Tel: +44 20 7594 3764 (Mon to Fri 09.00 – 17.00)

6. ASSESSMENT AND FOLLOW-UP

There will be no follow-up.

The study will end when 5 samples have been successfully analysed.

We cannot guarantee the study will help participants but the information we get may help improve the treatment of people with asthma. Patients will be informed if any results are published.

During the study incidental findings may be discovered that are of concern or importance to the participants health. The participant will be verbally informed of this if so.

7. STATISTICS AND DATA ANALYSIS

This is a laboratory study analysing responses to IL-5 in BECs and PBMCs. Standard parametric/non-parametric analyses will be performed comparing responses of cells stimulated with IL-5, with media control.

Previous research by Wark et al found a four-fold increase in IFN-β mRNA expression in 10 healthy control BECs 8 hours following RV-16 infection compared to no response in 14 asthma BECs following 8 hours of RV-16 infection. There was also a two-fold higher production of IFN-β protein in RV-infected BECs of 10 healthy compared to 14 asthmatic participants with moderately severe asthma.[1] Based on this, 5 successfully cultured samples will be required for data analysis.

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study.

8. REGULATORY ISSUES

8.1. ETHICS APPROVAL

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

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8.2. CONSENT

(If study does not involve consent, this section is not relevant)
Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the 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.

8.3. CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

Data will be pseudonymised. The research team and GlaxoSmithKline will have access to such data.

8.4. INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study/ Imperial College Healthcare NHS Trust holds standard NHS Hospital Indemnity and insurance cover with NHS Resolution for NHS Trusts in England, which apply to this study (delete as applicable)

8.5. SPONSOR

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

8.6. FUNDING

GlaxoSmithKline are funding this study. No participant payments will be made.

8.7. AUDITS

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

9. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through Dr Eva Fiorenzo.

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10. PUBLICATION POLICY

The study results will be submitted for scientific publication to a scientific journal once laboratory analysis is completed.

11.1 COLLECTION AND STORAGE OF DATA

Each participant will be assigned a unique participant identification code in place of their personal identifiers during consent (e.g. name and date of birth) which will be used to record all research data gathered – this will pseudonymise the research data. Assigning a unique participant identification code to research data gathered from each participant does not count as anonymising one's personal data (personal identifiers, research data, medical records or any other data that are regarded personal) according to the General Data Protection Regulation (GDPR), as the data can be related back to each participant given access to the appropriate information/key. Such information/key for the unique participant identification code will be stored electronically in the on-site computers which will only be accessible by clinical staff of the research team, and in a manual (paper) records stored securely in a locked room in the Imperial Clinical Respiratory Research Unit, St Mary's Hospital. Only the research staff of Imperial Clinical Respiratory Research Unit have access to this room via a manual keypad door lock. The code for the lock is known only to research staff in the Imperial College Respiratory Research Unit. All members of MAELABA's research team and the research staff of Imperial Clinical Respiratory Research Unit will follow the confidentiality procedures of Imperial College Healthcare NHS Trust and Imperial College London, whichever is applicable. The NHS Code of Confidentiality will be followed. Data will be treated in accordance with the Data Protection Act and GDPR.

Pseudonymised research data will be analysed by the research study team in the National Heart and Lung Institute, Imperial College London or by GlaxoSmithKline. Statistical analysis of pseudonymised research data will be undertaken by a third-party statistician contracted with Imperial College London or GlaxoSmithKline. All data, pseudonymised or otherwise, that are recorded on paper (CRF or others) will be stored in manual files in the above mentioned locked room in the Imperial Clinical Respiratory Research Unit, St Mary's Hospital.

All data (except medical records), pseudonymised or otherwise, stored in on-site computers will be located in files that are locked with access only granted to researchers within MAELABA's research team. Data stored in participants' medical records will be stored in the electronic medical records system of Imperial College Healthcare NHS Trust, which staff members of Imperial College Healthcare NHS Trust as well as MAELABA research team's clinical staff will be able to access. Medical records that are relevant to the study will be created, accessed and/or updated primarily by clinical staff of MAELABA's research team. However, other healthcare staff members who become involved in the care of the participants may

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access the data obtained from their medical records – participants' consent will be gained where appropriate. The information viewed in participants' medical records will be treated in strict confidence by the research team and other relevant staff, and confidentiality will be maintained in accordance with local and national requirements mentioned above.

Where research data from participants' medical records form source data such as x-ray and blood results, these will be printed out. All personal identifiers such as name, date of birth, address and hospital number will be replaced with the unique participant identification code on the printouts. These pseudonymised printouts will be added to the paper CRFs.

The use of personal identifiers such as participants' addresses, postcodes, faxes, emails or telephone numbers will be carried out with sensitivity and will only be used by the research team to contact participants while adhering to the local and national requirements mentioned above.

Imperial College London will keep identifiable information about participants from this study for 10 years after the study has finished. Institutions or organisations might be involved in sample processing in the future but will only have access to pseudonymous data meaning that this data will not be able to be used to identify participants personally. This is as per the policy of Imperial College London for retaining identifiable information about participants in research studies.

As per local guidance from Imperial College London, research data that are obtained about participants during this study will be retained for 10 years from the time that the study is completed. Clinical samples that are collected from participants during this study may be retained indefinitely. Participants will be informed of this in the Patient Information Sheet. Participants will be asked to consent to Imperial College London retaining and storing their clinical samples and any relevant clinical information. All samples will be stored in a laboratory at Imperial College London for up to 10 years, these laboratories will be the responsibility of Professor Sebastian Johnston. During or after 10 years the samples may be transferred to the Imperial College tissue bank. Samples may also be destroyed. Members of the research study team, including the Chief Investigator, will have access to the research data after the study has ended.

Imperial College London is the sponsor for this study based in the United Kingdom. Imperial College London will be using information from participants and participants' medical records in order to undertake this study and will act as the data controller for this study. This means that Imperial College London is responsible for looking after participants' information and using it properly. Imperial College London will keep identifiable information about participants that is obtained during this research study. The consent form that participants sign agreeing to participate in this study will be retained for 10 years. Research data that are obtained about participants during this study will be retained for 10 years from the time that the study is completed. Clinical

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samples that are collected from participants during this study may be retained indefinitely.

If the study team think that the participant is at serious risk during the study, confidentiality will need to be broken in the interests of participant safety. Where possible, the participant will be informed that confidentiality needs to be breached. When breaking confidentiality, only the minimum necessary number of persons will be informed and only relevant details will be shared.

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