1. Protocol details

1.1 PROTOCOL TITLE:

Beyond the **E**osinophil: **U**nderstanding the impact of eosinophil depletion on **T**2 **I**nflammation

Short Title: BEUTI

Design: Prospective open label phase IV study (single centre)

Intervention: Benralizumab

Target Disease: Severe Asthma

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1.3 Protocol details

BEUTI STUDY Jackson KCL

Version number: 1.0
Final/draft: Final
Date: 15/12/2022
IRAS: 310865

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2 Signature Page

The Chief Investigator and the R&D (sponsor office) have discussed this protocol. The investigators agree to perform the investigations and to abide by this protocol

The investigator agrees to conduct the trial in compliance with the approved protocol, EU GCP, the UK Data Protection Act (1998), the Trust Information Governance Policy (or other local equivalent), the Research Governance Framework (2005' 2nd Edition; as amended), the Sponsor's SOPs, and other regulatory requirements as amended.

15/12/2022

Chief investigator

Professor David Jackson

Signature Date

Sponsor Representative

R&D to Add
GSTFT

Signature Date

This Protocol template is intended for use with UK sites only.

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3 List of Abbreviations and Definitions

AE	Adverse Event
AR	Adverse Reaction
ASR	Annual Safety Report
CA	Competent Authority
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
DMC	Data Monitoring Committee
EC	European Commission
GAfREC	Governance Arrangements for NHS Research Ethics Committees
ICF	Informed Consent Form
MA	Marketing Authorisation
MS	Member State
Main REC	Main Research Ethics Committee
NHS R&D	National Health Service Research & Development
PI	Principle Investigator
QA	Quality Assurance
QC	Quality Control
Participant	An individual who takes part in a clinical trial
REC	Research Ethics Committee
SAE	Serious Adverse Event
SDV	Source Document Verification
SOP	Standard Operating Procedure
SSA	Site Specific Assessment
TMG	Trial Management Group
TSC	Trial Steering Committee

4 Summary/Synopsis

Title	**B**eyond the **E**osinophil: **U**nderstanding the impact of eosinophil depletion on **T**2 **I**nflammation
Protocol Short Title/Acronym	**BEUTI**
Protocol Version number and Date	1.0 7th October, 2022
Study Phase if not mentioned in title	Prospective open label phase IV study (single centre)
IRAS Number	310865
REC Reference	
Sponsor Reference	
Study Duration	24 months
Sponsor name	King's College London
Chief Investigator	Professor David Jackson
Funder	Astra Zeneca
Medical condition or disease under investigation	Severe Asthma
Purpose of research	Benralizumab is a relatively new treatment that is approved by NICE for patients with severe asthma with ongoing eosinophilic inflammation that remains poorly controlled despite high dose inhaled corticosteroid medication. Benralizumab targets a receptor on the surface of eosinophils called IL-5R leading to the rapid death of these cells and consequently a reduction in airways inflammation. In clinical trials, benralizumab has been shown to reduce both symptoms and the number of asthma attacks suffered by those with severe eosinophilic asthma. However, it remains unclear whether this clinical efficacy relates purely to the removal of the eosinophil, or additionally to the impact of this on other parts of the immune system. The BEUTI study will examine if benralizumab leads to a reduction in T2 mediators and/or activation in airway cells in patients with severe eosinophilic asthma. The aim is to take samples of cells from the airways during a bronchoscopy (a camera test looking into the lungs) before starting benralizumab and after 12 weeks of treatment. These investigations will allow us to better understand how benralizumab affects the cells within the airways and the pathways involved.
Primary objective	To determine if benralizumab leads to a reduction in T2 mediators and/or activation in airway cells in patients with severe eosinophilic asthma.
Secondary objective (s)	1.Determine any change in T2-related signalling pathways and cell populations in bronchial biopsies (including those relating to IL-4, IL-5, IL-13, TSLP, IL-25 or IL-33) in patients with severe eosinophilic asthma before and after 12 weeks of benralizumab using spatial transcriptomics.

	2. Determine any in vitro changes in bronchial epithelial antiviral responses to rhinovirus-16 and SARS-CoV-2 before and after 12 weeks of benralizumab. 3. Determine any transcriptomic changes in genes relating to airway remodelling before and after 12 weeks of benralizumab. 4. Determine the effect of benralizumab on small airway dysfunction using impulse oscillometry and MRI to measure the change in homogeneity of airways ventilation
Number of Subjects/Patients	12
Study Type	Observational. Prospective open label phase IV study (single centre)
Endpoints	1. Change in the number / proportion of T2 related cellular populations in BAL including ILC2, Th2, alveolar macrophages, eosinophils and basophils pre- and post benralizumab 2. Change in cellular populations and gene expression of T2-related signalling pathways in bronchial biopsies pre- and post benralizumab 3. Change in type 1 & 3 interferon production and viral replication following infection of cultured BECs and alveolar macrophages with RV-16 and examine the response of cells to SARS-CoV-2 collected pre- and post- benralizumab. 4. Change in cellular populations and gene expression of remodelling-related pathways in bronchial biopsies pre- and post benralizumab 5. Change in airway oscillometry measures and MRI measures of ventilation pre- and post benralizumab
Main Inclusion Criteria	1. Patients aged 18 and over with a diagnosis of severe eosinophilic asthma for at least the last 6 months 2. Eligible for benralizumab based on NICE criteria. 3. Poorly-controlled (ACQ-6 >1.5) 4. FeNO ≥50ppb at screening despite high dose inhaled corticosteroids (at least 1000mcg BDP equivalent) +/- maintenance prednisolone. 5. Adult-onset (18+) asthma in a minimum of 50% of the study subjects
Statistical Methodology and Analysis	The primary comparisons are pre- and post-benralizumab therapy. Descriptive statistics will be used for demographic and clinical characteristics of the study subjects. Clinical outcome measures will be compared using either parametric paired t-tests or non-parametric U-tests for continuous variables, and fisher tests for the analysis of proportions. All experimental analyses will be paired, considering patient-specific changes. All analyses involving the measurement and comparison of multiple variables (e.g. RNA-seq) will include a multiple test correction, such as false discovery rate / Bonferroni.
Human Tissue Samples (if applicable)	All human tissue samples will be processed and analysed by the main team and collaborators of co-investigator Dr Rocio Martinez-Nunez at King's College London. These samples will include blood, cells and tissues from the airways.
Data collected/storage (if applicable)	We will be using a combination of paper and electronic records. Pseudonymised data will be collected by the direct care team and KCL research team. All source documentation will be filed both physically

	and electronically in a secure location within GSTT and KCL sites and on password protected computers managed by GSTFT and KCL. The paper records will be filed in the study-specific patient folder and will also be scanned and filed electronically.

5 Introduction

Over 5 million people in the UK have asthma of varying severity. Despite maximum treatment (with high dose inhaled corticosteroids plus a second controller and/or systemic corticosteroids), ~5-10% of the patients remain symptomatic. These severe asthma patients have a high blood eosinophil count, frequent exacerbations and poor prognosis. Severe asthma remains an unmet need with significant impact on patients, society and healthcare.

Eosinophils are a type of white blood cell that are linked to allergy and inflammation and are raised in people with severe asthma. Increased levels of eosinophils can cause inflammation in the lungs, increasing the risk of asthma attacks. The standard treatment for asthma involves taking inhaled glucocorticosteroid medication which primarily work by suppressing eosinophilic inflammation in the lungs. With this treatment, the majority of people with asthma have no or very few asthma symptoms but those with severe asthma have ongoing eosinophilic inflammation despite this treatment and remain highly symptomatic and continue to experience frequent asthma attacks.

Commonly in severe asthma, there is bronchial epithelial damage (the junctions holding cells together on the airway lining is damaged), there is an increase in cells that make mucus, an increase in the thickness of the airway wall and an increase in immune cells. Granules produced by eosinophils (a type of white blood cell), can add to this damage and in particular increase the amount of mucus in the airways, which can block airways. Some studies have shown that increases in eosinophils in the airways can affect the immune responses to viruses and bacteria.

Benralizumab is a relatively new treatment that is approved by NICE for patients with severe asthma and ongoing eosinophilic inflammation that remains poorly controlled despite high dose inhaled corticosteroid medication. Benralizumab targets a receptor on the surface of eosinophils called IL-5R leading to the rapid death of these cells and consequently a reduction in airways inflammation. In clinical trials, benralizumab has been shown to reduce both symptoms and the number of asthma attacks suffered by those with severe eosinophilic asthma. However, it remains unclear whether this clinical efficacy relates purely to the removal of the eosinophil, or additionally to the impact of this on other parts of the immune system.

The clinical effectiveness of benralizumab evident across phase 3 and real world studies has reaffirmed the central role that the eosinophil plays in disease control and exacerbation pathogenesis in patients with severe asthma. However, whilst it is generally believed that this fundamentally reflects the removal of eosinophils and their toxic granules and mediators from the airway, the full impact of benralizumab on T2 immunity may be much broader. Firstly, benralizumab-treated patients stop exacerbating when they encounter respiratory viruses such as rhinovirus, suggesting that the deficient anti-viral immune responses present in many uncontrolled T2-high asthmatics are restored upon benralizumab. Secondly, our centres' finding that patients continue to remain well-controlled despite significantly reducing their inhaled corticosteroid (ICS) use following initiation of benralizumab suggests that the other elements of T2 inflammation (including IL-13 driven pathways) are either unimportant or suppressed (d'Ancona et al Allergy 2021). Thirdly our observation that benralizumab is very effective in FeNO-high patients (indicating increased IL-13-driven activation) and that treatment significantly reduces FeNO levels suggests that benralizumab directly or indirectly suppresses IL-13 pathways (Hearn et al JACI-IP 2021). Taken together, there is an increasing body of data supporting the notion that benralizumab has far-reaching anti-T2 effects that underpin its excellent efficacy in clinical practise. Through the use of novel techniques including spatial transcriptomics of bronchial biopsies this proposal aims to define and describe these effects.

The BEUTI study will examine how benralizumab affects the structure and function of the airway cells in people with severe eosinophilic asthma and how the immune function of these cells changes with treatment. The aim is to take samples of cells from the airways during a bronchoscopy (a camera test looking into the lungs) before starting benralizumab and after 12 weeks of treatment. These investigations will allow us to better understand how benralizumab affects the cells within the airways.

All potential participants with severe eosinophilic asthma (according to standard NICE criteria for biologic eligibility under the NHS) who have already been approved for this treatment in the severe asthma multidisciplinary meeting at Guy's & St Thomas' NHS Trust will be approached for this study. Agreement to take part in this study, will not significantly delay treatment initiation for these patients by more than a few weeks. Potential participants will be provided with further written information about the study in the form of the patient information sheet (PIS). They will be given ample time to read this and there will be a contact telephone number in the PIS should they wish to contact the research team for further information.

Hypothesis:
The depletion of eosinophils by benralizumab leads to additional inhibitory effects on T2 inflammation which collectively underpins the improvements in multiple clinical domains and reduction in T2 biomarkers observed in real world cohort studies.

The important research questions we will aim to answer:
i) What is the effect of benralizumab on T2 signalling. Can benralizumab modulate T2-signalling and/or gene expression in addition to triggering eosinophil and basophil depletion?
ii) What additional cell types (e.g. ILC2, alveolar macrophages, mast cells, bronchial epithelial cells, airway smooth muscle) are regulated by benralizumab?
iii) Does the marked reduction in exacerbations observed with benralizumab partly reflect restoration of the deficient anti-viral immune responses seen in patients with severe asthma?

6 Trial objectives and purpose

Primary Objective:
To determine if benralizumab leads to a reduction in T2 mediators and/or activation in airway cells in patients with severe eosinophilic asthma.
The effect of benralizumab on the airway inflammatory (BAL) cell profile of study subjects will be assessed using CyTOF. The CyTOF panel markers are: CD3, CD4, CD8, CD11c, CD14, CD16, CD19, CD20, CD25, CD27, CD28, CD38, CD45RA, CD45RO, CD56, CD57, CD66b, CD123, CD127, CD161, CD294, CCR4, CC46, CCR7, CXCR3, CXCR5, HLA-DR, IgD, TCRγδ, IL-33, IL-5RA/CD125, IL-6, IL-4, IL-5, IL-13.
These allow the identification of: CD4+ cells (Naïve, memory, and terminal effector CD4+ T cell, Th1, Th2, Th17, T regs), CD3+ CD4- (MAIT/NKT, γδ T Cell), CD3- CD8+ (Naïve and memory CD8+ T cell), CD3- Lymphocytes (NK cells - Early, late), B cells (naïve, memory, plasmablasts), monocytes, dendritic cells (myeloid, plasmacytoid), granulocytes (eosinophils, neutrophils, basophils), and ILCs.

Secondary Objectives
1. Determine any change in T2-related signalling pathways and cell populations in bronchial biopsies (including those relating to IL-4, IL-5, IL-13, TSLP, IL-25 or IL-33) in patients with severe eosinophilic asthma before and after 12 weeks of benralizumab using spatial transcriptomics

There has been recent progress in high-throughput techniques for single-cell genomic and transcriptomic analyses which has specifically led to spatially resolved transcriptomics, or spatial transcriptomics which consists of integrating the information gathered from the study of individual cells within a tissue as well as their cellular localisation within the same tissue (histology) together with the throughput of RNA-sequencing. This has opened up new possibilities to classify these into cell types, and characterize variations in their molecular profiles as a function of therapy in the case of our study but also in the context of genetics, the microbiome environment, cell–cell interactions, developmental processes, aging, and asthma disease.

Single cell sequencing methods allow for establishing the profile of individual cells leading to the discovery of novel cell populations and activation status. spatial transcriptomics has not been applied in patients with asthma as it has only recently been developed but the technology has been applied to post-mortem lung tissues from those who have died as a result of COVID-19 to better understand if smoking status or other lung diseases like COPD played a role in COVID severity. This method has never been used to study asthma or in understanding the full mechanism of action of benralizumab. For example, single cell RNA-seq has shown that IL-13 promotes remodelling in distinct populations of airway epithelial cells (Jackson et al. Cell Rep 2020). Spatial transcriptomics will allow identification of cellular populations that are modulated by benralizumab treatment - similar to single cell RNA-sequencing - the potential to reveal novel target populations of benralizumab treatment (including their spatial position), and the interactions between them in their tissue of origin. This is important because different milieu, provided by different surrounding cells, may differentially modulate one same cell type. It will also allow for analysing traditionally difficult to study cells such as mast cells and eosinophils present in the tissue, in their location of origin with their in situ interactions. We will validate our observations employing immunofluorescence to confirm the expression of novel markers/mediators uncovered. Our KCL Genomics Core has the technology to implement these experiments (Visium 10x Genomics). Polyadenylated RNA from tissue sections is captured in situ on arrays of spatially barcoded DNA probes, providing information of not only gene expression levels but also tissue localization. Two sequential cryosections from bronchial biopsies from each patient will be performed pre- and post-benralizumab treatment.

2. <u>Determine any in vitro changes in bronchial epithelial antiviral responses to rhinovirus-16 and SARS-CoV-2 before and after 12 weeks of benralizumab.</u>

Isolated primary bronchial epithelial cells from bronchial brushings will be cultured for a maximum of 4 passages and infected with rhinovirus 16 and SARS-CoV-2. We will measure transcriptional mRNA changes employing RNA-seq on uninfected vs infected bronchial epithelial cells, before and after 12 weeks of Benralizumab. Unstimulated cells will inform about baseline differences triggered by Benralizumab; rhinovirus- and SARS-CoV-2-infected cells will inform about changes triggered by Benralizumab that modulate the anti-viral immune response. We will employ these two viruses as they are both positive strand RNA viruses but elicit very different anti-viral responses. Moreover, rhinovirus is the major cause of asthma exacerbations while SARS-CoV-2 does not trigger as many, with underlying mechanisms still poorly understood. We will also assess bronchial epithelial integrity by assessing the transepithelial resistance of air-liquid interphase cultures or barrier permeability assays. In addition, we will be able to investigate the effects of different variants of SARS-CoV-2 that may arise, in addition to the RNA-seq cell data with SARS-CoV-2 omicron. We will aim to employ the most relevant variant of omicron for our RNA-seq experiments.

The Co-Investigator on this proposal Dr Martinez-Nunez led the KCL diagnostics response for COVID-19, including the diagnostics lab, and has already performed BEC infection with SARS-CoV-2 in our

institutions' Cat-3 lab. SARS-CoV-2 infections are routinely done in our School. Her lab also has expertise in rhinovirus infections and RNA-sequencing.

The RNA-seq datasets will be analysed to determine genes modulated by benralizumab, as well as enrichment of biological pathways employing Ingenuity Pathway Analysis (Qiagen) for which the College holds a licence. This will enable exploring other pathways modulated by Benralizumab in bronchial epithelial cells, such as those related to epithelial integrity (e.g. occludins, integrins), immune profile (alarmins) both at baseline and upon infection.

3. Determine any transcriptomic changes in genes relating to airway remodelling before and after 12 weeks of benralizumab

We will integrate the spatial-associated RNA-seq datasets from bronchial biopsies (spatial transcriptomics, secondary objective 1) with bulk RNA-seq in BECs (secondary objective 2) and investigate changes in gene expression that relate to airways remodelling (e.g. genes encoding for structural proteins, integrins, extracellular matrix proteins etc.). We will focus on analysing non-stimulated BECs from secondary objective 2, pre- and post- benralizumab, which will show the changes in epithelium responses upon benralizumab treatment.

We will further analyse extracellular matrix (ECM) deposition and degradation in biopsies using antibodies directed against ECM proteins as well as chemical trichrome staining (Masson's) to visualise connective tissues (collagen), cells and smooth muscle. We will also establish transepithelial integrity/permeability assays employing different methods (e.g. dextran permeability through the epithelial barrier).

Where feasible, we will dissect larger bronchial biopsies and attempt to isolate other structural cells (smooth muscle and fibroblasts) for functional assays associated with inflammation and bronchoconstriction and examine: cell contractility, proliferation, migration, protein expression and cytokine production.

4. Determine the effect of benralizumab on small airway dysfunction using impulse oscillometry and MRI to measure the change in homogeneity of airways ventilation

Impulse oscillometry is a far more accurate measure of small airways airflow obstruction than standard spirometry, whilst ventilation assessed by inhaled gas MRI has been recently shown to be sensitive to anti-T2 therapies and can be used to objectively evaluate the functional consequence of clearing eosinophils from the airway lumen (Svenningsen et al. CHEST Oct 2020).

7 Study design & Flowchart

7.1 Study Design

This is a prospective open label phase IV observational study

Primary Endpoint:

Change in the number / proportion of T2 related cellular populations in BAL including ILC2, Th2, alveolar macrophages, eosinophils and basophils pre- and post benralizumab (12 weeks).

Secondary Endpoints:

1. Change in cellular populations and gene expression of T2-related signalling pathways in bronchial biopsies pre- and post benralizumab
2. Change in type 1 & 3 interferon production and viral replication following infection of cultured BECs and alveolar macrophages with RV-16 and examine the response of cells to SARS-CoV-2 collected pre- and post- benralizumab.
3. Change in cellular populations and gene expression of remodelling-related pathways in bronchial biopsies pre- and post benralizumab
4. Change in airway oscillometry measures and MRI measures of ventilation pre- and post benralizumab.

Each recruited participant will be in the study for 3 months and will attend for 8 visits including screening and informed consent. Visits will include blood, breathing tests and bronchoscopy. Participants will undergo bronchoscopy twice in a 12 week period (pre- and 12 weeks post benralizumab treatment). Processing of biological samples and data from clinical measures will be mined throughout the study period of 24 months and also when patients revert to ongoing NHS clinical care that is standard for those on benralizumab treatment for their asthma.

Gantt Chart: The total study duration is 24 months.

Months	3	6	9	12	15	18	21	24
Ethics application and approval								
Patient recruitment, bronchoscopies and sample collection and laboratory procedures								
BEC and mac culture for anti-viral immunity								
BEC and Br biopsy Spatial transcriptomics								
Protein analysis								
Data analysis								
Manuscript(s) preparation								

Treatment Regimen:
Benralizumab will be commenced according to UK NICE severe asthma guidance with 30mg sc dosing every 4 weeks for first 3 doses, followed by every 8 weeks.

Design and patient flow:
Visit 1: Consent, Enrollment and schedule of visit dates
Visit 2: Day 1 Covid-19 test
Visit 3: Day 2 Bronchoscopy
Visit 4: Day 10 Subject receives benralizumab dose 1 (30mg subcutaneous injection)
Visit 5: Day 38 Subject receives benralizumab dose 2 (30mg subcutaneous injection)
Visit 6: Day 66 Subject receives benralizumab dose 3 (30mg subcutaneous injection)
Visit 7: Day 83 Covid-19 test
Visit 8: Day 84 Bronchoscopy

At visit 1, all eligible participants will give informed consent. They will then have baseline characteristics recorded, including information regarding their asthma history and symptoms, asthma control score and quality of life questionaire (ACQ-6/mAQLQ) to assess lung function, general medical history and all current medications. Breathing tests will be performed (spirometry, fractional exhaled nitric oxide (FeNO)). A blood sample will be taken and participants will also be asked for a urine sample (pregnancy test for female participants only).
At this visit, the bronchoscopy will be discussed in more detail, and a separate bronchoscopy consent form signed. A convenient date will be arranged for both the bronchoscopy and Covid-19 test.

Visit 2: This visit is one day before the bronchoscopy and participants will have a COVID-19 test and consent for bronchoscopy.

Visit 3 and visit 8: Participants will undergo a bronchoscopy, breathing tests, have a blood test, and then the bronchoscopy with samples taken of airway cells.

Visit 4/5/6: Participants will receive a dose of benralizumab at monthly intervals during the study and will subsequently continue to receive benralizumab every 8 weeks as per standard NHS guidelines. Patients will be followed up in the NHS severe asthma clinic as their clinical condition requires. Information regarding their clinical condition will be collected from their clinical notes over the duration of the study.

Visit 7: This visit is one day before the 2nd bronchoscopy and participants will have a COVID-19 test and consent for bronchoscopy.

At all visits unless otherwise stated, the following information and samples will be collected:
Information regarding their asthma symptoms and adverse events
Asthma control score and quality of life questionaire (ACQ-6/mAQLQ)
Breathing tests will be performed (spirometry, fractional exhaled nitric oxide (FeNO)).
A blood and urine sample (Visit 1 and 4 only) will be taken.

7.2 Flowchart

	Screening Visit	**Study visits**						
Visit Number	1	2	3	4	5	6	7	8
Day		1 COVID PCR	2 Bronch 1	10 benra dose 1	38 benra dose 2	66 benra dose 3	93 COVID PCR	94 Bronch 2
Full medical history, confirmation of SEA, medication review	X							
Physical examination	X	X	X	X	X	X	X	X
Current/recent medications, side effects	X	X	X	X	X	X	X	X
Nasal and/or throat swab for COVID-19 testing (PCR)		X					X	
Benralizumab				X	X	X		
Bronchoscopy			X					X

	Screening Visit	Study visits						
Visit Number	1	2	3	4	5	6	7	8
Day		1 COVID PCR	2 Bronch 1	10 benra dose 1	38 benra dose 2	66 benra dose 3	93 COVID PCR	94 Bronch 2
FeNO	X		X	X	X	X		X
Spirometry	X		X	X	X	X		X
Oscillometry	X		X	X	X	X		X
ACQ-6	X		X	X	X	X	X	X
mAQLQ	X		X	X	X	X	X	X
Blood	X		X	X	X	X	X	X
Urine pregnancy test (females only)	X			X				
Collection of adverse events (AE)		X	X	X	X	X	X	X

8 Subject selection

Population:
Adult patients with poorly-controlled severe eosinophilic asthma referred to Guy's Severe Asthma Centre, Guy's & St Thomas' NHS Trust, who meet NICE criteria to commence biologic therapy with benralizumab. Following review of eligibility criteria potential participants will be provided with further written information about the study in the form of the Patient Information Sheet (PIS). They will be given ample time to read this and there will be a contact telephone number in the PIS should they wish to contact the research team for further information.

This is a single centre study.

Each recruited participant will be in the study for 3 months and will attend for 8 visits including screening and informed consent. Visits will include blood, breathing tests and bronchoscopy. Participants will undergo bronchoscopy twice in a 12 week period (pre- and 12 weeks post benralizumab treatment). Processing of biological samples and data from clinical measures will be mined throughout the study period of 24 months and also when patients revert to ongoing NHS clinical care that is standard for those on benralizumab treatment for their asthma.

Retention:
The PIS will explain that the study involves 2 bronchoscopies and it is hoped that patients do not drop out of the study prior to the second bronchoscopy. We will work with our Patient Research Ambassadors to support retention. If patients decide to drop out they will continue to receive benralizumab as per the plan from the multidisciplinary team (MDT) and patients will be replaced until the desired paired sample numbers are reached. The clinical investigators are experienced in recruiting patients as well as performing bronchoscopies on patients with severe asthma and a standardised sedation and periprocedural protocol will be used to maintain consistency in sampling and patient experience.
Patients can withdraw from the study at any time. This will not affect their care or treatment within Guy's Severe Asthma Service and in particular will not affect ongoing delivery of benralizumab.

Sample Size Justification:
Based on the observed 85% responder rate to benralizumab in our centre (Kavanagh et al. CHEST 2021), statistically significant falls in FeNO observed in FeNO-high patients with benralizumab, and successful published transcriptomics studies in asthma of a similar number, we believe 12 subjects should yield at least 10 clinical responders to benralizumab. Twelve is also an achievable amount from a practical perspective to allow timely completion of this study in under 18 months from first subject in (FSI).

8.1 Subject inclusion criteria

1. Patients aged 18 and over with a diagnosis of severe eosinophilic asthma for at least the last 6 months
2. Eligible for benralizumab based on NICE criteria
3. Poorly-controlled (ACQ-6 >1.5)
4. FeNO ≥50ppb at screening despite high dose inhaled corticosteroids (at least 1000mcg BDP equivalent) +/- maintenance prednisolone
5. Adult-onset (18+) asthma in a minimum of 50% of the study subjects

8.2 Subject exclusion criteria

1. Other severe eosinophilic lung disease including EGPA, chronic eosinophilic pneumonia and ABPA
2. Maintenance daily oral corticosteroids (prednisolone)
3. Severe bronchiectasis on CT causing daily sputum production
4. Inability to give written informed consent
5. Current smoking or >20 pack year smoking history
6. Resting oxygen saturations <94% on air
7. Any severe cardiac or other non-asthma related co-morbidity that would make bronchoscopy and/or sedation high-risk
8. Symptoms suggestive of a respiratory viral / bacterial infection within the last 3 weeks
9. Acute exacerbations of asthma requiring high dose prednisolone within the last 3 weeks
10. A change in dose of maintenance inhaled and/or oral corticosteroid dose within the last 3 weeks
11. Positive strongyloides serology following screening
12. Pregnancy or lactation
13. Hypersensitivity to benralizumab or any of the excipients

9 Study procedures

9.1 Subject recruitment

Members of the patient's existing direct care team will have access to patient records for discussion at the Guy's and St Thomas' hospital NHS Trust (GSTT) severe asthma clinic meetings. Potential participants will be identified at site by the direct care team using clinic lists. Adult patients with poorly-controlled severe eosinophilic asthma referred to Guy's Severe Asthma Centre clinic at Guy's & St Thomas' NHS Trust, who meet NICE criteria to commence biologic therapy with benralizumab will be approached by the direct care team.

Following review of eligibility criteria potential participants will be provided with further written information about the study in the form of the Patient Information Sheet (PIS). They will be sent the PIS at least a week before their clinic visit and given as much time as they need read the PIS and to ask questions. The PIS and the Informed Consent Form (ICF) will be explained in clear, simple language. They will be provided with a contact telephone number should they wish to contact the research team for further information. The patients will have as much time as they require to decide whether to take part and will be given the opportunity to go home and discuss with family/friends if they want to.

The bronchoscopy procedure will also be discussed in detail prior to obtaining consent.
The asthma direct care team at GSTFT are integrated with the research team and these activities will be completed by both teams.

Informed Consent:

The process for obtaining participant informed consent will be in accordance with the REC guidance, and GCP and other regulatory requirements that might be introduced.
The chief investigator and/or the direct care team will conduct the informed consent process by explaining to the participant the nature of the study; its purpose; the procedures involved; the expected duration; the potential risks and benefits involved; and the fact that participation is completely voluntary and consent may be withdrawn at any time with no consequences for their subsequent medical care.
Participants will also be asked to review and sign a standard bronchoscopy consent form currently used by the respiratory department as per trust policy

The CI/PI/delegate and the participant or other legally authorised representative 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.

Participation in the study is voluntary and recruited patients will not be paid.

Participants will also be made aware of and consent to the fact that their GP will be informed of their participation. Each participant will authorise in writing that portions of their medical records and source data related to the project may be reviewed by a monitor, an auditor or a regulatory inspector, in accordance with applicable regulatory requirements. They will be made aware that Data protection will be handled in compliance with UK law.

9.2 Screening Procedures

Members of the patient's existing direct care team will have access to patient records for discussion at the Guy's and St Thomas' hospital NHS Trust (GSTT) severe asthma clinic meetings.

Where the participant has been approved to receive benralizumab for clinical reasons and they meet the inclusion criteria for the study, the identified participant will then be approached by a member of the GSTT asthma direct care team who will discuss the study in detail with their patient. The patient will be given a copy of the participant information sheet (PIS) or one will be sent to them by post or email to consider before their next scheduled clinic visit. The patient will be given adequate time to decide whether or not they wish to take part in the study and be encouraged to ask any questions.

9.3 Schedule of assessments for each visit

See also section 7.2

Design and patient flow:
Visit 1: Consent, Enrollment and schedule of visit dates
Visit 2: Day 1 Covid-19 test
Visit 3: Day 2 Bronchoscopy
Visit 4: Day 10 Subject receives benralizumab dose 1 (30mg subcutaneous injection)
Visit 5: Day 38 Subject receives benralizumab dose 2 (30mg subcutaneous injection)
Visit 6: Day 66 Subject receives benralizumab dose 3 (30mg subcutaneous injection)
Visit 7: Day 83 Covid-19 test
Visit 8: Day 84 Bronchoscopy

9.4 Follow up Procedures

The study participants will be referred back to the care of GSTT asthma clinic staff for continued standard care. There will be no study specific follow up.

9.5 End of Study Definition

The study is planned over a 2 year period. Last visit of the last subject will take place by 18 months of the study start date i.e. all 12 participants would have had 2 bronchoscopies by 18 months. After this point we have planned 6 months for processing of clinical samples, molecular work, data analysis and interpretation.

10 Laboratories

10.1 Central/Local Laboratories

All the samples collected in this study will be processed in the laboratories of Prof. David Jackson and Dr Rocio Martinez Nunez within King's College London at Guy's hospital campus.

10.2 Sample Collection/Labelling/Logging

Samples will be collected by the study direct care team. Samples will be immediately stored on ice or room temperature and labelled with patient code, visit number and the time of collection recorded on study worksheets, Each sample type will have specific SOPs and guidance on information that must be recorded at the appropriate environmental conditions to maintain sample integrity. The KCL

research team or delegated personnel who are appropriately trained will be contacted to inform them that the sample is ready for collection or that the sample is being delivered to the laboratory.

10.3 Sample Analysis Procedures

The aim is to study the influence of benralizumab on structural and inflammatory airway cells in patients with severe eosinophilic asthma.

Our primary outcome measure is:

The change in the number / proportion and/or activation status of Type-2 related cellular populations in samples derived from bronchoscopy including bronchial biopsies, bronchoalveolar lavage (BAL) and bronchial brushings. These cells include ILC2, Th2, alveolar macrophages, epithelial cells, eosinophils and basophils pre- and post- 12 weeks of benralizumab using molecular techniques.

Secondary Endpoints:

To investigate the following before and after completing treatment with 12 weeks of benralizumab.

1. Changes in epithelial barrier integrity using cell biology methods such as transepithelial resistance or barrier permeability.

2. Changes in epithelial antiviral responses in untreated vs respiratory virus-infected cells including rhinovirus-16 and SARS-CoV-2 using protein and nucleic acid analyses.

3. Changes in the antiviral responses of alveolar macrophages to rhinovirus-16 or other respiratory virus infection using protein and nucleic acid analyses.

4. Changes in epithelial responses to cytokines/chemokines and/or exposure to other cell types (co-cultures).

5. Changes in airway remodelling by histological examination.

6. Changes in the profile and function of isolated structural cells (such as airway smooth muscle and epithelial cells but not restricted to) dissected from larger biopsies by molecular and cellular methods.

7. To evaluate the impact on peripheral airways by obtaining bronchoalveolar lavage (BAL) and performing nucleic acid and protein analyses

8. Peripheral airway dysfunction using tests such as impulse oscillometry but not restricted to.

9. Change in FeNO levels.

<u>Study procedures-</u> Clinical Assessment

The CRF will be completed and include clinical details about asthma and other relevant medical conditions and medications.

Spirometry

Spirometry will be conducted in hospital using a spirometer conforming to ATS/ERS standards as specified by the manufacturer's instructions. FEV1 (L), FVC (L), FEV1/FVC ratio, FEF25-75 (% of predicted value) and PEF (L/min) will be recorded. FEV1, FVC and PEF will be documented as both absolute values and as a percentage of the predicted value.

Oscillometry

Oscillometry is a non-invasive measure of pulmonary resistance and reactance. By measuring oscillating pressures/flow signals of moving air within the lungs, when generated at different frequencies, it provides measures of airway mechanics from different lung regions. Generally lower frequencies, in particular frequency dependent changes in resistance between 5 and 20 Hz and capacitive reactance at 5 Hz are thought to reflect changes arising in the more distal airways. Oscillometry will be performed pre- and post-bronchodilator.

Fractional nitric oxide (FeNO)

FeNO is a surrogate marker of T2-airway inflammation. FeNO will be measured using a NIOX VERO device, as specified by the manufacturer's instructions. This includes collection by controlled exhalation at the recommended controlled expiratory flow rate of 50ml/s for greater than 6 seconds.

Blood sampling

Blood will be collected for routine full blood count (including blood eosinophil and basophils), biochemistry and coagulation measurements pre-bronchoscopy. In addition plasma/ serum will be stored for proteomic/nucleic acid analyses.

Bronchoscopy

Bronchoscopy will be conducted according to established guidelines, and will be performed by respiratory physicians experienced in bronchoscopy. Participants will receive nebulised salbutamol (2.5mg) and ipratropium bromide (500mcg) prior to the procedure. Local anaesthesia will be achieved with gel applied to the nasal passages and lignocaine spray to the throat, soft palate and pharynx. Intravenous sedation using midazolam (maximum 5 mg) and alfentanyl (maximum 500 mcg) will be given to achieve conscious sedation. A flexible bronchoscope will then be passed either through the nose or mouth into the lungs with additional lignocaine spray being applied to prevent coughing.

i) Bronchial brushings will be performed using single-use brushes. Up to a total of 6 brushes will be taken.

ii) Bronchial biopsies will be performed from the lower and middle lobes using single use forceps. Up to 6 evaluable biopsies will be taken.

iii) Bronchoalveolar lavage (BAL): the bronchoscope will be inserted in a sub-segmental bronchi and 60ml aliquots of pre-warmed 0.9% saline will be instilled followed by gentle suction to recover lining fluid. This will be repeated up to 3 times.

At the first bronchoscopy (Visit 3) samples will be collected from the right side of the lung and at second bronchoscopy (Visit 8) samples will be taken from the left side.

The bronchoscopy will be rescheduled if the patient has had an asthma exacerbation in the preceding 6 weeks.

Study Procedures- molecular

Epithelial brushes processing

Two brushings per patient will be stored at -80°C in RNA stabilising agent for future analysis. Four brushings will be processed for basal airway epithelial isolation by culturing in selective medium as previously described. Cultured cells, which are mainly basal epithelium, will be processed and infected/non infected with respiratory viruses including (but not restricted to) rhinovirus and SARS-CoV-2 to assess genome-wide changes, both at baseline and in their antiviral response upon benralizumab treatment. We will first set up time courses of 8,24 and 48h given that the responses to rhinovirus and SARS-CoV-2 in bronchial epithelium differs in its dynamics, and sequence a single time point that is appropriate. We will keep RNA samples for all time points assessed for future validation purposes, together with supernatants to enable analysis of cellular mediators.

Cells infected/uninfected with virus will be subjected to RNA-seq after treatment. Top candidates based on statistical and biological significance will be validated by qPCR; western blotting/ELISA or other protein detection approaches. In the validation experiments we will also employ UV-inactivated virus as additional control. Cell culture supernatants will be collected to assess virus titres and RNA, as well as for future protein expression analyses. Cell lysates will also be stored for future protein level assessment using different platforms (proteomics; western blotting; ELISA or similar such as Luminex, O-link, etc.). Same passage cells will also be frozen for future analyses and exploratory studies detailed above.

Endobronchial biopsies

Up to 6 biopsies will be obtained:

Spatial transcriptomics: the optimal conditions of hybridization and staining will be firstly titrated in the lab using currently existing biopsy samples, to determine optimal conditions to process all samples. Biopsies will be fixed and both cryopreserved (snap frozen) as well as processed in paraffin wax for histological applications such as Visium Spatial Gene Expression protocols.

Bronchoalveolar lavage processing

An aliquot of bronchoalveolar lavage (BAL) will be stored for microbiome analysis. The BAL will then be spun to obtain cell pellets. Supernatant will be aliquoted for future analyses including microbiome, RNA and protein expression.

An aliquot of at least 1 million cells will be frozen for future analyses. Cells will be plated to isolate alveolar macrophages as previously described. Purified alveolar macrophages will be infected/uninfected with respiratory viruses (including but not restricted to rhinovirus and SARS-CoV-2), processed for RNA and protein measurements and stored at -80C for future analyses. Supernatants will also be collected for future analyses.

Blood samples

Blood samples will be spun on the same day of extraction. Serum/plasma will be frozen for proteomics/nucleic acid analysis. An aliquot of whole blood cells will be stored in an RNA stabilising agent and aliquots of whole blood cells and peripheral blood mononuclear cells obtained by gradient centrifugation will also be frozen in freezing medium for future analyses.

10.4 Sample Storage Procedures

Samples will be appropriately stored in temperature controlled fridge, freezers and liquid nitrogen dewars which are remotely monitored 24hr. These temperature records will be filed for laboratory audit purposes to ensure and to show that the integrity of the stored samples have not been compromised. Inventory logs of all study samples will be maintained and storage positions in the freezers and dewar will be clearly recorded.

The samples will be stored in secure freezers in laboratories within the School of Immunology and Microbial Sciences at King's College London and GSTT sites linked to the direct care team.

Overall custodial arrangements will be with the Chief Investigator and access will only be provided to the study team or delegates of the study team (as decided by the chief Investigator).

The HTA site license number is 12521 for the KCL Guy's campus site. The designated individual is Cheryl Gillett.

10.5 Data Recording/Reporting

Enrolment into the study will be documented in each participant's medical notes. Data collection will comprise of a paper case report form (CRF) including participant demographics, asthma characteristics (e.g. age of onset, atopic status, T2 biomarker levels, presence of nasal polyposis etc), pulmonary function test results and record of bronchoscopy procedures.

All data will be handled within the conditions of the General Data Protection Regulations (GDPR) which came into force in May 2018 with the participant's informed consent form. Confidential information, both medical records and personal information will only be accessible by members of the GSTT direct care team. Recruited participants will be pseudonymised (given a code) and any data

records will be referred to by this code. Data will be archived according to current ICH GCP guidelines. Laboratory books containing results of the study will not contain personal information.

A combination of paper and electronic records will be used. The paper records will be filed in the study-specific folder and will also be scanned and filed electronically on KCL and GSTT servers on computers that are password protected.

Molecular data:

Sequencing datasets: sequencing datasets will be stored in local servers at King's College London (KCL) and in double-password protected hard drives which will be kept in a locked cabinet. Integration of the transcriptomics and clinical datasets will be done in local machines at KCL; if more computational power is required we will leverage on the cloud resources such as our local high Performance Computer (Rosalind) or Cyverse. Patient datasets will be pseudonymised and all data handling in the cloud will be done in accordance with GDPR regulations.

Strict measures will be in place to safeguard the confidentiality of all data collected. All laboratory specimens and forms, laboratory reports, study data collection tools and stored samples will be identified using unique study numbers alone, to maintain participant confidentiality. No personal identifier information will be shared or recorded on any of these forms or samples and their associated databases. Personal identifiers (e.g. name and contact details) will only be collected for (1) informed consent and (2) logistical purposes. Personal identifiers will appear on consent forms and paper enrolment logs only. These will NOT include any sensitive study information. The unique study number will link personal identifiers to study information. All electronic data will be stored in a password protected database systems on password protected computers, servers and networks. No individuals will ever be identified when reporting study findings or making sequencing data publicly available at the time of publication.

10.6 Sample Receipt/Chain of Custody/Accountability

Firstly, a materials transfer agreement will be in place between the GSTT and KCL site for sample transfer and chain of custody processes. Laboratory SOPs are in place to meet GCLP and HTA compliance. Sample receipt and processing worksheets will be in place for each sample type to record the patient code number, visit number, date, time and condition of the samples upon arrival to the laboratory. Sample labelling will be maintained throughout the processing and storing procedure.

10.7 Sample Transfer to sites outside the Organisation

Where study samples will need to be transferred outside the organisation to fulfil the endpoints of the study. These will be appropriately packed and sent with a medical courier with documentation of sample code, sample type, volume and safety guidelines and transported at an appropriate temperature.
In the study, biological samples [blood, lung biopsies and lavage fluid and airway cells] will be collected from patients in accordance with the patient consent form and patient information sheet and shall include all tissue samples or other biological materials and any derivatives, portions,

progeny or improvements as well as all patient information and documentation supplied in relation to them. Further, the custodian of the Materials will use the Materials for the Study only. For the avoidance of doubt Recipient shall only use the Material in accordance with the consent provided by the Study Donors (if applicable) and shall always use the Materials with dignity and respect and shall always use good laboratory practice in handling Materials

The chief investigator and study co-investigator [Dr Rocio Martinez-Nunez at King's College London] will process, store and dispose of the study samples [blood, lung biopsies and lavage fluid and airway cells] in accordance with all applicable legal and regulatory requirements, including the Human Tissue Act 2004 and any amendments thereto. While Provider uses reasonable endeavours to ensure the quality of the Materials, the Materials are provided 'as is' and it makes no representation and gives no warranty or undertaking in relation to the Materials, including but not limited to its safety, fitness for purpose or use of any kind.

After ethics approval for the study has expired, the study samples [blood, lung biopsies and lavage fluid and airway cells] will be disposed of in accordance with the Human Tissue Act 2004, and any amendments thereto, or transferred to a licensed tissue bank.

11 Assessment of Safety

An Adverse event (AE) is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (e.g. nausea, chest pain), signs (e.g. tachycardia, enlarged liver) or the abnormal results of an investigation (e.g. laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered. The term AE is used to include both serious and non-serious AEs.

An SAE is an AE occurring during any study phase (i.e., run-in, treatment, washout, follow- up), that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital anomaly or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above

An adverse drug reaction is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a medicinal product, suspected to be causally related to the product.

Participants will be asked about the occurrence of any AEs at each visit and will be asked to report AEs to the study direct care team between visits. AEs will be assessed by the CI/PI for causality, intensity, seriousness and expectedness. All AEs will be recorded in the CRF and reported to the Sponsor as per their guidelines. Any adverse events that do occur and are considered by the PI to be related to the investigations will be expedited to the Sponsor and REC within 7 days. Lists of the AEs will be provided to the Sponsor when requested.

All SAEs will be recorded on a Serious Adverse Event Form and will be reported within 24 hours of awareness to the Sponsor. All SAEs occurring to a research participant that the CI feels was related i.e. resulted from administration of any of the research procedures, and unexpected i.e. the type of event not listed in the protocol as an expected occurrence will be reported to the appropriate REC.

11.1 Ethics Reporting

Reports of related and unexpected SAEs will be submitted to the Main REC within 15 days of the chief investigator becoming aware of the event, using the NRES template. The form will be completed in typescript and signed by the chief investigator. The main REC will acknowledge receipt of safety reports within 30 days. A copy of the SAE notification and acknowledgement receipt will be sent to the R&D Directorate.

11.2 Trial Steering Committee (if applicable)

Please outline if there will be any data monitoring/steering/safety committees set up for this study. Describe the extent of the role of this committee and their involvement within the study.

11.3 Ethics & Regulatory Approvals

State the name and address of the REC to which the study protocol and other documentation will be submitted.

Which REC has not yet been determined.

12 Compliance and withdrawal

12.1 1Subject compliance

The direct care team will administer benralizumab subcutaneously therefore compliance will not be an issue.

12.2 Withdrawal / dropout of subjects

Treatment Regimen:
Benralizumab will be commenced according to UK NICE severe asthma guidance with 30mg sc dosing every 4 weeks for first 3 doses, followed by every 8 weeks.

No change in any background asthma medications (e.g change in ICS type or dose) is permitted during the study. Given the importance of measuring the effect of 3 doses of benralizumab following the first bronchoscopy, the subject will be excluded if they do not receive the second and third benralizumab dose at the correct time intervals (+/- 7 days for each dose) following the first benralizumab injection.

12.3 Protocol Compliance

Any protocol deviation will be recorded and discussed with the CI to identify if a serious breach has occurred.

13 Data

13.1 Data to be collected

The study is planned over a 2 year period.
Last visit of the last subject will take place by 18 months of the study start date i.e. all 12 participants would have had 2 bronchoscopies by 18 months.
After this point we have planned 6 months for processing of clinical samples, molecular work, data analysis and interpretation.
The laboratory measures gathered from blood and bronchoscopy samples will be analysed and correlated to clinical physiological parameters.
Main comparisons will be measures and pre- and post benralizumab treatment.

Epithelial brushes processing

Two brushings per patient will be stored at -80□C in RNA stabilising agent for future analysis. Four brushings will be processed for basal airway epithelial isolation by culturing in selective medium as previously described. Cultured cells, which are mainly basal epithelium, will be processed and infected/non infected with respiratory viruses including (but not restricted to) rhinovirus and SARS-CoV-2 to assess genome-wide changes, both at baseline and in their antiviral response upon benralizumab treatment. We will first set up time courses of 8,24 and 48h given that the responses to rhinovirus and SARS-CoV-2 in bronchial epithelium differs in its dynamics, and sequence a single time point that is appropriate. We will keep RNA samples for all time points assessed for future validation purposes, together with supernatants to enable analysis of cellular mediators.
Cells infected/uninfected with virus will be subjected to RNA-seq after treatment. Top candidates based on statistical and biological significance will be validated by qPCR; western blotting/ELISA or other protein detection approaches. In the validation experiments we will also employ UV-inactivated virus as additional control. Cell culture supernatants will be collected to assess virus titres and RNA, as well as for future protein expression analyses. Cell lysates will also be stored for future protein level assessment using different platforms (proteomics; western blotting; ELISA or similar such as Luminex, O-link, etc.). Same passage cells will also be frozen for future analyses and exploratory studies detailed above.

Endobronchial biopsies

Up to 6 biopsies will be obtained:
Spatial transcriptomics: the optimal conditions of hybridization and staining will be firstly titrated in the lab using currently existing biopsy samples, to determine optimal conditions to process all samples. Biopsies will be fixed and both cryopreserved (snap frozen) as well as processed in paraffin wax for histological applications such as Visium Spatial Gene Expression protocols.

Bronchoalveolar lavage processing

An aliquot of bronchoalveolar lavage (BAL) will be stored for microbiome analysis. The BAL will then be spun to obtain cell pellets. Supernatant will be aliquoted for future analyses including microbiome, RNA and protein expression.

An aliquot of at least 1 million cells will be frozen for future analyses. Cells will be plated to isolate alveolar macrophages as previously described. Purified alveolar macrophages will be infected/uninfected with respiratory viruses (including but not restricted to rhinovirus and SARS-CoV-2), processed for RNA and protein measurements and stored at -80C for future analyses. Supernatants will also be collected for future analyses.

Blood samples

Blood samples will be spun on the same day of extraction. Serum/plasma will be frozen for proteomics/nucleic acid analysis. An aliquot of whole blood cells will be stored in an RNA stabilising agent and aliquots of whole blood cells and peripheral blood mononuclear cells obtained by gradient centrifugation will also be frozen in freezing medium for future analyses.

13.2 Data handling and record keeping

The direct care team and KCL research team are responsible for data collection, recording and quality. All data will be handled within the conditions of the General Data Protection Regulations (GDPR) which came into force in May 2018 and the participant's informed consent form. Confidential information, both medical records and personal information will only be accessible by members of the clinical research team and any data removed from the site will be anonymous and de-identified. Data will be archived according to current ICH GCP guidelines. Laboratory books containing results of the study will not contain personal information.

Data collection forms:

Enrolment into the study will be documented in each participant's medical notes. Data collection will comprise of a paper case report form (CRF) including participant demographics, asthma characteristics (e.g. age of onset, atopic status, T2 biomarker levels, presence of nasal polyposis etc), pulmonary function test results and record of bronchoscopy procedures.

Strict measures will be in place to safeguard the confidentiality of all data collected. All laboratory specimens and forms, laboratory reports, study data collection tools and stored samples will be identified using unique study numbers alone, to maintain participant confidentiality. No personal identifier information will be recorded on any of these forms or samples and their associated databases. Personal identifiers (e.g. name and contact details) will only be collected for (1) informed consent and (2) logistical purposes. Personal identifiers will appear on consent forms and paper enrolment logs only. These will NOT include any sensitive study information. The unique study number will link personal identifiers to study information. All electronic data will be stored in a password protected database systems on password protected computers, servers and networks. No individuals will ever be identified when reporting study findings or making sequencing data publicly available at the time of publication.

Specified retention period: 15 years after the end of the study

14 Statistical considerations

Statistician

Name: Dr Rocio T Martinez-Nunez

Address: King's Centre for Lung Health, Faculty of Life Sciences & Medicine, School of Immunology and Microbial Sciences, 5th Floor Tower Wing, Guy's Campus, King's College London, London SE1 9RT

Email: rocio.martinez_nunez@kcl.ac.uk

14.1 Sample size calculation (some pilot/feasibility studies may not require a formal sample size calculation)

Sample Size Justification:
Based on the observed 85% responder rate to benralizumab in our centre (Kavanagh et al. CHEST 2021), statistically significant falls in FeNO observed in FeNO-high patients with benralizumab, and successful published transcriptomics studies in asthma of a similar number, we believe 12 subjects should yield at least 10 clinical responders to benralizumab. Twelve is also an achievable amount from a practical perspective to allow timely completion of this study in under 18 months from first subject in (FSI).

14.2 Statistical analysis

Descriptive statistics will be used for demographic and clinical characteristics of the study subjects. The primary comparisons are pre- and post-benralizumab therapy. Clinical outcome measures (asthma control scores, exacerbation frequency, medication use, lung function obtained at baseline and following benralizumab treatment will be evaluated using standard statistical tests. These statistical tests are either parametric paired t-tests or non-parametric U-tests for continuous variables, and fisher tests for the analysis of proportions. We will also leverage on local statisticians when needed.
Statistical comparisons in RNA-seq will be undertaken using currently available pipelines such as DESeq2. DESeq2 has in-built modelling based on binomial distributions of RNA-seq datasets and is well established and validated. Likewise we will follow the most up to date pipelines for analysis of spatial transcriptomics as per 10xgenomics, which include combining spatial information with single cell RNA-seq-type analyses.

Employing spatial transcriptomics, we will compare the effect of benralizumab on cell-specific gene expression changes, cell numbers and position within bronchial biopsies, as well as cell-to-cell interactions.
Employing RNA-seq on BECs, we will compare (1) unstimulated BECs pre- and post- benralizumab, and (2) SARS-CoV-2/RV-16 infected BECs pre- and post- benralizumab, determining the effect of benralizumab on the antiviral response of BECs to each one of these viruses separately.
All analyses will be paired, considering patient-specific changes.
All analyses involving the measurement and comparison of multiple variables (e.g. RNA-seq) will include a multiple test correction, such as false discovery rate / Bonferroni.

15 Ethical considerations

Study will go through R&D and REC approval.
Patients will give informed consent

16 Financing and Insurance

The study is being funded by Astra Zeneca.

The Chief Investigator is employed by KCL so additional indemnity cover is provided by the College As the study is co-sponsored by GSTFT, clinical negligence is provided via the NHS Indemnity Scheme.

17 Reporting and dissemination

Any pilot data arising from this study, and which is statistically robust will be presented at conferences. At the end of the study period, following data analysis completion, a manuscript will be prepared for peer review and publication.

Tables, Figures, References
Appendices
Including (where relevant):
Patient information sheet
Patient consent form
Data collection forms and validation information
Ethics form
Summary of product characteristics
Investigators brochure

Useful reading/websites

Integrated Research Application System (IRAS)
https://www.myresearchproject.org.uk/

Health Research Authority (HRA)
www.hra.nhs.uk

HRA Guidance for Patient Information Sheet and Informed Consent
http://www.hra.nhs.uk/research-community/before-you-apply/participant-information-sheets-and-informed-consent/

CONSORT statement

ICH Harmonised Tripartite Guidelines for Good Clinical Practice (1996)
http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R1_Guideline.pdf

Martin Bland et al, Statistical guide for research grant applications
http://www-users.york.ac.uk/~mb55/guide/guide.htm
Includes detailed information and definitions of many aspects required for a research protocol.

Declaration of Helsinki
(http://www.wma.net/en/30publications/10policies/b3/index.html)

Appendix 1 – Information with regards to Safety Reporting in Non-CTIMP Research

	Who	When	How	To Whom
SAE	Chief Investigator	-Report to Sponsor within 24 hours of learning of the event -Report to the MREC within 15 days of learning of the event	SAE Report form for Non-CTIMPs, available from NRES website.	Sponsor and MREC
Urgent Safety Measures	Chief Investigator	Contact the Sponsor and MREC Immediately Within 3 days	By phone Substantial amendment form giving notice in writing setting out the reasons for the urgent safety measures and the plan for future action.	Main REC and Sponsor Main REC with a copy also sent to the sponsor. The MREC will acknowledge this within 30 days of receipt.
Progress Reports	Chief Investigator	Annually (starting 12 months after the date of favourable opinion)	Annual Progress Report Form (non-CTIMPs) available from the NRES website	Main REC
Declaration of the conclusion or early termination of the study	Chief Investigator	Within 90 days (conclusion) Within 15 days (early termination) *The end of study should be defined in the protocol*	End of Study Declaration form available from the NRES website	Main REC with a copy to be sent to the sponsor
Summary of final Report	Chief Investigator	Within one year of conclusion of the Research	No Standard Format However, the following Information should be included:- Where the study has met its objectives, the main findings and arrangements for publication or dissemination including feedback to participants	Main REC with a copy to be sent to the sponsor