Effects of blocking Thymic Stromal Lymphopoietin (TSLP) on airway inflammation and the epithelial immune response to exacerbation triggers in patients with COPD

(UPSTREAM-COPD)

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Sponsor (Global)	Bispebjerg Hospital, Copenhagen, Denmark
Sponsor Trial Reference:	0886
Funder:	AstraZeneca

SIGNATURE PAGE

The undersigned confirms that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and any subsequent amendments of the clinical trial regulations, GCP guidelines, the Sponsor's (and any other relevant) SOPs, and other regulatory requirements.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the trial publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the trial will be given. Any discrepancies and serious breaches of GCP from the trial as planned in this protocol will be explained.

Sponsor: Bispebjerg Hospital, Copenhagen, Denmark

Signature:	Date:
Name (please print):	
Position:	
Chief Investigator (UK): Professor Chris Brightling	
Signature:	Date:
Co-ordinating investigator: Dr Asger Sverrild	
Signature:	Date:

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1 Contents

2 LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Event of Special Interest
AH	Airway Hyperresponsiveness
AR	Adverse Reaction
ASM	Airway Smooth Muscle
ATS	American Thoracic Society
BAL	Broncho-alveolar lavage
BD	Bronchodilator
BRC	Biomedical Research Centre
CAT	COPD Assessment Test
CHMP	Committee for Medicinal Products for Human Use
CI	Chief Investigator
(e)CRF	(electronic) Case Report Form
COPD	Chronic Obstructive Pulmonary Disease
CTU	Clinical Trials Unit
DLCO	Diffusing Capacity for Carbon Monoxide
ECP	Eosinophil Cationic Protein
ERS	European Respiratory Society
ETR	End of Trial Report
FEG	Free eosinophil granules
FeNO	Fraction of exhaled nitric oxide
FEV1	Forced Expiratory Volume in 1 second
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
GLI	Global Lung Function Initiative
HRCT	High Resolution
ICF	Informed Consent Form
ICS	Inhaled corticosteroid

ILC	Innate Lymphoid Cells
IMP	Investigational medicinal product
ISF	Investigator Site File
LABA	Long-acting beta2-agonist
LAMA	Long-acting muscarinic antagonist
OCS	Oral cortico-steroid
PBMC	Peripheral Blood Mononuclear Cells
PI	Principal Investigator
PIC	Participant Identification Centre
PIS	Participant Information Sheet
PROM(s)	Patient Reported Outcome Measure(s)
QC	Quality Control
Q4W	4-weekly
RBG	Reticular basement membrane
RCT	Randomised Control Trial
REC	Research Ethics Committee
RNA	Ribonucleic acid
RNASeq	RNA Sequencing
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SC	Sub-cutaneous
SGRQ-C	St Georges Respiratory Questionnaire for COPD patients
SMA	Smooth muscle actin
SNOT-22	Sino-Nasal Outcome Test-22
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
T2	Type-2
Th	T-helper cell
TLC	Total Lung Capacity
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee

TSLP	Thymic stromal lymphopoietin
TSLP-R	Thymic stromal lymphopoietin Receptor
VOC	Volatile Organic Compounds
WOCBP	Woman of child-bearing potential

KEY WORDS

Tezepelumab, COPD, TSLP, randomised, inflammation, exacerbations

1 Trial summary

Trial Title	Effects of blocking TSLP on airway inflammation and the epithelial immune response to exacerbation triggers in patients with COPD
Trial Acronym	UPSTREAM-COPD
Clinical Phase	Phase 2
Trial Design	A randomized double-blind, placebo-controlled trial of Tezepelumab in COPD patients
Trial Participants	COPD patients with at least one exacerbation in last 12 months
Planned Sample Size	80
Treatment duration	Screening period for 4 weeks followed by treatment period of 20 weeks (24 weeks)
Planned Trial Period	2023 - 2026
Follow up duration	No follow up period
Study	Objectives
Primary	To evaluate the effect of tezepelumab on eosinophilic bronchial mucosal tissue inflammation
Secondary	To evaluate the effect of tezepelumab on bronchial mucosal tissue inflammation
Exploratory (Immuno-histochemistry)	To explore the effect of tezepelumab including but not limited to the following: 1. RBM thickness 2. Airway integrity 3. SMA staining 4. Markers of airway healing 5. Additional T2 and inflammatory markers 6. Breathprint 7. Gene expression by RNAseq in airway and peripheral blood 8. Leukocyte phenotyping

Exploratory (HRCT)	To explore the effect of tezepelumab including but not limited to the following: 1. Airway geometry 2. Lung densitometry 3. Lung vasculature
Exploratory (Clinical outcome measures)	To explore the effect of tezepelumab on measures including but not limited to: 1. Lung function 2. Symptoms and quality of life
Exploratory sub-group analysis comparison	To identify subgroups of patients with COPD based on the measures above and analyse 1. Bronchial epithelial immune response to triggers at baseline 2. Wound healing capacity at baseline
Investigational Medicinal Product(s)	Tezepelumab Vs Placebo
Formulation, Dose, Route of Administration	Tezepelumab (210mg, sterile, preservative-free, solution in vials for SC administration) Vs Placebo (sterile, solution in vials for SC administration)

3 Funding and support in kind

Funder(s)	**Financial and non-financial support given**
AstraZeneca	Financial and Trial IMP support
Central Pharmacy, Copenhagen, Denmark	IMP labelling
NIHR Leicester Biomedical Research Centre - Respiratory Theme	Infrastructure support (UK)
NIHR Leicester Clinical Research Facility	Infrastructure support (UK)

4 Role of trial sponsor

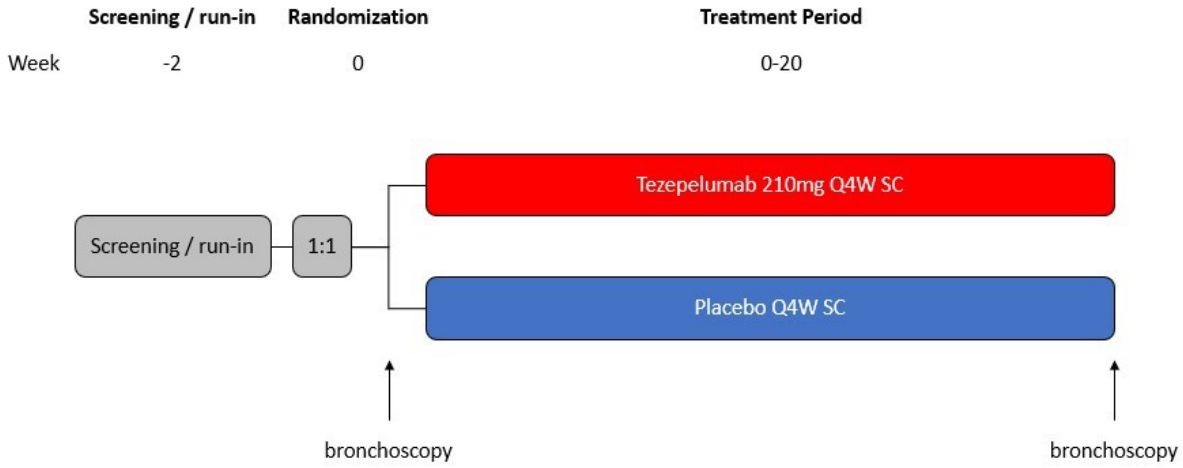
The Sponsor of this research is Bispebjerg Hospital, Copenhagen, Denmark. The CI and PIs delegated by the sponsor, are responsible for the proper conduct and management of the trial.

5 Roles and responsibilities of trial management Committees

Trial Management Group (TMG)

The TMG will comprise of Co-ordinating investigator, Chief Investigators, Research Fellow(s), Trial Manager and Trial Statistician. Members of the site recruiting team will attend meetings as observers and contribute to recruitment discussions. The TMG will oversee the operational aspects of the trial, which include the processes and procedures employed, and the day-to-day activities involved in trial conduct. The Trial Manager will undertake the day-to-day management of the trial. The TMG is responsible for overseeing all aspects of the trial (including protocol compliance, safety reporting, recruitment rate, budget management, etc.) and for ensuring appropriate action is taken to safeguard trial participants and the quality of the trial. Significant issues arising from TMG meetings will be referred to the Trial Steering Committee, Sponsor or Investigators, as appropriate.

6 Trial flow chart



7 Background

COPD is a common chronic lung disease with a prevalence of 251 million cases globally and is projected to be the third leading cause of death by 2030(1). A significant subgroup of patients develops frequent exacerbations despite maintenance treatment with long-acting bronchodilators, with or without inhaled corticosteroids. Frequent exacerbations are associated with increased disease burden in terms of reduced quality of life, increased mortality, increased health care utilization and hospitalizations, and hence with high

economic burden, absorbing around 50% of direct cost associated with COPD care (1, 2). Important triggers of exacerbations in both asthma and COPD are respiratory viruses, with rhinovirus in particular responsible for up to 20-55% of COPD exacerbations (3, 4). New treatment strategies to prevent exacerbations are urgently needed in order to improve patient outcomes and reduce costs.

The pathogenesis of COPD is complex, and the exact underlying cellular and molecular mechanisms remain unclear(5). Chronic airway inflammation forms the basis for the development of COPD and is characterized by increased numbers of neutrophils, macrophages, mast cells and lymphocytes(6). Although COPD is not considered a T2-high disease, between 13 and 40% of patients with COPD also demonstrate eosinophilic inflammation (7).

Thymic stromal lymphopoietin (TSLP) is a pleiotropic, upstream epithelial cytokine released in the airways in response to various triggers such as viruses, allergens, bacteria, physical injury and smoke (8, 9) TSLP drives a broad range of T2 and non-T2 processes affecting a wide range of cells that express the TSLP receptor including eosinophils, basophils, mast cells, airway smooth muscle cells, group 2 innate lymphoid cells (ILC2s), lymphocytes, dendritic cells and macrophages(10). This in turn induces the production of a wide range of downstream interleukins including classic Type 2 cytokines like IL-4, IL-5 and IL-13 but also a Th17 cell expansion responsible for neutrophil recruitment(11) making it likely that TSLP plays a role in a number of diseases including COPD.

Although the inflammatory effects of TSLP are described in much more detail in asthma(8), a role for TSLP more specifically in COPD is supported by observations of increased TSLP protein levels in induced sputum and broncho-alveolar lavage (BAL) as well as increased epithelial TSLP mRNA expression in non-exacerbating patients with COPD ((12, 13). Further, airway epithelium stimulated with virus mimic or live rhinovirus produce higher levels of TSLP in both asthma and COPD compared to healthy controls (14).

8 Rationale

Tezepelumab is a human monoclonal antibody (mAb) immunoglobulin G2λ (IgG2λ) that binds to TSLP and thus prevents interaction with the TSLP-receptor and downstream signalling. Due to the upstream role of TSLP in initiating and maintaining airway inflammation, blocking TSLP has provided an opportunity to interfere with the multiple downstream effects of asthma and COPD triggers.

The current clinical evidence largely stems from trials in patients with asthma, but a study evaluating the effect of 420mg tezepelumab Q4W on exacerbations in patients with COPD is currently being conducted (NCT04039113).

A proof-of-concept phase II study showed that tezepelumab attenuated the early and late phase fall in FEV1 as well as the expected increase in FeNO and sputum eosinophils to allergen challenge in mild steroid-free asthmatics (15). A more general effect on airway hyper responsiveness in patients with moderate-to-severe asthma has since been confirmed (16, 17).

In a phase 2b dose ranging, double blind, randomized, placebo-controlled study of patients with asthma on moderate-to-high doses of ICS, it was shown that tezepelumab reduced the

annualized asthma exacerbation rate by 71%(18). This effect on reducing exacerbations was later confirmed in a phase III trial.

Importantly, the clinical efficacy of tezepelumab in severe asthma seems to be independent of the level of maintenance treatment with inhaled glucocorticoids as well as the level of total IgE, FeNO or eosinophils in blood(18, 19) . It suggests that the efficacy obtained by blocking TSLP in asthma possibly stretches beyond classic Type 2 features like allergy and eosinophilia, and that blocking TSLP could play a role in treatment of COPD.

Two studies have investigated the effects of tezepelumab on airway inflammation showing a marked and consistent suppression of eosinophilic inflammation(16, 17). Whereas the one study (28 weeks treatment with 210mg SC Q4W) found no effect on number of sub epithelial mast cells, the other (12 weeks treatment with 700mg iv Q4W) identified a near-significant reduction in mast cells of 25% warranting further investigation. There was no effect on airway sub epithelial CD3+ T-cells, CD4+ T-cells or neutrophils. The effects on CD8+ T-cells and macrophages were not reported.

Finally, unpublished data presented at the European Respiratory Society International Congress 2021 (Sverrild and Cerps et al. abstract number: 3890) on epithelial cells from asthma patients treated with tezepelumab for 12 weeks showed a significant reduction in IL33, IL-4 and IL-5 at in vitro virus stimulation. This together with viral stimulation-induced overproduction of TSLP in bronchial epithelium from asthmatics as well as COPD donors (14) suggest that TSLP regulates host tolerance to virus which could explain in part the effect of tezepelumab on exacerbations.

Overall, the clinical effect in the phase II and III trials in asthma was seen irrespectively of type-2 biomarker status and accounting for the similarities between asthma and COPD at the level of epithelial responses to virus, it is reasonable to consider similar effects in patients with COPD.

9 Aim and Hypothesis

With this study we aim to investigate the effects of blocking TSLP signalling in patients with COPD on airway inflammation and triggers of exacerbations such as virus. The hypothesis for the mechanism of action of tezepelumab in COPD is twofold. Firstly, given that TSLP is an upstream cytokine that affects numerous downstream signalling pathways, the blockage of TSLP is anticipated to have an impact on the airway inflammation seen in COPD. Secondly, since TSLP is one of the earliest responses to airway damage, inhibition of TSLP is expected to leave the airway epithelium less susceptible to airway virus by improving host tolerance to exacerbation triggers such as the virus.

10 Assessment and management of risk

In order to evaluate the clinical benefit-risk balance for tezepelumab, preclinical and clinical data from studies in asthma have been taken into consideration. Benefits for tezepelumab over placebo include a clinically meaningful reduction in asthma exacerbations, as well as improvements in lung function and asthma control metrics.

Tezepelumab has been well tolerated and there have been no associated identified risks up to this point. In the asthma trials, the commonest adverse events were asthma,

nasopharyngitis, bronchitis, and headache but these occurred with similar rates across intervention and placebo groups, with decreased exacerbation frequency in the treatment group. Of note, no treatment-related anaphylactic reactions have been reported in the Phase II or Phase III studies.

Although TSLP suppression could theoretically have unanticipated immune-related side effects impairing host defense against certain infections, there is no clear preclinical or clinical evidence supporting such a role, and no safety signals related to infections have been detected to date in the tezepelumab program.

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of tezepelumab may be found in the Investigator's Brochure.

Two bronchoscopies including mucosa biopsies, brush biopsies and BAL will be performed as part of the trial. All procedures will be carried out by qualified medical staff experienced in research bronchoscopies and with all precautions taken to minimize the risk to the patient in fully set up and professional wards for invasive procedures. The hospitals also provide sufficient back-up in terms of a full-scale medical ward and Intensive Care Units in case of emergencies.

The safety of flexible bronchoscopy has been reported to be similar in patients with and without COPD in a study including 1,400 patients Menzies-Gow et al. N Engl J Med 2021;384:1800-9 (20) and is in general considered a safe procedure (20-23). It has been suggested that bronchoscopies can be performed safely in COPD patients with lung function as low as 25% and an absolute FEV_1 of 0.64L (24), but in the present study – for safety reasons – we plan only to include patients with $FEV_1 \geq 30\%$ and $\geq 1L$). Common occurring risks (>10%) associated with the procedure are: Transient fever, cough, chest discomfort and a transient decline in FEV_1(20-22). Potentially more severe complications are rare and include major bleeding (<1%) and pneumothorax (<0.2%) (20, 21).

Safety follow-up visits (phone) after bronchoscopies have been implemented in the study plan.

This trial is categorised as:

- Type B = Somewhat higher than the risk of standard medical care

11 Objectives and outcome measures/endpoints

11.1 Primary objective

To evaluate the effect of tezepelumab on eosinophilic bronchial mucosal tissue inflammation

Secondary objective

To evaluate the effect of tezepelumab on bronchial mucosal tissue inflammation

Safety Objective

To evaluate the safety and tolerability of tezepelumab

12 Table of endpoints/outcomes

	Objectives	Outcome measures
Primary	To evaluate the effect of tezepelumab on eosinophilic bronchial mucosal tissue inflammation	The change, expressed as ratio, in eosinophil cell counts per mm2 from baseline to week-20
Key Secondary	To evaluate the effect of tezepelumab on bronchial mucosal tissue inflammation	The change, expressed as ratio, in inflammatory cell counts (including neutrophils, mast cells, CD4+, CD8+ and macrophages) from baseline to week-20
Exploratory (Immuno-Histochemistry)	To explore the effect of tezepelumab including but not limited to the following: 1. RBM thickness 2. Bronchial epithelial integrity 3. Airway smooth muscle (ASM) mass 4. Markers of airway healing 5. T2 biomarkers e.g., sputum cell counts, FeNO and other markers in airway samples 6. Gene expression by RNAseq of airway samples and peripheral blood 7. Breathomics 8. Leukocyte phenotyping from blood, airway samples and tissue	Changes in measures of the followings from baseline to week-20. 1. RBM thickness 2. % Epithelial integrity, expressed in ratio 3. % ASM mass 4. Markers of airway healing 5. Inflammatory cell counts, FeNO and other markers measured in the airway samples 6. Altered gene expression in blood, sputum and airway 7. Breathomics 8. Changes in leukocyte frequency, count or status in blood, airway and tissue samples
Exploratory (HRCT)	To explore the effect of tezepelumab including but not limited to the following: 1. Airway geometry 2. Lung densitometry 3. Lung vasculature	Changes from baseline to week 20 in: 1. Airway luminal area 2. Standardised airway wall thickness 3. Mean lung density 4. Parametric response mapping 5. Total blood volume 6. Proportion of blood in small blood vessels 7. Small blood vessel density
Exploratory (Clinical outcome measures)	To explore the effect of tezepelumab on measures including but not limited to: 1. Lung function	Changes from baseline to week 20 in: 1. Pre and Post bronchodilator FEV1, FVC, TLC, DLCO

	2. Symptoms and quality of life	2. PROMs (CAT, SGRQ-C, SNOT-22)

13 Trial Design

This study is a randomized, double-blind, placebo-controlled trial. It includes enrolment period of maximum 4 weeks, 20 weeks of treatment (five doses of either 210mg tezepelumab or placebo SC) and no follow-up period.

The study aims to recruit patients with moderate-to-severe COPD on standard of care (LABA+LAMA±ICS) who have had at least one exacerbation the past 12 months. Baseline treatment with ICS is not a requirement for participating in this trial, as the plan is to recruit patients across the spectrum of blood eosinophils with a baseline exacerbation rate of ≥1 within 12 months that may not fulfil criteria for ICS according to current guidelines (*GOLD 2021 Report*). They must have been stable on their current inhaled therapy for at least 3 months.

Patients will undergo stratified randomisation based on their baseline blood eosinophil count and smoking status. The median eosinophil count in blood from patients with COPD in Denmark (main trial site) has been reported to be 0.20 $x10^9$/L in patients with COPD and at least one exacerbation the past 12 months (25). Based on this we aim at recruiting 50% of patients with blood eosinophils <0.20 $x10^9$/L and 50% of patients with blood eosinophils ≥0.20 $x10^9$/L.

The primary endpoint in the trial will be change in sub epithelial eosinophils, expressed as a ratio, from baseline to week-20. Secondary outcomes will include changes in sub epithelial neutrophils, mast cells, macrophages, CD4+ and CD8+ T cells.

This is a mechanistic study designed to better understand how tezepelumab works in patients with COPD, and RNA sequencing of airway biopsies is one of the means to reach this goal.

We plan to approach these data in an unbiased fashion with an exploratory purpose as set out in the relevant sections of this protocol.

14 Trial Setting

Patients will be randomized 1:1 to receive either tezepelumab or placebo on top of their regular therapy that is, otherwise considered standard-of-care. By making the study randomized, double blind, placebo-controlled we introduce a minimum of bias in the treatment groups and treatment evaluation.

15 Participant eligibility criteria

15.1 Inclusion criteria

- Willing and able to consent to participate in the trial

- Clinically diagnosed chronic obstructive airway disease with post bronchodilator FEV_1 ≥ 30% to 80% predicted value (and ≥ 1.0L)
- Age ≥ 40 years old
- Current or ex-smokers with ≥ 10 pack years past smoking history
- Stable airway disease status on maintenance inhaled therapy (LAMA+LABA±ICS) for at least 3 months prior to screening
- History of ≥1 moderate to severe exacerbation event treated with prednisolone and/or antibiotic in the past 12 months
- Good compliance with daily inhaler regime (≥ 70% adherence rate) at screening

15.2 Exclusion criteria

- History of unstable or severe cardiac, hepatic, thyrotoxicosis, concomitant respiratory or renal disease, or other medically significant illness, which the investigator believes, would be a contraindication to study participation.
- Significant concomitant respiratory disease such as cystic fibrosis, pulmonary fibrosis, aspergillosis, active or untreated primary tuberculosis.
- Any significant abnormal laboratory results at screening, which in the opinion of the investigator, may put the subject at risk to take part in the study,
- Current diagnosis of Asthma
- Previous Lung volume reduction surgery for the indication of COPD
- Any use of home oxygen therapy
- Patients with clinically significant laboratory abnormalities (not associated with the study indication) at screening
- Recent acute exacerbation event requiring oral corticosteroids or antibiotics (any dose for more than 3 days) or respiratory tract infection 4 weeks prior to screening
- History of active Malignancy in any organ system (diagnosis within last 12 months or ongoing active cancer treatment such as chemotherapy, radiotherapy, or immunotherapy.
- History of treatment with biologics within four months or five half-lives (whichever is longer) prior to screening.
- History of anaphylaxis to any biologic therapy or sensitivity of any component of IMP formulation
- Have been involved in another medicinal trial (CTIMP) within the past 28 days
- Women who are pregnant, lactating or intend to become pregnant during the study period
- Planned surgical procedures requiring general anaesthesia or in-patient status for > 1 day during the conduct of the study.
- Receipt of any live or attenuated vaccines within 15 days prior to screening
- Patients whose treatment is considered palliative (life expectancy < 6 months).
- History of chronic alcohol or drug abuse within 12 months prior to screening
- Receipt of immunoglobulin or blood products within 30 days prior to screening

- Use of immunosuppressive medication (e.g., methotrexate, troleandomycin, oral gold, cyclosporine, azathioprine, intramuscular long-acting depot corticosteroid) within 3 months prior to screening.
- History of any known primary immunodeficiency disorder excluding asymptomatic selective immunoglobulin A or IgG subclass deficiency.
- Subject taking antiretroviral medications, as determined by medical history
- History of human immunodeficiency virus (HIV) or hepatitis B or C.

16 Trial procedures

Recruitment & Participant identification

Patients will be recruited from specialist led COPD clinics identified opportunistically during clinic visit and/or by screening patient lists by study team. The search criteria will include 1) those having a clinical diagnosis of COPD, 2) post bronchodilator FEV1 ≥ 30% predicted (and ≥ 1.0L) 3) regular LAMA+LABA ± ICS inhaler prescription with good compliance. Study will look to recruit patients who have had one or more moderate exacerbations in 12 months prior to screening visit.

Potential participants will be given written information about the study (including the approved PIS leaflet), and how they can access further information and register their interest in participating.

All participants will have as much time as they like (at least 24 hours) to process the information and discuss the study with a research nurse or doctor, before deciding whether they wish to participate in the screening process. Potentially interested participants at this stage will then be offered an appointment for an onsite screening visit.

Screening

Each potential participant will provide written informed consent at screening before starting any study related procedures. Participants will be assessed for eligibility as per inclusion/exclusion criteria at initial screening visit and each subsequent treatment visit for any changes.

Payment

Participants will be reimbursed for all travel expenses for research visits. This includes provision of reimbursement for fuel, bus fares, car parking charges, or taxi fares. Participants will be asked to provide their local study team with receipts. They will also receive payments to cover inconveniences. This will be £200 for visits involving bronchoscopy, £40 for other visits involving clinical tests and £10 for visits where only medication is given. This is a total of £520.

17 Consent

During the screening visit, study nurses at trial sites will outline trial processes and procedures to participants with written and verbal information. This will include what is involved for participants; the implications and constraints of the protocol; and potential side effects and risks involved in taking part. Women of childbearing potential will be explained in greater depth regarding the requirement of pregnancy test, reporting measures of pregnancy and contraception methods. It will be clearly stated that the participant is free to withdraw from the trial at any time and for any reason, without prejudice to future care, and with no obligation to give the reason for withdrawal.

Participant's permission to share the past medical records from primary and secondary care will be requested for the purpose of thorough risk assessment for study treatment.

Consent will also be obtained for the storage and sharing of pseudonymised and anonymised data and samples among trial sites, and for their GP/healthcare professional to be informed of their participation in the trial. The informing study investigator will explicitly mention and explain that a comprehensive mapping of genes and bronchoscopy procedures are planned for the study.

The signed informed consent form will then be obtained. A unique subject identification number will be assigned to each subject screened.

Participants who are deemed not to be eligible in the trial at screening visit will be screen failed and their screening log entry identified as a screen failure along with the details of their ineligibility/declined invitation.

The process for obtaining informed consent will be in accordance with the REC guidance, and GCP and any other applicable regulatory requirements, which may be introduced. Trial procedures will not be undertaken until the informed consent form has been signed and dated by the participant. Should there be any subsequent amendment to the final protocol or participant-facing documents including updates regarding safety information, which might affect their participation in the trial, these will be discussed with the participant and, where applicable, participants will be re-consented using the updated information and consent.

17.1 Re-Enrolment

Subjects who fail screening can be re-enrolled once if relevant (as judged by the investigator). If withdrawal during screening due to an exacerbation (treated with oral prednisolone), a minimum of 4 weeks waiting time will be obligated before re-enrolment.

17.2 Randomisation

Participants will be randomised in a 1:1 ratio to IMP Vs placebo. The trial team and participants will be blinded to treatment assignment. Randomisation will be stratified based on blood eosinophil counts at baseline (< or ≥ 0.2 $x10^9$/L) and smoking status.

Blocked randomisation method will be used in this study. Pre-defined clusters of randomised strata will be distributed from Danish site along with study medication and randomisation in sealed envelopes prior to the start of the study. Once all eligibility criteria are met, study nurse will enter the data pertaining smoking status and baseline eosinophil count. The assigned strata with randomised ID will be subsequently generated.
The central pharmacy from Denmark site will be responsible for receiving and labelling IMP for all sites according to a randomization-list prepared by a computerized system. They will inform local pharmacy/team which number vial to administer to each patient as randomised. They will centrally monitor stock of IMP and placebo in each site, ensuring appropriate stocks are always available. Clinical teams at all sites will be blinded to intervention.

17.3 Blinding

Participants, investigators, and all involved in trial conduct, sample analysis, or with any other interest in this trial will remain blind to the randomised treatment assignments until after final analysis is complete. The exception(s) to this are as follows;

- The Central Pharmacy team from Denmark will be unblinded and have responsibility for storing and dispensing IMP and placebo as per local SOPs. The former will also be responsible for creating randomisation strata list and trial drug preparation. They will liaise with individual site pharmacy leads to facilitate ordering and delivery.
- The Danish Central Pharmacy will prepare the IMP/placebo.
- Blinded trial personnel from the research site will have responsibility for dosing the participants. The blinded trial personnel will collect and transfer the prepared IMP/placebo to the clinical area for administration.
- All documentation of IMP/placebo preparation (including a participant accountability log), randomisation CRF and prescription containing information about randomisation for each participant, will be kept confidential from Denmark trial site. A label will be added directly to the syringe containing only the trial ID, initials, a statement that it is ‘either drug IMP or placebo within’, date, time of preparation, and the initials of the person preparing it.
- There may be slight colour and viscosity differences between the IMP and placebo.
- The treatment allocation will remain blinded to all other trial team members until after database lock.

17.4 Emergency Unblinding

The requirement for unbinding will, if possible, be discussed with the Chief Investigator. The Principal Investigator or delegated Co-Investigator at site will authorise the code break and decide on further management including the need to inform regulatory authorities. Emergency unblinding will be available to the investigator /pharmacist / Sponsor. Unblinding will only occur in the case of a medical emergency when the identity of the allocated treatment must be known in order to provide appropriate medical treatment.

If unblinding occurs, the investigator must record the reason for unblinding, as well as the date and time of the event in the site file and medical notes. Corresponding information will be recorded in the CRF by the investigator. It will also be documented at the end of the trial in any final trial report and/or statistical report. The PI/Investigating team will notify the Sponsor in writing as soon as possible following the code break, detailing the necessity. In the case of unblinding an individual participant for safety reasons, appropriate follow-up of safety related events is required until the event(s) has satisfactorily resolved.

Unblinding of all participants will be undertaken by the trial statistician after the last participant has completed their final visit, and the database has been locked.

18 Trial Assessment

18.1 Visit 1 (Screening visit, -14 days)

The following procedures will be done during screening visit.

- Informed Consent
- Eligibility Assessment
- Baseline Assessment
    - Height and Weight, Demographic information, Medical History, History of exacerbations, Concomitant medication check
- Baseline measures for Efficacy assessment
    - Lung function tests: Pre and Post Bronchodilator FEV1, FEV1/FVC, TLC and DLCO, FeNO testing, Questionnaires, sputum, Exhaled breath print, Blood samples: FBC, LFT, Urea and Electrolytes, Bone Profile, Creatine Kinase, INR)
      High Resolution Computed Tomography (HRCT) scan
- Blood samples (details explained in trial procedure section)
- Safety assessments
    - Vital Signs, Physical Examination
    - ECG
    - Urine Analysis (dipstick and Pregnancy test for Women of Childbearing Potential only)

18.2 Visit 2 (Week 0 + 14 days)

The period from enrolment (V1) to randomization (V2) and 1st dose of tezepelumab / placebo (V2) will be 14 days (maximum 28 days). The following procedures will be performed.

- Review of inclusion and exclusion criteria
- Randomisation
- IMP dosing
- Review of concomitant medication

- Safety and tolerability
    - Vital Signs, Review of AE and SAEs
- Baseline measures for efficacy assessment
    - Bronchoscopy, Nose brush (details explained in trial procedure section)

18.3 Visit 3, 4, 5 & 6 (Weeks 4, 8, 12 & 16; ± 3 days)

The following procedures will be performed.

- Review of concomitant medication
- Safety and tolerability
    - Vital Signs, Review of AE and SAEs, ECG
    - Physical examination
    - IMP administration
- Efficacy assessment
    - Blood samples: FBC, LFT, Urea and Electrolytes, Bone Profile, Creatine Kinase, INR
- Blood samples (Visit 3 and 6 Leicester only) (details explained in trial procedure section)

18.4 Visit 7 (End of treatment) (Week 20 ±3 days)

The following procedures will be performed.

- Safety and tolerability
    - Vital Signs, Review of AE and SAEs
    - Physical examination ECG
    - Urine Analysis (dipstick and Pregnancy test for Women of Childbearing Potential only)
- Efficacy assessment
    - Lung function tests: Pre and Post Bronchodilator FEV1, FEV1/FVC, TLC and DLCO, FeNO testing, Questionnaires, sputum, Exhaled breath print, Blood samples: FBC, LFT, Urea and Electrolytes, Bone Profile, Creatine Kinase, INR)
- Blood samples (details explained in trial procedure section)
- High Resolution Computed Tomography (HRCT) scan

18.5 Visit 8 (Bronchoscopy visit at the end of treatment) (Week 20 ±7 days)

The following procedures will be performed.

- Vital Signs, Review of AE and SAEs
- Concomitant medication check
- Bronchoscopy
- Nose Brushing

18.6 Safety visits (Visit 2.1 & Visit 8.1)

All subjects will be contacted by phone within 3 working-days after both bronchoscopies. This will be a short (15 min) conversation asking about any AE/SAE following the procedure.

19 Trial procedures

19.1 Vital Signs

Heart rate, respiration rate, blood pressure, pulse oximetry, body temperature. No discomfort associated with this. Risk: No particular risk expected.

19.2 ECG

A conventional 12 lead ECG will be performed. The following parameters will be recorded for each ECG: date and time of ECG, heart rate (beats/min), QT (ms), QTcB (ms), sinus rhythm (yes/no), and overall evaluation (normal/abnormal). Risk: No particular risk expected.

19.3 Urine Analysis

(Dipstick and Pregnancy test for Women of Childbearing Potential only)

Urine for Protein/Albumin, Hb/Erythrocytes/Blood, and Glucose will be collected
Risk: No discomfort associated with this.

19.4 Lung function tests

Lung function test will be measured according to ERS/ATS recommendations (26, 27). FEV1, FVC and FEV1/FVC, TLC and DLCO will be recorded in the CRF. Spirometry references: The Global Lung Function Initiative (GLI) equations (28).

At screening V1 and V7 (end of treatment visit), FEV1 will be recorded before and after 4 puffs of salbutamol administered via spacer. Two (instead of four) puffs are allowed if any concern about side effects or safety of the subject.

% Reversibility = (post-BD FEV1- pre-BD FEV1) × 100/pre-BD FEV1

Subjects should withhold their usual maintenance therapies on the day(s) when lung function testing is being performed as below:

- SABA and SAMA: 6 hours
- Twice daily LABA or ICS/LABA: 12 hours
- Once daily LABA and LAMA: 24 hours
- Theophylline within 24 hours

Risk/discomfort: Possible dizziness and light-headedness

19.5 FeNO testing

Measurement of fractional exhaled nitric oxide (FeNO) as per trial SOP manual and ERS/ATS guidelines (30)

Risk/discomfort: No risk or discomfort are associated with this

19.6 Blood Samples

Samples will be processed in accordance with SOPs. Blood will also be collected and processed for research purposes including but not limited to leukocyte phenotyping and transcriptomics. The amount of blood at any given visit will not exceed 70mL and the total amount of blood over the course of the study will not exceed 250mL.
Risk/discomfort: Low risk: May cause transient itching, minor bruise and or discomfort.

19.7 Questionnaires

The following patient reported outcomes will be collected and then transfer to eCRF or entered directly onto eCRF after paper record.

- St. George's Respiratory Questionnaire COPD patients (SGRQ-C)
- COPD Assessment Test (CAT)
- Sinonasal disease: Sino-Nasal Outcome Test-22 (SNOT-22)

Risk/discomfort: No risk

19.8 Exhaled breath print

Breath samples will be taken according to a previously validated protocol (29) that involves 5 minutes of normal breathing and one deep in- and expiration. A total of 500mL exhaled air will be collected for later analysis.

Risk/discomfort: No risk.

19.9 Sputum sample

A sputum sample will be collected via an induced sample collection method, unless this is contraindicated or not tolerated, in which case spontaneous sputum will be obtained. If collected via the induced method, the sample will be collected after inhalation with nebulized saline. Selected sputum plugs will be stored for later mRNA analysis. Sputum processing follow a protocol that secure supernatant before and after adding 0.1% dithiothreitol (details will be outlined in a SOP).

Samples to be collected:

- Supernatant: maximum of 6ml in total Sputum cell pellet: maximum of 8 x 0.5ml in total Sputum plugs: maximum of 2ml

Risk/discomfort: No risk or discomfort are associated with this.

19.10 High Resolution Computed Tomography Scan (HRCT)

HRCT of thorax will be performed at enrolment in the trial and at the end of treatment visit. End of treatment CT scan can be scheduled before the end of treatment bronchoscopy or 5-10 days after the end of treatment bronchoscopy. This will be a standardised protocol between sites and will enable confirmation of underlying diagnosis along with exclusion of alternative diagnosis. It may also allow us to stratify subgroup analyses by CT findings. This will be performed as an outpatient scan with our local CT scanner and reported as a research scan. The CT Chest examination will be done during inspiratory and expiratory phases. The exposure required by the study is additional to routine clinical care.

These scans have a radiation dose of 9.7mSv. The total protocol dose is therefore 19.4 mSv.This is equivalent to 8 years of average natural background radiation in the UK.

Risk/discomfort: Ionising radiation can cause cancer which manifests itself after many years or decades. The risk of developing cancer because of taking part in this study is estimated as 0.1%. For comparison, the natural lifetime cancer incidence in the general population is about 50%.

19.11 Bronchoscopy

Bronchoscopies will be carried out in line with international standards (31) and local guidelines and safety protocols. All participants will receive at least 0.4 mg of salbutamol through a spacer before the bronchoscopy procedure. BAL, brushings and mucosa biopsies will be collected as per the SOP. Subjects will be closely monitored for at least 1 hour after bronchoscopy and will only be discharged when the effects of sedation and local anaesthesia have weaned off. Subjects will be provided with information regarding 24-hour emergency procedures. All subjects will be contacted by phone within 3 working-days after a bronchoscopy.

Risk/discomfort: Common occurring risks (>10%) associated with the procedure are transient fever, cough, chest discomfort and a transient decline in FEV1 (22-24). Potentially more severe complications are rare and include major bleeding (<1%) and pneumothorax (<0.2%) (22,23).

Bronchial biopsy

During bronchoscopy, bronchial biopsies will be obtained. Biopsies (maximum n=8 per patient per visit) will be collected and processed for immunohistochemistry and RNA sequencing. Details for sample collection, handling, processing and storage transportation will be described in a separate SOP.

Bronchial brushings

During bronchoscopy, bronchial brushings will be obtained. Bronchial brushings (a maximum of n=6 per patient per visit) will be collected and processed for cell culture studies and RNA sequencing. Details for sample collection, handling, processing and storage transportation will be described in a separate SOP.

Broncho-alveolar lavage (BAL)

During bronchoscopy BAL (a maximum instillation of 2x50mL per visit) will be collected. BAL will be processed for differential count and explorative analysis as set out under objectives. Details for sample collection, handling, processing and storage transportation will be described in a separate SOP. *Any remaining material to be used for exploratory analysis including but not limited to leukocyte phenotyping.*

19.12 RNA transcriptomics

Brush biopsies, bronchial biopsies, BAL and blood samples will be subject to RNA sequencing and analysed according to objectives and outcome measures and procedure details are described in separate SOPs. Analysis of RNA transcriptomics include unbiased analysis looking into transcripts most changed with treatment with no pre-selection of genes. This is intended to be performed on whole genome.

19.13 Biomarker Analysis

The sample materials will be stored within an HTA accredited laboratory at the research site and processed. The treatment, communication, and the transfer of samples will at all times comply with the Data Protection Act as well as the provisions of Regulation (EU) 2016/679 (General Data Protection Regulation (GDPR)) and national legislations.

Should any excess material be left, this will be stored in the Danish National Biobank (tissue bank) for future analysis in relation to COPD. Maximum storage duration will be 15 years. At this point all material will be destroyed. The use of biobank material will only be used in accordance with the Informed Consent given by the patient. In case a subject withdraws Informed Consent for donated biological samples, any samples or data donated up to that point will be kept but no further samples will be obtained.

20 Withdrawal criteria

Every effort should be made by the research team to keep the participants in the trial. Withdrawal of consent details and reasons (if known) should be recorded on the CRF and in the medical notes.

The investigator may discontinue treatment of a participant at any time if they are not compliant with the trial protocol or to protect the participant's safety and well-being after consultation with the CI.

If a participant fails to attend the clinic for trial visit, the research team should contact the participant and re-schedule the missed visit within a week. If the participant cannot be contacted or misses his/her next appointment, the research team should make every effort to contact the participant again by phone and letter. If the participant still cannot be contacted, then he/she will be recorded as 'lost to follow-up'. A participant will be considered to have completed the trial if they continue to take trial treatment until final visit.

<u>Criteria for withdrawal</u>

1. Subject is unwilling to continue in the clinical trial

2. Continuing participation in the study may be medically harmful to the subject (as judged by the investigator) (e.g., deterioration in lung function)

3. Lost to follow-up

4. Pregnancy or lack of adequate contraception in women of childbearing potential

7. Unblinding

In case of hospitalization, the investigator should be notified immediately. Withdrawal due to admission will be evaluated case by case based on the potential harm to the subject by continuing in the study, and potential influence on defined endpoints (as judged by the investigator).

In case of withdrawal, the subject will be asked if they want to attend a visit in the clinic within two weeks from the date of withdrawal. At this visit, a study nurse will include (but not restricted to):

CAT questionnaire, Vital signs, Physical examination, FeNO, Spirometry, Urine Pregnancy test in (WOCBP), Urine analysis, Blood samples, Concomitant medication, Review of AE/SAEs, ECG

21 Pandemic Adaptations

Local adaptations have been implemented at all sites to minimise risk for COVID-19 and possible future pandemics. Home monitoring has been introduced to reduce reliance on in-clinic assessments. Tests such as blood tests could be switched to home visits if required in the future. If we were to experience another pandemic or further risks of COVID-19, then a risk assessment will be conducted in partnership with the Sponsor. Amendments and appropriate strategies will be put in place accordingly.

22 Storage and analysis of research samples

The following blood samples will be processed by the research site central pathology labs and will be collected at room temperature from the trial site. These samples are not stored for the purposes of the study and are either used or destroyed during the testing process.

Haematology/Haemostasis (whole blood)	Clinical Chemistry (serum or plasma)
Haemoglobin (Hb)	Creatinine
Leukocyte differential count (absolute count)	Bilirubin, total
Leukocyte count	Alkaline phosphatase (ALP)
Platelet count	Alanine transaminase (ALT)
INR	Albumin
	Potassium
	Calcium
	Sodium

	Creatine kinase (CK)

The following samples will be analysed for research purposes and processed at the respective research site or laboratory nominated by the Sponsor. Samples will be kept on ice or stored in monitored -20°C or -80°C or -150°C freezers prior to analysis, unless contraindicated. All samples are stored in accordance with the Human Tissue Act 2014.

- Urine sample
- Sputum sample (collected on ice and processed at 4°C as soon as possible and within 2 hours of expectoration) including RNA
- Nasal brush sample
- Blood samples
- Bronchoscopy samples

23 End of Trial

This trial will end when the specified number of participants have been recruited, all participants have completed their last follow up visit, data validation has taken place and the database is locked and statistical analysis complete.

The trial will be stopped prematurely if:

- Mandated by the Ethics Committee or the Sponsor
- Mandated by the Medicines and Healthcare products Regulatory Agency (MHRA)
- Funding for the trial ceases

24 Trial Treatments

24.1 Name and description of investigational medicinal product(s)

The investigational medicinal product (IMP) for this trial is tezepelumab. It is a human monoclonal antibody which blocks thymic stromal lymphopoietin, an epithelial cytokine implicated in the pathogenesis of asthma and COPD.

IMP	Dosage Form and Strength	Manufacturer
Tezepelumab	Sterile, preservative-free solution in vials for SC administration	Amgen/AstraZeneca

24.2 Regulatory status of the drug

Tezepelumab does not currently have a marketing authorisation in the UK. It is under review by NICE for use in asthma. In July 2022 it was recommended for EU approval by the Committee for Medicinal Products for Human Use (CHMP) for use as an add-on maintenance treatment in adults and adolescents 12 years and older with severe asthma who are inadequately controlled despite high dose inhaled corticosteroids plus another medicinal product for maintenance treatment.

24.3 Drug storage and supply

Tezepelumab and placebo will be issued by the international manufacturer AstraZeneca. It will be delivered to the central pharmacy for the Capital Region of Denmark, who will be responsible for receiving and labelling IMP for all sites. This will be according to a stratified randomisation list prepared by computerised system. Both placebo and drug will be relabelled at the central pharmacy according to a randomisation code and will therefore be received blinded to the study site.

Tezepelumab should be stored in a fridge between 2°C to 8°C. If needed it may be kept at room temperature for a maximum of 30 days. Once it has reached room temperature it should not be returned to the fridge. It should be stored in the original carton to protect from light until time of use.

24.4 Preparation and labelling of Investigational Medicinal Product

The Central Pharmacy in the Region of Copenhagen, Denmark will receive and label the study drug and placebo and will label in accordance with Good Manufacturing Practice (GMP). The IMP will be sourced from a site outside EU and a QP-declaration will be issued by the Central Pharmacy.

24.5 Dosage schedules

IMP or placebo will be administered subcutaneously (SC) at visits 2, 3, 4, 5 and 6. This is 5 doses in total, 4-week interval between each. The dosage will remain the same through the trial. Participants should not receive any two consecutive doses of IMP less than 3 weeks apart. Each visit window includes 3 days either side, except visit 2 as this is the first IMP dose.

If a visit is missed and cannot be rearranged within the visit window, one dose of IMP can be skipped. If a further dose is missed, the PI must be contacted to decide if the participant can continue in the trial.

24.6 Dosage modifications

There are no dose modifications for tezepelumab. There are no intended treatment breaks/drug free holidays. If a dose cannot be given within the study window it can be

skipped. If a participant has missed two consecutive doses, then the PI will be contacted to decide on ongoing involvement in the trial.

24.7 Known drug reactions and interaction with other therapies

No formal drug interaction studies have been performed with tezepelumab. However, no interactions have been reported in previous trials.

24.8 Concomitant medication

Prohibited Medication/Class of drug:

- Immunosuppressive medication (e.g., methotrexate, troleandomycin, oral gold, cyclosporine, azathioprine, intramuscular long-acting depot corticosteroid, or any experimental anti-inflammatory therapy)
- Any marketed (including omalizumab) or investigational biologic agent
- Allergen immunotherapy (new or not on stable doses)
- Phosphodiesterase 4 inhibitors
- Regular oral glucocorticoids as per exclusion criteria
- Use of anticoagulation treatment (e.g., warfarin, clopidogrel) that cannot be withheld prior to bronchoscopy

24.9 Trial restrictions

There are some restrictions on usual medication therapies on visit dates which involve lung function testing (visit 1 and visit 7).

The following medications should be withheld:

- SAMA and SABA: 6 hours
- Twice daily LABA or ICCS/LABA: 12 hours
- Once daily LABA or LAMA: 24 hours
- Theophylline: 24 hours

Females of childbearing potential who are sexually active with a non-sterilized male partner must use a highly effective method of contraception from the time informed consent is obtained and must agree to continue using such precautions through Week 20 of the study; cessation of contraception after this point should be discussed with a responsible physician. Periodic abstinence, the rhythm method, and the withdrawal method are not acceptable methods of contraception (see Table 1 for highly effective methods of contraception). Females of childbearing potential are defined as those who are not surgically sterile (i.e., bilateral tubal ligation, bilateral oophorectomy, or complete hysterectomy) or postmenopausal (defined as 12 months with no menses without an alternative medical cause).

Barrier Methods	Hormonal methods
• Vasectomised partner	• Combined (oestrogen and

• Intrauterine device • Sexual abstinence • Bilateral tubal occlusion • Intra-uterine hormone-releasing system (IUS)	progesterone containing) hormonal contraception associated with inhibition of ovulation • Oral • Transdermal • Intravaginal • Progestogen-only hormonal contraception associated with inhibition of ovulation • Oral • Injectable • Implantable

24.10 Assessment of compliance with treatment

The IMP will be administered by a qualified healthcare professional at each study site. The injection site will be recorded in the eCRF each visit.

Participants will be observed for a minimum of 1 hour after administration of the first two doses of IMP. For the remaining doses, participants will be observed for a minimum of 30 minutes post dose.

24.11 Late or missed IP dosing

Patients should not receive any two consecutive IP doses closer than 3 weeks apart. If it is not possible to keep a study visit within the specified time window the IP administration should be skipped. If a subject skips 2 consecutive IP administrations, the PI should be contacted to discuss further participation.

24.12 Name and description of each Non-Investigational Medicinal Product (NIMP)

The matching placebo is presented as a sterile, single-dose, solution in a vial, APFS or AI identical to that for tezepelumab. The placebo composition is 0.7% (w/v) sodium carboxy methyl cellulose, 10 mM acetate, 250 mM L-proline, 0.01% (w/v) polysorbate 80, pH 5.0. Tezepelumab/placebo will be administered by a member of the study team.

25 Safety Assessments

25.1 Operational definitions for adverse events

All adverse events should be accurately recorded on the Adverse Event Form once the participant has provided their consent to be screened for the trial.

Adverse Event (AE) is any untoward medical occurrence in a patient, not associated with the medicinal product/study medication, occurring prior to administration of study medication.

Events meeting the definition of an AE include:

- Exacerbation of a chronic condition excluding asthma or COPD as the primary cause
- New conditions detected or diagnosed after trial treatment administration even though it may have been present prior to the start of the trial
- Signs, symptoms, or the clinical sequelae of a suspected interaction
- Signs, symptoms, or the clinical sequelae of a suspected overdose of trial treatment

Events that do not meet the definition of an AE include:

- Medical or surgical procedure (e.g., endoscopy, appendicectomy); the condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (social admission to a hospital).

Adverse Reaction (AR) is any untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant.

Serious Adverse Event (SAE) is defined as any adverse event in a trial participant that:

- Results in death
- Is life threatening (the subject was at risk of death at the time of event), with the exclusion of COPD exacerbations
- Requires hospitalisation or prolongation of an existing hospitalisation, with the exclusion of COPD exacerbations
- Results in persistent or significant disability or incapacity
- Consists of a congenital anomaly or birth defect
- Other serious Important Medical Event - an event that may not be immediately life threatening or result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the outcomes listed above should be considered.

The following events will be excluded from SAE reporting:

- Routine elective/social admission not linked with exacerbation of airway disease
- Any emergency room visit due to worsening airway disease symptoms but not resulting in a hospital admission.

Adverse event of special interest (AESI): An adverse event of special interest is one of scientific and medical concern specific to the study product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor can be appropriate. Such an event might warrant further investigation in order to characterize and understand it. Depending on the nature of the event, rapid communication by the trial sponsor to other

parties (e.g., regulators) might also be warranted.

- Episodes of pneumonia (with proven evidence of radiological consolidation changes) will be recorded as AESI.

Suspected Unexpected Serious Adverse Reaction (SUSAR) is defined as an adverse reaction that is both unexpected (not consistent with the approved Reference Safety Information) and meets the definitions of a Serious Adverse Event**.**

25.2 Recording and reporting of SAEs, SARs AND SUSARs

All SAEs / SUSARs occurring following first administration of IMP until the final trial visit (week 20) or 30 days post cessation of trial treatment where this occurs earlier than intended must be recorded on the SAE Form. This should be sent to the Sponsor immediately and within 24 hours of the research staff becoming aware of the event. The Sponsor will review and track all SAEs. Once all resulting queries have been resolved, the Sponsor will send an acknowledgement of the closure of the SAE, all correspondence and signed SAE forms will be retained in the Investigator Site File, within the Trial Master File and electronically on the database.

SAEs will be reported to AstraZeneca at the time they are reported to any health authority, and at least within three-month intervals.

For each SAEs / SUSARs the following information will be collected:

- Full details in medical terms and case description
- Event duration (start and end dates when deemed serious, if applicable)
- Action taken
- Outcome
- Severity criteria
- Causality (i.e., how directly related to trial treatment/investigation)
- In the opinion of the investigator whether the event would be considered anticipated (i.e., expected).

Any change of condition or other follow-up information should be sent to the Sponsor promptly and within 24 hours of the information becoming available. Events will be followed up until the event has resolved or an outcome has been reached.

All SAEs assigned by the CI or delegate (or following Sponsor review) as both suspected to be related to IMP-treatment and unexpected will be classified as SUSARs and will be subject to expedited reporting to the Medicines and Healthcare Products Regulatory Agency (MHRA). The sponsor will inform the MHRA and the REC of SUSARs within the required expedited reporting timescales. Fatal or life-

threatening SUSARs must be reported within 7 days and all other SUSARs within 15 days.

26 Responsibilities

Coordinating Investigator (CI) / delegate or independent clinical reviewer:

1. Clinical oversight of the safety of patients participating in the trial, including an ongoing review of the risk / benefit.
2. Using medical judgement in assigning AE severity, causality and whether the event was anticipated (in line with the Reference Safety Information) where it has not been possible to obtain local medical assessment.
3. Immediate review of all SUSARs.
4. Review of specific SAEs and SARs in accordance with the trial risk assessment and protocol as detailed in the Trial Monitoring Plan.
5. Assigning Body System coding to all AEs, SAEs and SARs.
6. Preparing and final sign off the Development Safety Update Report (DSUR).

Chief Investigator (CI) or suitably trained delegate:

1. Checking for AEs when participants attend for treatment / follow-up.
2. Using medical judgement in assigning seriousness, causality and whether the event was anticipated using the Reference Safety Information approved for the trial.
3. Ensuring that all SAEs are recorded and reported to the sponsor immediately and within 24 hours of becoming aware of the event and provide further follow-up information as soon as available. Ensuring that SAEs are chased with Sponsor if a record of receipt is not received within 7 working days of initial reporting.
4. Ensuring that AEs and ARs are recorded and reported to the sponsor in line with the requirements of the protocol.

Sponsor: (NB where relevant these can be delegated to CI)

1. Central data collection and verification of AEs, ARs, SAEs, SARs and SUSARs according to the trial protocol onto a database.
2. Expedited reporting of SUSARs to the Competent Authority (MHRA in UK) and REC within required timelines.
3. Notifying Investigators of SUSARs that occur within the trial.
4. Checking for (annually) and notifying PIs of updates to the Reference Safety Information for the trial.
5. Reviewing the DSUR in collaboration with the CI and ensuring timely submission to the MHRA and REC.

27 Trial oversight committee

Trial Management Group (TMG)

The TMG will be responsible for oversight of the day-to-day management of the trial through feedback and input from each participating site. The TMG is responsible for all aspects of the trial (including recruitment rate, budget management, safety reporting, protocol compliance etc.) and for ensuring appropriate action is taken to safeguard trial participants and the quality of the trial. The TMG will be chaired by the Chief Investigator.

Role of Sponsor

The sponsor for the whole trial (Copenhagen site) will be responsible for all aspects of the trial as per ICH-GCP. Data and samples will be shared between the Sponsor and Funder(s) who will both be provided with all Protocols and SOPs for their comment and input and regular reports.

The Sponsor will have overall responsibility for the trial tasks, obtaining and complying with the requirements of the relevant regulatory bodies; collection, management and analysis., The Sponsor will cooperate with the University of Leicester with interpretation of data; writing of any reports; the decision to submit reports for publication, including who will have ultimate authority over each of these activities. It will work closely with the Chief Investigators, all members of the Trial Management Group (TMG) and Industrial collaborators

28 Notification of deaths

Deaths will be reported to the Sponsor promptly and certainly within 24 hours of the investigator becoming aware of it. Severe exacerbation events are associated with a mortality rate of 12%.

29 Pregnancy reporting

Participants will not be eligible to continue in this trial if they are pregnant, breastfeeding or intend to become pregnant.

A woman is defined as being of childbearing potential (WOCBP), (i.e., fertile), following menarche and until becoming post-menopausal, unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.

Limited data is available on the use of the IMPs during pregnancy. Animal studies indicate reproductive toxicity at exposures which are not clinically relevant. The potential for harm to a human foetus is unknown. The manufacturer advises to avoid use during pregnancy unless the benefit outweighs risk.

MHRA guidelines (http://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf accessed from https://www.gov.uk/government/publications/common-issues-identified-during-clinical-

trial-applications/common-issues-clinical) For use of IMPs likely to carry a low risk of human teratogenicity/fetotoxicity advise that women of child-bearing potential (WOCBP) must have a negative highly sensitive urine pregnancy test or negative blood serum pregnancy test performed prior to first administration of trial treatment, a negative urine pregnancy test at each subsequent visit prior to dosing and must agree to use an acceptable method of birth control for the duration of the trial.

However, given the average age range of the disease population, this will not be a significant proportion of participants. Additional testing will also occur at any time during the trial if a menstrual period is missed or as required by local law.

Acceptable birth control methods that result in a failure rate of more than 1% per year include:

- progestogen-only oral hormonal contraception, where inhibition of ovulation is not the primary mode of action
- male or female condom with or without spermicide
- cap, diaphragm, or sponge with spermicide

Additional, highly effective contraceptive measures (i.e., a failure rate of less than 1% per year when used consistently and correctly) may also be used. These are defined as:

- combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
    - oral
    - intravaginal
    - transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation:
    - oral
    - injectable
    - implantable
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)
- bilateral tubal occlusion
- vasectomised partner (if partner is the sole sexual partner of the WOCBP trial participant and that the vasectomised partner has received medical assessment of the surgical success)
- Sexual abstinence (defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments).

All pregnancies within the trial (either the trial participant or the participant's partner, with consent) should be reported to the PI and the Sponsor using the relevant Pregnancy Reporting Form immediately and within 24 hours of notification.

Pregnancy is not considered an AE unless a negative or consequential outcome is recorded for the mother or child/foetus. If the outcome meets the serious criteria, this would be considered an SAE. The pregnancy must be followed up to determine outcome (including premature termination) and status of mother and child in accordance with Sponsor and local reporting requirements and timelines.

Any SAE occurring in association with a pregnancy, brought to the investigator's attention after the participant has completed the trial, and considered by the investigator as possibly related to the trial treatment, must be promptly reported to sponsor in accordance with Sponsor and local reporting requirements and timelines.

30 Overdose

In case of overdose (or suspected overdose), the subject will be thoroughly evaluated by the investigator, and, if necessary, monitored for as long as needed (as judged by the investigator). There is potential for hepatic function abnormality (3-fold or greater elevations of the upper limits of normal of alanine transaminase or aspartate transaminase), which will be reported.

31 Reporting Urgent Safety measures

If any urgent safety measures are taken the CI/Sponsor shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the MHRA and the relevant REC of the measures taken and the circumstances giving rise to those measures.

32 Follow-up after adverse reactions

Following an adverse reaction (AR) the participants will be followed-up for an appropriate length of time as dictated by the nature of AR and their clinical needs. ARs will continue to be recorded and reported for up to 4 weeks after the last dose of IMP has been administered. Any SUSAR will need to be reported to the Sponsor irrespective of how long after IMP administration the reaction has occurred until resolved.

33 Development safety update reports

Within 60 days following the anniversary of the authorisation date for the trial, a Development Safety Update Reports (DSURs) will be sent by the Chief Investigator to the MHRA and the Research Ethics Committee. A copy of the report will also be sent to the trial funder/drug supplier, and all host organisations. The Trial Management team will prepare the DSUR report on behalf of the CI and submit to the Sponsor who is responsible for reporting to the Competent Authority (MHRA) within the specified time frame.

34 Statistics and Data Analysis

34.1 Sample size calculation

The change in cell counts per mm2 will be defined as the ratio of the treatment period (change in geometric means from baseline to week-24). The sample size estimate is calculated with respect to change in eosinophil cells per mm2. We assume that the distribution of (log transformed) eosinophil cell counts is approximately normal and that the standard deviation in the different treatment groups is approx. equal. In the UPSTREAM

study (17) examining changes in submucosal eosinophils with tezepelumab in patients with asthma a pooled SD=2.17 for the change in log eosinophils was observed. In the CASCADE study (18) also studying the change in submucosal eosinophils with tezepelumab in asthma the sample size calculation was based on a pooled SD=1.97. The ratio between geometric means in eosinophils in the overall population in the two studies were 0.20 and 0.15, respectively, corresponding to fold changes in effect of 5 and 6.67 comparing tezepelumab to placebo. In subgroups of patients with low eosinophils the ratios between geometric means were 0.15 and 0.08, corresponding to a fold change in effects of 6.72 and 12.5, in the two studies, respectively. The constant of 1 will be added prior to the log transformation to avoid zero values.

We plan to randomize 80 patients, 40 patients in the active group and 40 patients in the placebo group. This calculation is based on assuming a common SD of 2.17 on the log scale, a fold change in effect of 4 and using two-tailed t-tests of difference between means (on the log scale) with 80% power and $\alpha = 0.05$.

34.2 Methods for statistical analyses

34.2.1 Analysis of the primary variable(s)

The primary endpoint will be analysed by calculating the within-subject change from baseline to week 20, expressed as a ratio, in number of airway submucosal inflammatory cells. The null hypothesis is that the ratio between tezepelumab and placebo equals 1 and will be tested against the alternative hypothesis that the ratio is not equal to 1. The test will be performed using an ANCOVA model with blood eosinophil strata (high/low), treatment, and baseline value.

All outcomes will be log-transformed, and we will estimate geometric means and ratios between geometric means with 95% confidence intervals.

The data will undergo intention to treat analysis.

34.2.2 Analysis of the secondary variable(s)

Secondary endpoints will be analysed in similar models as described for the primary outcome. For normally distributed variables, we will analyse mean changes from baseline to week 20 and differences in means with 95% confidence intervals. For time course data such as lung function and PROMs we may use mixed effects methods for analysis.

34.2.3 Interim analysis

No interim analysis is planned.

34.3 Participant population

Definitions of analysis sets

Populations for analysis: Patients who have completed 20 weeks of treatment and with evaluable bronchoscopy biopsies at baseline and week-20 will be included in the primary analysis. Only patients with complete data for an endpoint will be included in the analysis of the specific endpoint.

Safety population: All subjects who receive at least one dose of tezepelumab/placebo.

35 Data management

Bispebjerg Hospital Site, Denmark will be the Data Controller for the study and will be responsible for data management for the trial. A REDCap electronic CRF will be hosted by Bispebjerg Hospital.

35.1 Data collection tools and source document identification

Source Data is defined as the first-place data is recorded; this will include:

- Medical Records
- Paper CRFs
- Electronic CRFs via REDCap
- Laboratory Reports
- Printouts from equipment (i.e., spirometry)
- Participant reported outcome questionnaires

Data collection tools will comprise of:

- REDCap Database (transcribed from CRFs) and direct source data entry
- Participant reported outcome questionnaires

The research team will seek consent from participants to re-contact them about taking part in future ethically approved research, some of which may be based on their results, phenotype, genotype, and response to treatment following the completion of the current trial. This is outlined in the PIS and consent form and participants will be able to opt out without affecting their involvement in the trial.

35.2 Data handling and record keeping

For the purposes of this clinical trial, Bispebjerg Hospital will be the Data Controller across all study sites. Before research collaborations can be initiated and before transfer of data or biological samples, a collaboration agreement, a data processor agreement, and a material transfer agreement in accordance with guidelines, legislation and after approval by The Danish Data Protection Agency will be signed by Bispebjerg Hospital and the collaborator. Any transfer or storage of data will be handled after approval from and according to guidelines and legislation by The Danish Data Protection Agency, including the Data

Protection Act and GDPR and with respect to chapter 5 of the GDPR for transfers to United Kingdom. All agreements relating to the study will be available in the TMF.

Records of trial participant data will be made on trial specific CRFs. A sticker will be placed on the cover of the notes (or inside cover) detailing the trial title, contact details of the PI and the fact that the notes should not be destroyed for 25 years from the end of the trial. All trial visit summaries and AEs will be recorded in the hospital notes (if recruited from secondary care).

During the trial all eCRFs will be stored in a secure online web, REDCap, accessible to trial site and sponsor staff. Each enrolled participant will be allocated a unique trial ID so that the eCRFs and electronic database remains pseudonymised.

36 Documentation storage, access, and security

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections- in line with participant consent.

All study documentation containing identifiable patient data will be managed in accordance with ICH GCP, the UK Policy Framework for Health and Social Care, the Data Protection Act and the General Data Protection Regulation (GDPR).

Information will only be obtained from the participant if necessary for the trial. The trial team will use the participant's name, NHS number and contact details, to contact them about participating in the trial and to make sure that relevant information about the trial is recorded, to ensure patient care and for quality assurance purposes. The trial team will pass these details to the Sponsor, along with the information collected from the participant and their medical records. The only people in the Sponsor organisation who will have access to identifiable information will be those requiring it for trial purposes or for audit of the data collection process. Those trial team members only involved in data analysis will not have access to identifiable information.

37 Archiving

Data will be entered by trained members of the research team at site via a browser-based web application, Research Electronic Data Capture (REDCap). The latter will be used for storing electronic CRF across all study sites. The study sites in the UK will have user-access to source data files in REDCap and will be able to enter new data but will not have user rights or be able to delete or export data; this right will be held by the Data Controller.

38 Monitoring, audit & inspection

The Sponsor operates a risk-based monitoring and audit programme, to which this trial will be subject.

As part of the quality management process, the trial will be subject to a risk assessment developed by the Sponsor in collaboration with the Chief Investigator and in accordance with the level of risk identified to participant safety, integrity of the trial and trial data validity.

The trial may also be subject to triggered monitoring conducted by the Sponsor, and/or audit by the Sponsor delegate in accordance with the monitoring plan.

All source data, trial documents, and participant notes will be made available for monitoring, audits, and inspections by the Sponsor (or their delegate), NHS Host Organisation, and the regulatory authorities.

39 Ethical and regulatory considerations

39.1 Research Ethics Committee (REC) review & reports

Before the start of the trial, approval will be sought from a REC and the HRA for the trial protocol, informed consent forms and other relevant documents e.g., advertisements. Any amendments that require review by REC will not be implemented until the relevant approvals have been issued and Sponsor Green Light has been provided.
All correspondence with the REC and HRA will be retained in the Trial Master File. The CI will be responsible for informing the REC of the end of trial. After completion of the trial, CI will submit a final report with the results to the REC.

The University of Leicester, as delegated by the Sponsor, is responsible for obtaining regulatory approvals for the study and subsequent amendments. This incorporates authorising the submissions and amendments on IRAS on behalf of Sponsor.

39.2 Peer review

The trial set up and conduct has been collaboratively reviewed by trial scientific steering group organised by Copenhagen site that includes clinicians and senior scientists. This review team have had the protocol reviewed by their internal senior management along with two external and independent colleagues and approved for support.

39.3 Public and Patient Involvement

The trial will be included amongst the trials and studies discussed with the PPI group within the NIHR Leicester Respiratory BRC.

39.4 Regulatory Compliance

The trial will not commence until Clinical Trial Authorisation (CTA) is obtained from the MHRA, HRA and REC, and Sponsor Green Light has been issued following Confirmation of Capacity and Capability by each host NHS Organisation and PIC. The protocol and trial conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004 and any relevant amendments.

The trial will be conducted in accordance with the UK Policy Framework for Health and Social Care Research (2017) and the Medicines for Human Use (Clinical Trials) Regulations, 2004. The Sponsor will be responsible for checking research governance arrangements.

40 Protocol compliance

Participants are required to complete the scheduled clinic visits within the time windows allowed in the protocol.

Any visits completed outside of the trial window period must be recorded as protocol deviations. Participants will be permitted where required, up to a 14-day treatment delay to allow for adverse events, holidays or other unexpected events.

Depending on the nature of an AE/SAE it will be left to the clinician's discretion to determine if the patient should be withdrawn from the study. Participants that are delayed for more than 21 days will be withdrawn.

Persistent deviations would indicate potential non-compliance and will have to be discussed with the Chief Investigator and the Sponsor regarding possible withdrawal. Those participants who are planning to take more than a 21-day consecutive holiday will not be included in the trial, and this will be determined at screening.

41 Notification of Serious Breaches to GCP and/or the protocol

Any serious breach (a breach which is likely to effect to a significant degree the safety or physical or mental integrity of the participants of the trial; or the scientific value of the trial) will be reported to Sponsor immediately and within 24 hours of discovery.

42 Data protection and patient confidentiality

All investigators and trial site staff will comply with the requirements of relevant legislation with regards to the collection, storage, processing, and disclosure of personal information for the Sponsor.

The personal information that is collected will be kept secure and maintained by:

- The creation of a unique trial ID number, depersonalised data where the participant's identifying information is replaced by an unrelated sequence of characters;
- Secure maintenance of the data, in both electronic and paper forms and the linking code in separate locations;
- Limiting access to the minimum number of individuals necessary for quality control, audit, and analysis;
- Paper based pseudonymised trial records will be stored in locked filing cabinets within a locked office. Electronic records will be stored on secure trial web database
- The database will be password protected and only researchers collecting data will have access. All data collected during the trial will be stored pseudonymously;
- Participants' contact details will be held separate to the trial visit data and used to arrange data collection visits by the research team or direct care team.

43 Indemnity

Insurance for the trial will be provided by the University of Leicester on behalf of the Sponsor. If a trial participant wishes to make a complaint about any aspects of the way they have been treated or approached during the research project, the standard National Health Service complaint system will be available to them, the contact details for which are in the participant information sheet.

44 Post-trial care

Incidental findings from routine assessments (e.g., abnormally elevated LFTs) will be referred to the participant's GP or suitable clinician for follow up. Incidental findings from non-routine/exploratory assessments, particularly those analysed after the completion of the trial, will not be referred for investigation.

45 Access to the final trial dataset

PI and their appointed deputies will have access to the analysed trial dataset following execution of the SAP and completion of the ETR (end of trial report).

46 Dissemination policy

A publication plan will be written by the TMG during the trial with the sponsor and funder approvals. It is envisaged that the results of the trial will be published in the relevant peer-reviewed journals. Acknowledgement of any supporting organisations, including funders and University of Leicester will be included.

At the end of the trial participants will be invited, where possible, to attend an online dissemination event to inform them of the results of the trial and to thank them for their participation.

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48 Appendix

Schedule of trial procedures

Activity	**Screening/ run-in**	**Randomisation**		**Treatment period**				**End Of Treatment**		
Visit	**V1**[1]	**V2¤**	**V2.1¤**	**V3**	**V4**	**V5**	**V6**	**V7**[1]	**V8**	**V8.1¤**
Week	-2	0	**V2¤** +3Ds	4	8	12	16	20		**V8** +3Ds
Visit window (days)	-14	+14		±3	±3	±3	±3	±3	±7	
IMP administration		x		x	x	x	x			
Informed consent	x									
Eligibility/inclusion & exclusion criteria	x	x								
Demographic information	x									
Randomization		x								
Medical history	X									
Exacerbation Log	x	x		x	x	x	x	x		
Weight and height	x									
Physical examination	x			x	x	x	x	x		
Vital signs	x	x		x	x	x	x	x	x	
Spirometry (Pre-BD)	x[4]							x		
Spirometry (Post-BD)	x							x		
TLC, DLCO	x							x		
FeNO	x							x		
Exhaled breathprint	x							x		

Sputum sample	x							x[2]		
Blood samples - FBC, LFT, U&E, bone profile, CK, INR	x			x	x	x	x	x		
Blood samples	x			x[3]			x[3]	x		
Bronchoscopy		x							x[#]	
Nasal brush		x							x	
Questionnaires incl. CAT, SGRQ-C, SNOT-22	x							x		
Pregnancy test (for WOCBP only)	x							x		
ECG	x			x	x	x	x	x		
HRCT*	x							x		
Urine analysis	x							x		
Concomitant medication	x	x		x	x	x	x	x	x	
Review AE/SAE		x	x	x	x	x	x	x	x	x
eCRF completion including data transfer	x	x	x	x	x	x	x	x	x	x

[1]Visit 1 and 7 can be split in 2 visits

#Procedures will only be performed at week-20 if done and of acceptable quality at screening/run-in.

* Can be performed on a separate day from the other V1 and V7 procedures.

[2]Can be repeated as part of a second V7 in case the EOT sputum was unsuccessful on first try

[3] Leicester only

[4] Participants need to withhold inhalers prior to pre-bronchodilator spirometry. At visit 1 participants will be given a choice of either i) Providing verbal consent to attend visit 1 after withholding inhalers. Full documentation will be made in the participant medical records and

CRF, or ii) Visit 1 can be split over 2 visits. Participant completes informed consent on the first visit and pre-bronchodilator spirometry is completed at the second visit.

¤Patients will receive a call within 3 working-days after bronchoscopy for safety monitoring

Ds = Days

48.1 Appendix 2: Trial Data Flow Diagram

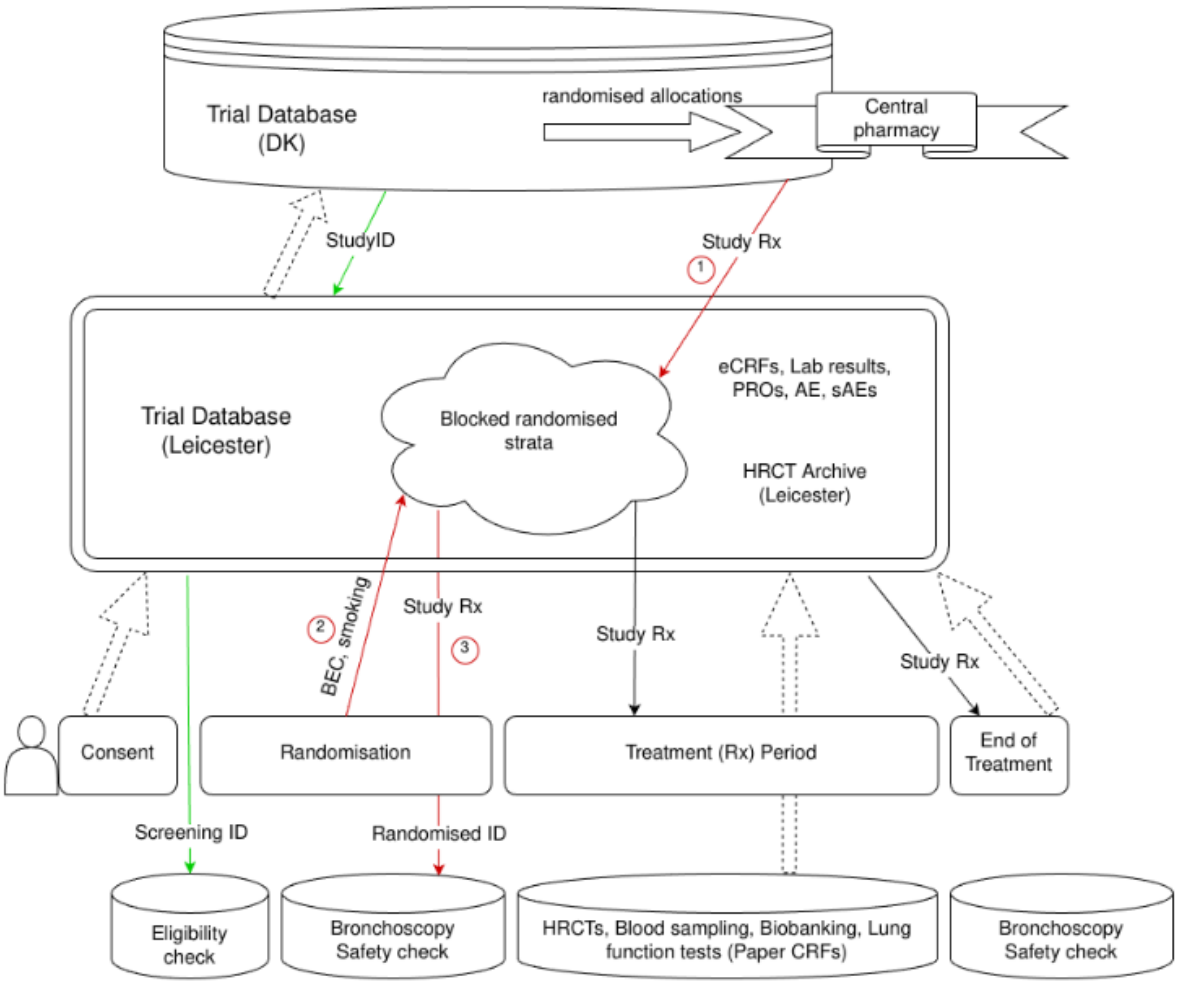

Figure: Data flow Chart

Abbreviations: BEC = Baseline Eosinophil Count, AE = Adverse events, sAEs = Serious Adverse Events, CRF= Case Report Forms, PROs = Patient Reported Outcomes, HRCT = High-Resolution Computed Tomography