Imperial College London

Imperial College Healthcare NHS Trust

ICSAVA

Understanding the role of inhaled corticosteroids (ICS) on vascular ageing and cardiovascular comorbidities in COPD

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STUDY COORDINATION CENTRE: ICL Research Management team

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Sponsor

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

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This protocol describes the ICSAVA study and provides information about procedures for entering participants. The protocol should not be used as a guide for the treatment of other participants; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study, but centres entering participants for the first time are advised to contact the trials centre to confirm they have the most recent version.

Problems relating to this trial should be referred, in the first instance, to the study coordination centre.

This trial 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 the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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GLOSSARY OF ABBREVIATIONS

AIx	Augmentation Index

BOEC	Blood Outgrowth Endothelial Cells
BP	Blood pressure
CAT	COPD Assessment Test
COPD	Chronic Obstructive Pulmonary Disease
CVD	Cardiovascular Disease
CXR	Chest X-Ray
DDR	DNA damage response
DLCO	Diffusing capacity of the lungs for carbon monoxide
EC	Endothelial cells
ECFC	Endothelial Colony Forming Cells
ECHO	Echocardiogram
EGM-2	Endothelial growth medium-2
EndoPAT	Endothelial peripheral arterial tonometry
FBS	Fetal bovine serum
FEV1	Forced expiratory volume in the first second
FRC	Functional Residual Capacity
FVC	Forced vital capacity
HRCT	High-resolution computed tomography
HUVEC	Human umbilical vein endothelial cells
IF	Immunofluorescence
miRNA	microRNA
MRC	Medical Research Council
MRI	Magnetic resonance imaging
RAAS	Renin Angiotensin Aldosterone System
RHI	Reactive Hyperaemia Index

SAP	Statistical analysis plan
TLC	Total lung capacity
WB	Western blotting

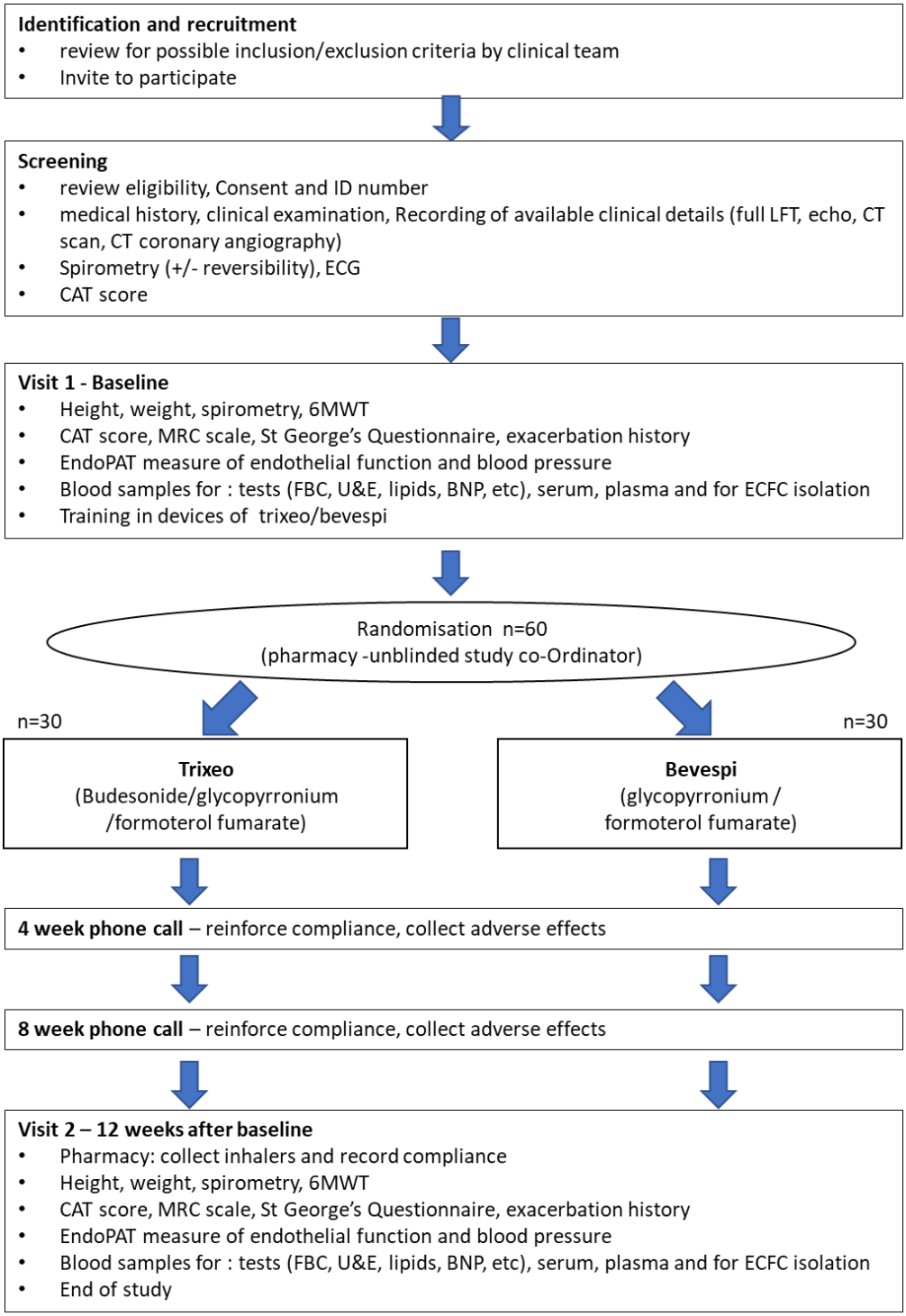
KEYWORDS

Endothelial progenitor cells, COPD, ageing, senescence, DNA damage, endothelial dysfunction, cardiovascular disease, EndoPAT, endothelial colony forming cells

STUDY SUMMARY

TITLE	**Understanding the role of inhaled corticosteroids (ICS) on vascular ageing and cardiovascular comorbidities in COPD**
DESIGN	An exploratory, assessor blind, parallel group, randomised controlled trial
AIMS	To investigate whether inhaled corticosteroids (budesonide) exhibit a protective effect on senescence in endothelial progenitors in COPD, promoting endothelial homeostasis and vascular health and protecting against cardiovascular disease.
OUTCOME MEASURES	1. To investigate the protective effect of ICS/triple combination on endothelial senescence in patients with COPD using ECFC, and correlate molecular with clinical readouts of endothelial dysfunction. 2. To identify secretome and epigenetic dysfunction linked to senescence and effect of treatment with ICS. 3. To confirm the protective effect of ICS/triple combination on endothelial senescence *ex-vivo* using ECFC (2D and 3D culture models).
POPULATION	60 COPD patients with ≥10 on the COPD Assessment Test (CAT), FEV1<80%, who are ICS naïve.
ELIGIBILITY	COPD patients with a score of ≥10 on the COPD Assessment Test (CAT), FEV1<80%, who are ICS naïve. We will exclude patients with asthma, other comorbidities including significant cardiovascular disease, cancer, neurological and renal disorders, and patients with acute worsening of COPD in the 6 weeks prior to screening resulting in treatment with oral corticosteroids or antibiotics.
TREATMENT	COPD patients will be randomized to receive either triple therapy (ICS/LAMA/LABA - Budesonide/glycopyrronium/formoterol) or LAMA/LABA combination (glycopyrronium /formoterol) (n=30 per group) for 12 weeks.
DURATION	Two years

REFERENCE DIAGRAM



1. INTRODUCTION

1.1 BACKGROUND

Cardiovascular disease (CVD) is a major cause of morbidity and mortality in COPD. Even though the cardiovascular co-morbidities are well documented in COPD, the molecular pathways linking endothelial dysfunction and CVD in COPD remain elusive.

Cellular senescence is a fundamental mechanism that contributes to tissue degeneration and drives the pathophysiology of age-related disorders, such as CVD and chronic obstructive pulmonary disease (COPD)[1,2]. Premature cellular senescence occurs in response to various stimuli, such as persistent activation of DNA damage response (DDR)[3]. In the vascular system, accumulation of senescent endothelial cells (EC) promotes CVD[4]. Senescent EC are dysfunctional, exhibit impaired angiogenic ability and secrete inflammatory cytokines, promoting atherogenesis and thrombosis[5].

The use of circulating endothelial progenitors, named **endothelial colony forming cells (ECFC)** or blood outgrowth endothelial cells (BOEC), is a unique tool for molecular studies in patients, providing non-invasive access to EC[6]. Few groups worldwide have the expertise to work with this rare cell population isolated from peripheral blood and currently, there is no other available non-invasive method to isolate endothelial cells from patients. Isolation of ECFC is a challenging, expensive and time-consuming assay. The rate of successful isolation of ECFC is about 75% in COPD patients and healthy controls[7], and the time from blood sample collection to harvest of cells for various assays is about 6-8 weeks but may take more than two months in some instances. Nevertheless, the benefits of using ECFC for molecular studies, personalised medicine and future regenerative approaches clearly outweigh the difficulties[6].

1.2 RATIONALE FOR CURRENT STUDY

Using ECFC, we have provided **evidence of accelerated endothelial senescence** in smokers and COPD patients due to epigenetic dysfunction, supporting the concept of accelerated ageing of the endothelium as a contributor to CVD in COPD[7,8]. Importantly, targeted pharmacological treatment of the identified molecular dysfunction could inhibit the increased endothelial senescence, suggesting novel targets for CVD therapy in this group of patients[7,8]. Subgroup analysis of previous data published in Stem Cells 2013[7], demonstrates that **ECFC from COPD patients on inhaled-corticosteroids (ICS) exhibit significantly reduced senescence** (Senescence-associated-beta galactosidase activity, p21), markers of DNA damage response (DDR) and IFN-γ-inducible-protein-10 (IP-10) compared to COPD patients not on ICS[9].

In vitro studies using human-umbilical-vein-endothelial-cells (HUVEC) showed a protective effect of ICS (budesonide) on the DDR, senescence and apoptosis caused by oxidative-stress, suggesting a protective molecular mechanism of action of corticosteroids on endothelium. In summary, we have shown that ICS reduce senescence and the senescence-associated pro-inflammatory phenotype in circulating endothelial progenitors in COPD patients, suggesting a novel protective molecular mechanism of action of ICS on endothelium[9].

2. STUDY OBJECTIVES

Primary Objective

• To investigate the protective effect of ICS/triple combination on endothelial senescence in patients with COPD using ECFC.

Secondary Objectives

• To correlate molecular with clinical readouts of endothelial dysfunction.

• To identify secretome and epigenetic dysfunction linked to senescence and effect of treatment with ICS.

• To confirm the protective effect of ICS/triple combination on endothelial senescence ex-vivo using ECFC (2D and 3D culture models).

3. STUDY DESIGN

This is an exploratory, assessor blind, parallel group, randomised controlled clinical trial. The study is primarily one of tissue sample (blood samples) collection and study *ex-vivo* and *in vitro*. Blood samples will be collected from patients with COPD. Participants will also perform lung function tests and will be assessed for their vascular function with non-invasive clinical methods. Molecular findings will be correlated with clinical parameters.

Duration: 2 years.

COPD patients will be randomized to receive triple therapy (ICS/LAMA/LABA - Budesonide /glycopyrronium /formoterol) or LAMA/LABA combination (glycopyrronium /formoterol) (n=30 per group) for 12 weeks.

Number and type of subjects: Sixty (60) COPD patients will be recruited with a score of ≥10 on the COPD Assessment Test (CAT), FEV1<80%, who are ICS naïve.

COPD patients will be with no significant cardiac comorbidities, as recruitment of patients with cardioprotective treatment (e.g. antiplatelet treatment) could introduce confounding factors and bias the results. However, we will include patients with hypertension and hypercholesterolemia, which are common comorbidities in COPD. We will exclude very severe disease FEV1<30%, and patients with more than 2 moderate exacerbations, or 1 severe (requiring hospitalisation) within the last year, to avoid disruption of the study protocol due to exacerbations. We will also exclude patients with asthma, other significant comorbidities including cancer, neurological and renal disorders, and patients with acute worsening of COPD in the 6 weeks prior to screening resulting in treatment with oral corticosteroids or antibiotics.

Subjects may be discontinued from the study at any time at the discretion of the investigator. Subjects are free to discontinue their participation.

3.1 Study outcome measures

Primary Endpoint

• Degree of senescence in ECFC, measured by markers of senescence and DNA damage response, isolated from COPD patients before and after treatment with budesonide/ glycopyrronium/ formoterol or glycopyrronium / formoterol

Secondary Endpoints

• Endothelial function assessed using the EndoPAT device

• Quality of life (CAT score)

• Mean arterial pressure

• Prediction algorithms for CVD (Framingham risk score and QRISK3)

• Blood eosinophils

• Blood cardiovascular markers [high-sensitivity C-reactive protein, brain-natriuretic-peptide, troponin, fibrinogen, activation of the RAS system (renin to aldosterone ratio)]

• Proteomic analysis of ECFC, serum/plasma for studying the senescence-associated secretome

• miR-126-3p and other miRNA dysregulation in ECFC

• markers of senescence and DDR in treated ECFC ex-vivo in 2D culture models

• permeability and markers of senescence in treated microvessels (3D culture models) using ECFC

Table of Objectives and outcome measures

Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure (if applicable)
Primary Objective	Characterisation of ECFC for	Baseline and 12 weeks post-

To investigate the protective effect of ICS/triple combination on endothelial senescence in patients with COPD using ECFC.	markers of senescence and DNA damage response, isolated from COPD patients before and after treatment with budesonide/ glycopyrronium/ formoterol or glycopyrronium / formoterol. Change from baseline in all above markers will be evaluated.	treatment
Secondary Objective 1 • To correlate molecular with clinical readouts of endothelial dysfunction.	• Endothelial function assessed using the EndoPAT device • Quality of life (CAT score) • Mean arterial pressure • prediction algorithms for CVD (Framingham risk score and QRISK3) • Blood eosinophils • Blood cardiovascular markers (high-sensitivity C-reactive protein, brain-natriuretic-peptide, troponin, fibrinogen, activation of the RAS system (renin to aldosterone ratio) Change from baseline in all above will be assessed. Correlation of clinical parameters to molecular markers of senescence will be evaluated.	Baseline and 12 weeks post-treatment
Secondary Objective 2 • To identify secretome and epigenetic dysfunction linked to senescence and effect of treatment with ICS.	• Proteomic analysis of ECFC, serum/plasma for studying the senescence-associated secretome • miR-126-3p and other miRNA dysregulation in ECFC Change from baseline in all above will be assessed.	Baseline and 12 weeks post-treatment
Secondary Objective 3 • To confirm the protective effect of ICS/triple combination on endothelial senescence ex-vivo using ECFC (2D and 3D culture	• change in markers of senescence and DDR in treated ECFC ex-vivo in 2D culture models • change in permeability and markers of senescence in treated microvessels (3D culture models)	Baseline and 12 weeks post-treatment

models).	using ECFC	

4. Participant Entry

4.1 Pre-screening evaluations

Stable, adult patients with COPD, who are ICS naïve will be considered for evaluation.

4.2 Inclusion Criteria

1. Adults over 18 years of age

2. Diagnosis of COPD

3. COPD Assessment Test (CAT) score of ≥10

4. FEV1<80%

5. No ever history of allergic or other respiratory disease

6. Able to demonstrate adequate inhaler technique with a pressurised metered dose inhaler (pMDI) and willing to take study medications as instructed.

7. Patients are defined as ICS naïve according to the following, have not been prescribed regular ICS treatment before.

4.3 Exclusion Criteria

1. Subjects unable to give informed consent form

2. Pregnancy or breast feeding

3. Patients on regular treatment with ICS

4. Very severe disease FEV1<30%,

5. Patients with more than 2 moderate exacerbations, or 1 severe (requiring hospitalization) within the last year

6. Patients with significant cardiac comorbidities and/or on antiplatelet treatment. Participants with ECG QTcF interval (corrected for heart rate using Fridericia's formula [QTcF]) >480 msec will be excluded. Participants with high degree atrioventricular block II or III, or with sinus node dysfunction with clinically significant pauses will also be excluded.

7. Patients with acute worsening of COPD in the 6 weeks prior to screening resulting in treatment with oral corticosteroids or antibiotics

8. Patients with asthma, other significant comorbidities including cancer, neurological, hepatic, unstable endocrine, renal (creatinine clearance < 30mL/min) disorders, narrow-angle glaucoma or prostatic hypertrophy.

9. Patients who are taking part in interventional clinical trials

10. Patients with known hypersensitivity to budesonide, glycopyrronium or formoterol.

11. For women of childbearing potential only (defined as: fertile, following menarche and until becoming post-menopausal, unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. Postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.) – currently pregnant, breast feeding, or planned pregnancy during the study or not using acceptable contraception. Acceptable contraception methods are outlined below, patients must adhere to this for the entirety of the study

- combined, or progestogen only pill- female
- intrauterine device (IUD) (copper coil)- female
- intrauterine system (IUS) (hormonal coil)- female
- contraceptive implant- female
- contraceptive injection- female
- contraceptive patch- female
- vaginal ring- female
- condoms- male or internal condoms- female
- female sterilization and vasectomy (of the male partner).
- sexual abstinence (a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject." in line with the Clinical Trials Coordination Group guidance).

12. Patients with allergy to latex

13. As a result of the medical interview, physical examination or screening investigations, the Physician Responsible considers the volunteer unfit for the study.

14. Patients prescribed Theophylline or any other prohibited medicatons listed in section 6.4 below.

15. Patients taking HIV inhibitors

4.4 Withdrawal criteria

Withdrawal from the study refers to discontinuation of study intervention and study procedures and can occur for the following reasons:

- Participant decision
- Loss to follow-up

Participants may discontinue study intervention for the following reasons:

- At the request of the participant.
- Adverse event/ Serious Adverse Event
- If the investigator considers that a participant's health will be compromised due to adverse events or concomitant illness that develop after entering the study.
- If a participant becomes pregnant during the study, study intervention should be discontinued.

If a participant withdraws from trial procedures, an assessment must be made as to whether trial data and samples collected to date can be retained and analysed for the trial.

The decision to withdraw from further trial procedures will be documented on the electronic case report form (eCRF) and in the medical notes.

If the participant withdraws consent to further be contacted at all for the study purposes, this will be documented on the electronic case report form (eCRF) and in the medical notes. Data up to the time of withdrawal can be included in the study if pseudonymised.

5. RANDOMISATION AND ENROLMENT PROCEDURE

5.1 RANDOMISATION OR REGISTRATION PRACTICALITIES

Patient identification (PIC) sites for the identification of participants will be set up at the following NHS Trusts, Guys and St Thomas's and Kings College London. Patients will also be recruited from COPD clinics and TLHC services at the Royal Brompton Hospital Trial participation will also be advertised at local GP surgeries and on social media (Facebook/Twitter). Patients will be seen at the clinical research facility at the Royal Brompton Hospital, all patient visits will be conducted at this location as this is a single centre trial.

Participants will be assigned a unique trial identifier (ID number) at the stage of screening, which will be used for all data collection including spirometry data, EndoPAT measurements, blood test results and ECFC characteristics.

Participants will be made aware that they will receive treatment that should not be revealed to the study team (study team should remain blinded) at any stage of the study.

An unblinded study co-ordinator based in the pharmacy, will facilitate the randomisation and delivery of medication. The unblinded study co-ordinator will be independent from the study team.

Randomisation will be carried out using a web-based randomisation and Electronic Data Capture (EDC) system, called OpenClinica. This will be programmed with a variable block randomisation schedule, stratified by age and gender. Trial data will also be collected on OpenClinica EDC.

Participants will receive treatment (3 inhalers of allocated drug) having a unique treatment code linked to the treatment allocation from the pharmacy after completion of visit 1, in cooperation with the unblinded study co-ordinator.

Participants will be identified by the ID number and each treatment will be identified with a unique treatment code linked to the allocation and trial identification (ID number).

5.2 UNBLINDING

Each participant will be assigned a unique trial ID which will be used for the collection of the data. Each inhaler dispensed will be identified by a unique treatment code which is linked to the treatment allocation. The treatment code and treatment must not be revealed to the study team except in medical emergencies when the appropriate management of the participant necessitates knowledge of the treatment, or in the event that expedited reporting to the Research Ethics Committee (REC) of an unexpected and related Serious Adverse Event (SAE) is required.

In the event that emergency unblinding of an individual participant is required, the unblinded trial co-ordinator and authorised staff (as documented on the delegation log) will follow trial procedures to unblind the participant in question and proceed with expedited reporting if required. The trial EDC system will also include an automated unblinding facility, in case unblinding is required.

Unblinding should only be considered if management of the participant would differ depending on whether they are on budesonide/glycopyrronium/formoterol or glycopyrronium/formoterol treatment.

5.3 VISIT SCHEDULE

Eligible subjects will have to fulfil the inclusion and exclusion criteria, and to give written informed consent. Recruited subjects will be asked to attend for 2 or 3 visits. The first visit will be the screening visit (standard screening procedure will be done for new patients/subjects only who have not been screened before within the last year). Screening visit can be combined with the study visit 1.

Screening Visit

- Informed Consent of every subject will be taken by one of the Investigators before any study

procedures are performed.

- Participants will be assigned a unique trial identifier (ID number)
- The subject's medical history, smoking history and current medications will be documented in the hospital notes and electronic case report form. Subjects will be asked in particular about relevant pulmonary and cardiovascular history. The specific parameters recorded will include age, family history of pulmonary and cardiovascular disease in first degree relatives, hypertension and family history of hypertension, hypercholesterolaemia, diabetes mellitus (types I and II) and family history of diabetes, smoking history, and exercise history (hours per week). A basic physical examination will be performed comprised by the assessment of vital signs and cardiovascular and respiratory evaluation by auscultation (assessment of heart and breath sounds by stethoscope). Female participants of reproductive age will be asked to verbally confirm absence of pregnancy. A urine pregnancy test will be also performed.
- Spirometry will be performed at screening to determine eligibility for the study
- A standard reversibility test will be performed if has not been performed before (new patients/subjects only)
- Respiratory symptoms will be assessed by the COPD Assessment Test (CAT)
- A baseline ECG will be performed at the screening visit.
- Existing Imaging will be recorded: CXR, HRCT scan (with expiratory scan if possible), echocardiograms (ECHO), MRI, CT coronary angiography
- Eligible patients will be recruited (60 in total; 30 per group)

Study Visit 1

The following tests will be done:

- Subjects will have their height, body weight and BMI measured.
- Spirometry test
- Exercise: 6 minute walk distance, this will be administered according to the ATS standard, the patient will perform 2 tests with a 30 minute rest period in between to eliminate learning effect.
- Dyspnoea and respiratory symptoms, quality of life and exacerbation history will be assessed by the questionnaires:
  - Symptoms: COPD Assessment Test (CAT), MRC dyspnoea scale
  - Quality of life: St George's Respiratory Questionnaire
  - Exacerbation history: moderate (antibiotics, oral steroids), severe (hospitalisation)
- Endothelial function and blood pressure (BP) will be measured by EndoPAT, as patients will have to be fasted and relaxed before the test this will be completed at the start of each visit to

minimize patient burden.

- Blood tests (30ml): full blood count [white blood cells, eosinophils (absolute and %), haemoglobin, platelets], glucose (fasting), urea and electrolytes, liver function, cholesterol, lipoproteins, high-sensitivity assays for C-reactive protein, brain-natriuretic-peptide, troponin, fibrinogen, homocysteine, serum and plasma for storage (-80°C)
- 50 mL of blood will be taken for ECFC isolation (alternatively named BOEC)
- For women of childbearing potential, a urine pregnancy test will be performed.
- Participants will be trained to appropriate inhaler technique using placebo inhaler devices. Patients will be directed to the Asthma+Lung UK website for inhaler technique videos.
- Participants will be informed that the study team will be blinded to the treatment that they will receive. Any communication between the study team and the participant during the study, including the 4-week and 8-week phone calls, should not reveal the medication/ device that the participant will receive.
- At the end of the study visit 1, participants will go to the pharmacy and will receive the medication (3 inhalers either Bevespi or Trixeo) for the duration of the study. They should start to take daily treatment from the following day (two inhalations twice daily).

4-week phone call Used to reinforce compliance and collect information about any adverse events.

8-week phone call Used to reinforce compliance and collect information about any adverse events.

Study Visit 2 (12 weeks)

- Participants will first attend to the pharmacy before meeting the study team, to hand-over empty and remaining inhalers. Compliance will be recorded.
- Participants will perform all tests / interventions including in study Visit 1: BMI, spirometry, 6MWT, CAT score, MRC scale, St George's Questionnaire, exacerbation history, EndoPAT measure of endothelial function and blood pressure and blood samples for: clinical laboratory tests (FBC, U&E, lipids, BNP, etc), serum and plasma storage and for ECFC isolation. For women of childbearing potential, a urine pregnancy test will be performed.
- Participants will exit trial.

5.4 STUDY MEASUREMENTS

Spirometry The diagnosis of COPD will depend on clinical diagnosis confirmed by spirometry caried out according to ATS/ERS guidelines. Forced expiratory spirometry maneuvers for derivation of FEV1 and

FVC will be assessed using a spirometer that meets or exceeds minimum performance recommendations of the ATS. Outcomes will be FEV1, FVC, FEV1/FVC

Body mass index (BMI) - This is calculated as weight in kilos divided by height in meters squared. Height measured at the start of the study will be used for both measures. Electronic scales will be used to measure weight.

The COPD assessment (CAT) score will be administered to evaluate quality of life – this 8-item symptom score has been shown to be responsive both to exacerbations and to pulmonary rehabilitation. Each item is scored 0-5 giving a score from 0 to 40 with 40 being the worst possible health status.

Medical Research Council Dyspnoea Scale (eMRC): Breathlessness will be assessed using extended version of the eMRC[10]. Participants be asked to select which of the following descriptions they feel best applies to themselves recently. The extended version is scored 1 to 5, with 5 indicating "Too breathless to leave the house". In the extended version 5 has two subdivisions; 5a independent in washing and/or dressing and 5b dependent in washing and dressing.

St. George's Respiratory Questionnaire (SGRQ). This is a well-established questionnaire, comprising 50 questions designed to measure Quality of Life in patients with diseases of airway obstruction. It contains 50 items divided into 3 domains: "Symptoms" assessing the respiratory symptoms, their frequency and severity; "Activity" referring to activities that cause or are limited by breathlessness; and "Impacts" which covers a range of aspects related to social functioning and psychological disturbances resulting from airway disease. A "Total" score combining each domain will be calculated. In each case the lowest possible value is zero and the highest is 100. Higher values correspond to greater impairment of quality of life.

Exacerbation history: The number of exacerbations in the last year, number of times admitted to hospital with exacerbation the last year and number of times admitted to hospital with exacerbations overall will be recorded.

The **6 Minute Walk Test (6MWT)** measures the distance that a patient can quickly walk on a flat, hard surface in a period of 6 minutes. It is a sub-maximal exercise test used to assess aerobic capacity and endurance. The participant will perform two tests according to the gold standard guidelines, with a 30 minute rest interval between each test.

Endothelial function will be assessed by measuring pulsatile arterial volume changes using finger plethysmography (**Endothelial Peripheral Arterial Tone: EndoPAT** Itamar Medical device). EndoPAT calculates the endothelium flow mediated dilation caused by a 5-minute occlusion of the brachial

artery, using a standard blood pressure cuff. It provides a **Reactive Hyperaemia Index (RHI)**, which is the ratio of the post-to-pre occlusion peripheral arterial tonometry amplitude of the occluded arm versus control arm. An RHI of 1.67 and below correlates to endothelial dysfunction, whereas an RHI of 1.67 and above is considered within normal range, as determined by previous studies[11]. It also calculates the **PAT-Augmentation Index (AIx75),** calculated by averaging the PWA data over 3.5 minutes using the following formula: PAT-AI = (augmentation pressure (ΔP)/ pulse pressure) × 100, where P1 = pulse pressure and P2 = pressure corresponding to the inflection point. These values are then corrected for a standard heart rate of 75 beats/min (AIx75).

All individuals should fast for at least 4 hours, and should refrain from caffeine, tobacco, vitamins or medications that affect vascular tone for 8 hours, unless contraindicated.

Participants will sit comfortably on an armchair or be laid in a supine position. The index or middle fingers (the same in each participant) will be attached to specially designed sensor probes. These probes comprise a system of inflatable latex air cuffs connected by pneumatic tubes to an inflating device controlled through a computer software. Pulsate volume changes of the distal digit induce pressure alterations in the finger cuff, which are sensed by pressure transducers and transmitted to and recorded by the EndoPAT device. One arm of each study participant will be occluded using the blood pressure cuff, while the contralateral arm will be used as a control. Baseline signal will be recorded for 5 minutes. Subsequently, blood pressure cuff on the test arm will be inflated to 60 mm Hg above baseline systolic BP or at least 200 mm Hg. After 5 minutes, the cuff will be deflated, and the signal will be recorded for 5 more minutes. The EndoPAT test is both operator and interpreter independent[12].

Due to the fasting requirement the EndoPAT will be completed at the start of each study visit to minimize patient burden.

The values of the Reactive Hyperaemia Index (RHI) and Augmentation Index (Alx75) will be recorded in the eCRF using the ID number of participant.

5.5 LABORATORY EVALUATIONS

Blood samples will be obtained from participants for Full Blood Count (FBC), biochemistry, analysis of lipid and cardiovascular biomarkers, serum and plasma storage and for isolation of ECFC. We will perform the following:

Clinical Laboratory Tests – Haematology/biochemistry analysis

Full blood count [white blood cells, eosinophils (absolute and %), haemoglobin, platelets], glucose (fasting), urea and electrolytes, liver function, cholesterol, lipoproteins, high-sensitivity assays for C-reactive protein, brain-natriuretic-peptide (BNP), troponin, fibrinogen and homocysteine will be performed by the clinical laboratory at Royal Brompton Hospital or Imperial College Healthcare Trust (ICHT) using standard methods. Results will be recorded in the eCRF using the ID number of participant.

Exploratory tests / Research samples

ECFC isolation

Blood samples of up to 50mls will be collected in CPT vacutainers (BD Biosciences) and will be pseudonymised with the participant's ID number. Peripheral blood mononuclear cells will be isolated from the blood samples and will be seeded in appropriate endothelial growth medium (EGM)−2 supplemented with serum, onto six-well plates precoated with type I rat tail collagen, as previously described[7]. After 24 hours, nonadherent cells and debris will be aspirated, adherent cells will be washed once with EGM-2 medium, and fresh EGM-2 will be added to each well. Medium will be changed daily for 7 days and then every 2 days. Colonies of ECFC will appear between 7 and 22 days in culture as discrete colonies of cells with cobblestone morphology and will be enumerated by visual inspection using a ×4 objective lens. Endothelial cells derived from the colonies will be passaged for 2–3 weeks after appearance and grown to confluence. Aliquots will be stored in liquid nitrogen facilities.

ECFC characterisation: ECFC between Passage 3 and 6 will be used for experiments. ECFC will be characterised for the Senescence-associated β-galactosidase **(SA-β-Gal) activity, a marker of senescence**, by histochemical detection of β-gal activity at pH:6.0 as previously published[7-9]. The number of blue (senescent) cells relative to the total cell number will be counted in two to four different optic fields, using ×10 or ×20 objective lens and at least 200 cells will be counted per sample. The percentage of SA-β-Gal positive cells will be recorded in the eCRF.

Further characterisation of the ECFC will be performed for a) additional markers of senescence such as for p21, p16 and SIRT1 by western blotting (WB) and/or immunofluorescence (IF); b) markers of DNA damage response (DDR) including γ-H2AX, 53BP1, p-ATM by IF and WB; c) proliferation: [5-Ethynyl-2'-deoxyuridine (EdU) assay and/or Ki-67 by IF.

The ECFC secretory phenotype for several pro-inflammatory cytokines related to senescence (senescence associated secretory phenotype – SASP) will be performed including IL-6, IL-8, IL-1β and IP-10 by **proteomic analysis** in cell lysates and conditioned media. Selective cytokines, such as **CXCL10 (IP-10)** will be also confirmed by Elisa or IF as previously published[9]. Alternatively to proteomics, a Luminex assay may be performed as previously published[9].

MicroRNA profile has been identified dysregulated in the COPD group[8]. We will measure **miR-126-3p** levels, previously demonstrated to augment DDR and ECFC senescence in COPD patients[8], and the small nucleolar RNAs RNU44 and snoRNA135 as reference genes, by TaqMan assays Life Technologies) and will be recorded in the eCRF. Additional miRNA that have been found dysregulated in preliminary studies in COPD, will be also measured.

Serum/ plasma samples

Additional exploratory cardiovascular biomarkers will be measured in the laboratory, such as activation of the RAS system (renin to aldosterone ratio), IL-6, DNA extraction.

Serum and plasma will be stored in -80°C, for **proteomics**, selective **miRNA studies** and other exploratory analysis.

Ex-vivo studies to investigate the effect of budesonide, glycopyrronium/formoterol and combination in 2D and 3D culture models.

ECFC from COPD patients isolated at baseline – visit 1, will be treated ex-vivo with i) budesonide, ii) glycopyrronium/formoterol fumarate combination or iii) budesonide/glycopyrronium/formoterol using traditional 2D-cultures. Outputs will be the (SA-β-Gal) activity and other markers of senescence and DDR as outlined above and published in previous studies[7-9].

3D cell culture models offer a novel alternative approach to model human disease for drug development and for personalized medicine[13,14]. We have recently used a high-throughput 'organ-on-a-chip' microfluidic platform called OrganoPlate from MIMETAS that allows the long-term culture of EC and formation of microvessels for functional and immunofluorescent (IF) analysis and screening assays[13-16]. We have been able to confirm reproducible formation of microvessels by ECFC and perform IF and functional studies of vascular integrity (perfusion and permeability assay).

We will also compare in 3D models the function (permeability) of microvessels formed using ECFC from the same COPD patients isolated in visit 1 vs visit 2. These studies will be performed with selected samples after the end of the trial and unblinding of participants, as cannot be performed in a large scale, to allow comparison between a small group of COPD patients treated with glycopyrronium/ formoterol vs budesonide/ glycopyrronium/ formoterol combination.

Sample storage and analysis

Baseline (Visit 1) and visit 2 blood samples for haematology and biochemistry analysis will be transferred to the clinical local laboratories immediately for analysis using participants' details. All other blood samples will be named with the unique trial identifier (ID number) and will be transferred to Dr Paschalaki's lab at Imperial College London for immediate processing or storage.

Fresh blood samples for ECFC isolation will be pseudonymised and processed immediately within Dr Paschalaki's lab.

Samples will be kept beyond the end of the trial and stored in accordance with the Human Tissue Act. These will be banked in a HTA licensed Imperial College Healthcare Tissue Bank for use in future ethically approved research, except for samples whose' participants did not consent to this on their consent form – these will be safely disposed of.

Incidental findings

Incidental findings will be reviewed by the research team referred to the patient's clinical team and General Practitioner as appropriate.

6. TREATMENTS

6.1 TREATMENT ARMS

Trixeo Aerosphere (formoterol fumarate / glycopyrronium/ budesonide - 5 micrograms/7.2 micrograms/160 micrograms pressurised inhalation, suspension) or Bevespi Aerosphere (formoterol fumarate / glycopyrronium 5 micrograms / 7.2 micrograms pressurised inhalation, suspension) will be provided from pharmacy after randomisation and in collaboration with the unblinded study co-ordinator after completion of the study visit 1. Randomisation will be performed using the OpenClinica system. Participants will be assigned randomly to the treatment group (Trixeo) and the control group (Bevespi) in a 1:1 ratio. Treatment for 12 weeks will be provided (3 inhalers per participant).

Participants will be taking two inhalations twice daily (two inhalations in the morning and two inhalations in the evening). Patients are advised not to take more than 2 inhalations twice daily. If a dose is missed, it should be taken as soon as possible, and the next dose should be taken at the usual time. A double dose should not be taken to make up for a forgotten dose.

Participants will be advised to retain all inhalers when finished and return them to the pharmacy at study visit 2.

Drugs will be provided by AstraZeneca for free.

6.2 DOSE MODIFICATIONS FOR TOXICITY

Budesonide, glycopyrronium and formoterol all have well established safety profiles. In case of suspected toxicity, treatment should be halted (see section of adverse events). It is not endorsed to amend recommended dose.

6.3 PREMEDICATION

N/A

6.4 INTERACTION WITH OTHER DRUGS

Co-administration of Trixeo or Bevespi Aerosphere with other anticholinergic and/or long-acting β_2-adrenergic agonist containing medicinal products is not recommended as it may potentiate known inhaled muscarinic antagonist or beta2-adrenergic agonist adverse reactions (see AE section).

Concomitant treatment with methylxanthine derivatives, steroids, or non-potassium-sparing diuretics may potentiate the possible initial hypokalaemic effect of β_2-adrenergic agonists, therefore, caution is advised in their concomitant use.

A list of concomitant use of respiratory medication during the study is below:

Prohibited Respiratory Medications during the study
Class of Medication
• LAMAs (Tiotropium, Aclidinium, Glycopyrronium, Umeclidinium) • LABAs • Fixed-combinations of LABA/LAMA • Fixed-combinations of LABA/ICS • Oral β-agonists 2 days • Theophylline

Trixeo and Bevespi Aerosphere should be administered with caution to patients being treated with medicinal products known to prolong the QTc interval.

The metabolism of budesonide (included in Trixeo) is primarily mediated by CYP3A4. Co-treatment with strong CYP3A inhibitors, e.g. itraconazole, ketoconazole, HIV protease inhibitors and cobicistat-containing products, are expected to increase the risk of systemic side effects, and should be avoided unless the benefit outweighs the increased risk of systemic corticosteroid adverse reactions, in which case patients should be monitored for systemic corticosteroid adverse reactions.

A list of prohibited medication is described below:

Prohibited Medications
Class of Medication
• Any drug with potential to significantly prolong the QT interval • Other investigational drugs • Non-selective β-blocking agents (Except Carvedilol) • Cardiac antiarrhythmics Class Ia, III (amiodarone) • Anticonvulsants • Tricyclic antidepressant • Monoamine oxidase inhibitors • Anti-tumor necrosis factor α antibodies (eg, infliximab) • Monoclonal antibodies • Antipsychotic drugs • Systemic calcineurin inhibitors, protease inhibitors • Systemic anticholinergics • Antiplatelet treatment • Systemic treatment with strong CYP3A4-inhibitors (e.g., ketoconazole, itraconazole, and ritonavir) • Live attenuated vaccines (emergent vaccines before or during the study need to be discussed at study level.)

6.5 DISPENSING AND ACCOUNTABILITY

AstraZeneca will supply both medications. The medications being investigated are fully licensed for use within the COPD population and will be provided for as commercial stock. The Pharmacy department at the Royal Brompton Hospital will be responsible for handling, storage and dispensing of the medications. AstraZeneca and Imperial College will be responsible for the transportation of the medications.

Participants will return all three inhalers to the pharmacy before attending for procedures of study visit 2.

Pharmacy will note compliance (remaining doses in provided inhalers).

7. Pharmacovigilance

7.1 Definitions

Adverse Event (AE): any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. *An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product (IMP), whether or not considered related to the IMP.*

Adverse Reaction (AR): all untoward and unintended responses to an IMP related to any dose administered. *All AEs judged by either the reporting investigator or the sponsor as having reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.*

Unexpected Adverse Reaction: an AR, the nature or severity of which is not listed in the reference safety information (RSI) e.g. list of expected medical events within section 5.6 of the investigator's brochure*. When the outcome occurs, this adverse reaction should be considered as unexpected.*

Serious Adverse Event (SAE) or **Serious Adverse Reaction:** any untoward medical occurrence or effect that at any dose:

- **Results in death.**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.*
- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation.**
- **Results in persistent or significant disability or incapacity.**
- **Is a congenital anomaly or birth defect.**

The NCI CTCAE V5.0 will be used to grade the scale of an AE within this clinical trial and medical judgement should be exercised in deciding whether an AE/AR is serious in other situations. Important AE/ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Suspected Unexpected Serious Adverse Reaction (SUSAR): any suspected adverse reaction related to an IMP that is both unexpected and serious.

7.2 Causality

Most adverse events and adverse drug reactions that occur in this study, whether they are serious or not, will be expected treatment-related toxicities due to the drugs used in this study. The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions in the table below.

If any doubt about the causality exists the local investigator should inform the study coordination centre and Chief Investigators. The pharmaceutical companies and/or other clinicians may be asked to advise in some cases.

In the case of discrepant views on causality between the investigator and others, all parties will discuss the case. In the event that no agreement is made, the MHRA will be informed of both points of view.

Relationship	Description
Unrelated	There is no evidence of any causal relationship

Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

7.3 Reporting Procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the study coordination centre in the first instance. A flowchart is given below to aid in the reporting procedures.

AEs will be collected from the time a subject consents to participate in the study and until the exit visit (visit 2). SAEs will be collected over the same period of time as stated above for AEs. All SAEs will be reported within 24 hours, as indicated in the below section 7.3.2.

7.3.1 Non serious AR/AEs

All such toxicities, whether expected or not, should be recorded in the toxicity section of the relevant case report form and sent to the study coordination centre within one month of the form being due.

7.3.2 Serious AR/AEs

Fatal or life threatening SAEs and SUSARs should be reported on the day that the local site is aware of the event. The SAE form asks for nature of event, date of onset, severity, corrective therapies given, outcome and causality (i.e. unrelated, unlikely, possible, probably, definitely). The responsible

investigator should sign the causality of the event. Additional information should be sent within 5 days if the reaction has not resolved at the time of reporting.

SAEs

An SAE form should be completed and faxed to the study coordination centre for all SAEs within 24 hours. However, hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

SUSARs

In the case of suspected unexpected serious adverse reactions, the staff at the site should:

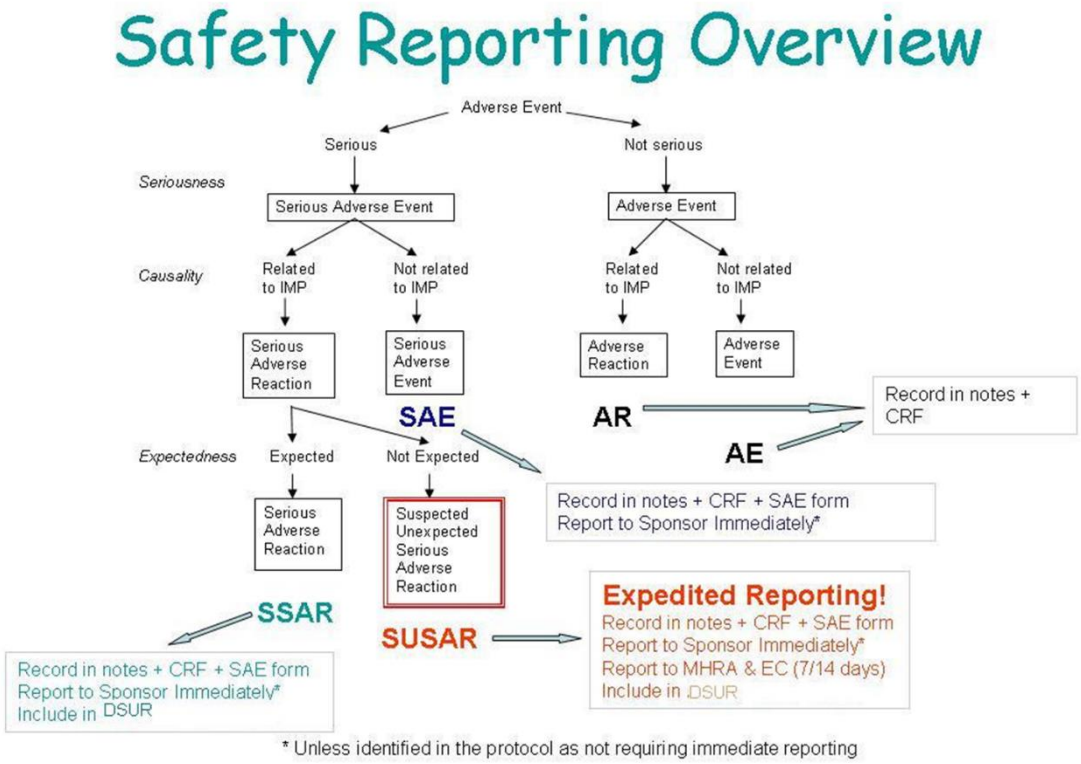
Complete the SAE case report form & send it immediately (within 24 hours,), signed and dated to the study coordination centre together with relevant treatment forms and anonymised copies of all relevant investigations.

Or

Contact the study coordination centre by phone and then send the completed SAE form to the study coordination centre within the following 24 hours as above.

The study coordination centre will notify the MHRA, REC and the Sponsor of all SUSARs occurring during the study according to the following timelines; fatal and life-threatening within 7 days of notification and non-life threatening within 15 days. All investigators will be informed of all SUSARs occurring throughout the study.

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



Contact details for reporting SAEs and SUSARs

RGIT.ctimp.team@imperial.ac.uk

CI email (and further details below).

Dr Koralia Paschalaki

attention email: k.paschalaki@imperial.ac.uk

Please send SAE forms to: k.paschalaki@imperial.ac.uk

Tel: +44(0)20 75942728 (Mon to Fri 09.00 – 17.00)

8. ASSESSMENT AND FOLLOW-UP

Follow up of participants is not expected after the end of the study. It is advised to attend the regular follow up clinic appointments.

Incidental findings

Incidental findings will be reviewed by the research team referred to the patient's clinical team and General Practitioner as appropriate.

loss to follow-up

Participants lost to follow up will be withdrawn from the study as discussed in section 4.4.

trial closure

The end of the trial will be defined as entry of the final data item following last patient last visit.

9. Statistics And Data Analysis

Sample size and power considerations

This is a pilot mechanistic study, and therefore exploratory analysis will be performed, with the sample size mainly determined by the feasibility of the laboratory work.

However, based on our previous studies from COPD patients without ICS and SA-β-gal 50.77±17% (mean+/-SD), a sample size of 10 ECFC samples from each group would give 90% power to detect a difference of 26 (~ 50% reduction) and 80% power for a difference of 22.5. As 54% from COPD patients' samples yielded high proliferative ECFC colonies in our previous studies[7-9], 30 subjects per group should be adequate to generate at least 10 paired samples needed for this project. Based on the literature[17], such sample size is adequate to also detect differences on RHI measurements by EndoPAT.

Statistical analysis

Data will be entered into a validated eCRF developed by ICTU.

The web-based database (eCRF) will contain all data points and will include the following:

- medical history, concomitant medications, exacerbation history and adverse events
- CAT score

- Spirometry: FEV1, FVC, FEV1/FVC
- BMI
- SGRQ
- eMRC
- 6MWT
- Blood pressure
- EndoPAT measurements: RHI and AIx
- Clinical blood sample results: Full blood count [white blood cells, neutrophils, eosinophils (absolute and %), haemoglobin, platelets], glucose (fasting), urea and electrolytes, liver function, cholesterol, lipoproteins, high-sensitivity assays for C-reactive protein, brain-natriuretic-peptide (BNP), troponin, fibrinogen and homocysteine
- Prediction algorithms for CVD (Framingham risk score and QRISK3)
- ECFC senescence as expressed as % of SA-β-gal positive cells.

Each patient will be given a unique number (ID), which will be linked to a paper record of the participants' names and addresses. This will only be accessible by trial personnel seeing patients at the study site.

Analysis of molecular parameters, such as characterization of ECFC for markers of senescence, DDR, SASP, miRNA etc, will be performed by a research associate who will be only given the ID number of the samples and will be blinded to the treatment group.

Analysis will be carried out by the Research Team and in collaboration with a trial statistician. Continuous variables will be presented as means and standard deviations if normally distributed, and as medians and inter-quartile ranges for skewed data, whilst categorical variables will be presented as frequencies and percentages. Normality will be checked, and appropriate transformation performed if not normally distributed. All statistical tests will be two-tailed with a 5% significance level.

In case of treatment discontinuation prior to the timepoint, all observed values from the participant will be recorded and will be evaluated to be included in the analysis. ECFC samples isolated from visit 1 can be used for ex-vivo studies and drug responses in 2D and 3D cultures.

A detailed description of all the analyses will be given in a detailed statistical analysis plan (SAP) will be prepared and finalised prior to database lock. Any deviations from the SAP will be justified and documented in the final report.

All primary and secondary outcomes with clinical outputs will be analysed at the end of the study. Exploratory outcomes will be analysed after the biological samples' analysis.

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

of the study.

10. Monitoring

Risk assessment

A study-specific risk assessment will be performed prior to the start of the study to assign a risk category of 'low', 'medium' or 'high' to the trial. Risk assessment will be carried out by the CI in collaboration with the ICTU and the result will be used to guide the monitoring plan.

Study oversight and management will be conducted by Dr Koralia Paschalaki as trial manager and Dr Parris Williams as research fellow.

The Research and Governance Integrity Team at the Royal Brompton Hospital will be responsible for onsite monitoring at RBH as per their local monitoring plan.

The risk assessment will consider all aspects of the study and will be updated as required during the course of the study.

monitoring at study coordination centre

All monitoring will take place at the Royal Brompton hospital (Local Site)

monitoring at local site

The Research and Governance Integrity Team (RGIT) at the Royal Brompton Hospital (RBH) will be responsible for onsite monitoring at RBH as per their local monitoring plan. The study will be monitored periodically by local trial monitors to assess the progress of the study, verify adherence to the protocol and other national/international requirements and to review the completeness, accuracy, and consistency of the data.

Monitoring procedures and requirements will be documented in a monitoring plan, in accordance with the risk assessment, in close collaboration with the RGIT team at RBH and the research management team (Dr Paschalaki and Dr Williams).

11. Regulatory Issues

11.1 CTA

This study has Clinical Trials Authorisation from the UK Competent Authority; MHRA. Reference: xxx

11.2 Ethics approval

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

11.3 Consent

Informed consent will be obtained from all participants using REC approved Participant Information Sheet (s) (PIS) and Consent Forms (CF) during the baseline visit.

The participant will be informed of about the trial by responsible clinician or a member of the research team and given a copy of the PIS. Informed subjects will be given an adequate amount of time to consider their participation in the trial. If the subject decides to participate in the trial they will be asked to sign the CF which will then be countersigned by the responsible clinician/researcher/ The patient will obtain one copy of the signed CF. Another copy will be place in the participant's medical records whilst the original will be retained in the participant's research record, at site.

The right of the participant to refuse to participate without giving reasons must be respected. All participants are free to withdraw at any time from the protocol intervention without giving reasons and without prejudicing further treatment.

11.4 Confidentiality

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

Data will be pseudonymised. On the CRF or other documents submitted to the Sponsors, participants will be identified by a participant ID number only. Documents that are not submitted to the Sponsor (e.g., signed informed consent form) should be kept in a strictly confidential file by the investigator.

The investigator shall permit direct access to participants' records and source document for the purposes of monitoring, auditing, or inspection by the Sponsor, authorised representatives of the

Sponsor, NHS, Regulatory Authorities and RECs.

The investigators and study site staff will comply with the requirements of the Data Protection Act 2018 concerning the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.

11.5 Indemnity

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

11.6 Sponsor

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

11.7 Funding

ASTRAZENECA UK LIMITED is funding this study.

11.8 Audits and Inspections

The study may be subject to inspection and audit by Imperial College London under their remit as Sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to GCP.

12. Trial Management

A Trial Management Group (TMG) will be a will be convened including the Chief Investigator, co-investigators and key collaborators and will be responsible for overseeing the progress of the trial. The day-to-day management of the trial will be co-ordinated through the TMG.

13. Publication Policy

The results of the study will be published in high impact peer-reviewed open access journals. This will be augmented by other dissemination strategies including blogs/editorials and press release supported by the media teams at Royal Brompton and NHLI, Imperial College London.

All publications and presentations relating to the study will be authorised by the Trial Management Group. The first publication of the trial results will be in the name of the Trial Management Group, if this does not conflict with the journal's policy. If there are named authors, these will include at least the trial's Chief Investigator, Statistician and Trial Coordinator. Members of the TMG and the Data Monitoring Committee will be listed and contributors will be cited by name if published in a journal where this does not conflict with the journal's policy. Authorship of parallel studies initiated outside of the Trial Management Group will be according to the individuals involved in the project but must acknowledge the contribution of the Trial Management Group and the Study Coordination Centre.

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Appendix 1. Summary of investigations

PROCEDURES	SCREENING VISIT	VISIT 1 (Baseline)	4 weeks	8 weeks	VISIT 2 (12 weeks from baseline)
Informed Consent	X				
History, Physical examination	X				
Recording of available clinical data from existing files (LFT, Chest X-Ray, HRCT scan, ECHO, CT coronary angiography)	X				
Spirometry +/- reversibility test	X	X			X
CAT score	X	X			X
Baseline ECG	X				
Pregnancy test (women of childbearing potential)	X	X			X
BMI (height, body weight)		X			X
6 Minute Walk Test		X			X
Respiratory symptoms and Quality of life assessment (MRC scale, St George's Questionnaire, exacerbation history)		X			X
Endothelial function assessment (Blood pressure, EndoPAT)		X			X
Blood Samples (80 ml in total) • Blood tests (FBC, U&E, lipids, BNP, etc) • serum, plasma • Up to 50ml for ECFC		X			X
Phone call to reinforce compliance and collect AE/SAE**			X	X	

* Screening visit and visit 1 can be combined.
** AE/SAE to be collected from screening visit (signing of consent) till Visit 2 week 12