Evaluation of **T**riple T**h**erapy using Magnetic Resonance Imaging in **A**sthma
ETHA

Sponsor:

Robarts Research Institute,

Schulich School of Medicine & Dentistry
Western University London CANADA

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Study site(s) and number of participants planned
1 site: 30 participants

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PROTOCOL SYNOPSIS

Achieving and maintaining asthma control is a primary goal of asthma treatment. Despite inhaled corticosteroid (ICS)/long-acting β_2-agonist (LABA) therapy, some participants with asthma remain with poorly controlled symptoms.[1]

A recent study in asthma participants of triple therapy [ICS/LABA and a long-acting muscarinic antagonist (LAMA)] demonstrated a statistically significant improvement in the forced expiratory volume in 1 second (FEV_1) after 24 weeks of treatment as compared to dual therapy.[2] In the same study, the annualised rate of moderate/severe exacerbations also improved, but this improvement was not significantly different as compared to dual therapy.[2]

The objective of this proof-of-principle, investigator-initiated project is to measure the effect of six weeks of triple therapy with fluticasone furoate, an ICS; umeclidinium (UMEC), a LAMA; and vilanterol (VI), a LABA, delivered in a single daily inhalation via an Ellipta inhaler on ^{129}Xe magnetic resonance imaging (MRI) ventilation defect percent (VDP), oscillometry and multiple breath nitrogen washout (MBNW) measurements in asthma participants with poor disease control while prescribed low or medium dose ICS/LABA inhaled therapy.

By measuring ^{129}Xe MRI VDP, the function of small and large asthma airways can be directly evaluated which may help uncover the mechanism of action by which triple therapy may improve FEV_1 and asthma control. **Our overarching hypothesis** is that six weeks of once daily therapy with FF/UMEC/VI (200/62.5/25 µg) will result in significantly improved and clinically-relevant changes in ^{129}Xe MRI VDP as well as MBNW and oscillometry measurements of airway function in participants with uncontrolled asthma while prescribed low or medium dose ICS/LABA inhaled therapy.

TITLE	ETHA: **E**valuation of **T**riple T**h**erapy using Magnetic Resonance Imaging in **A**sthma
SPONSOR	Robarts Research Institute, Western University, 1151 Richmond St. N. London, ON, N6A 5B7
INDICATION	Uncontrolled asthma
OBJECTIVE	To measure the effect of six weeks of triple therapy with fluticasone furoate - an inhaled corticosteroid (ICS); umeclidinium (UMEC), a long-acting muscarinic antagonist (LAMA); and vilanterol (VI), a long-acting β_2-adrenergic agonist (LABA), delivered in a single daily inhalation via Ellipta inhaler on ^{129}Xe MRI ventilation defect percent (VDP), oscillometry and multiple breath nitrogen washout measurements in asthma participants with poor disease control while prescribed low or medium dose ICS/LABA inhaled therapy.
STUDY DESIGN	Six-week treatment study with no washout from ICS/LABA, open label, no control arm
PLANNED TOTAL SAMPLE SIZE	30 participants
PARTICIPANT SELECTION CRITERIA	Thirty participants between 18-75 years of age with a diagnosis of uncontrolled GINA 2-4 asthma, while prescribed low or medium dose ICS/LABA inhaled therapy under the care of a respirologist; ACQ-6≥1.5

TREATMENTS	1) hyperpolarized ^{129}Xe gas for MRI, 2) FF/UMEC/VI (200/62.5/25 µg QD) via Ellipta inhaler
DOSES	1) 400 ml inhaled from Tedlar bag, 2) FF/UMEC/VI (200/62.5/25 µg QD)
ROUTE OF ADMINISTRATION	Ellipta inhaler for FF/UMEC/VI
MAIN PARAMETERS OF:	
- SAFETY	Heart rate, respiration and oxygen saturation during imaging. Adverse events throughout study
- EFFICACY	MRI VDP Pulmonary function tests • Spirometry • Plethysmography • Oscillometry • Multiple breath nitrogen washout CBC for blood eosinophils FeNO ACQ-6/AQLQ/SGRQ questionnaires
FIRST PARTICIPANT FIRST VISIT	Q2 2022
LAST PARTICIPANT LAST VISIT	Q2 2023

Study design

This is an open-label, single arm pilot study in participants prescribed low or medium dose ICS/LABA inhaled therapy and uncontrolled asthma to quantify hyperpolarized ^{129}Xe MRI VDP before and after six weeks of therapy with FF/UMEC/VI 200/62.5/25 µg QD. Male and females between 18 and 75 years of age will provide written informed consent to two visits including screening and baseline (Visit 1 week 0), and study end (Visit 2 week +6). The general study procedures are the same for Visit 1/Day 0 and Visit 2/Day 42.

For Visits 1 and 2, participants are to withhold their medication as previously described.[3] Vital signs will be recorded at the beginning of the visit. FeNO, spirometry, plethysmography, forced oscillation technique (FOT), MBNW, ^{1}H and ^{129}Xe MRI will be performed pre-bronchodilator. FeNO will be performed before all other pulmonary function testing. Following ^{129}Xe MRI, all participants will inhale 4 puffs (100 mcg each) of a bronchodilator and quietly rest for 15 minutes. After 15 minutes, participants will undergo post-bronchodilator ^{1}H and ^{129}Xe MRI, and once MRI is complete, participants will undergo post-bronchodilator spirometry, plethysmography, FOT and MBNW. St. George's respiratory questionnaire (SGRQ), asthma control questionnaire (ACQ-6) and asthma quality of life questionnaire (AQLQ) will be administered after post-bronchodilator assessments are completed. Adverse events will be recorded at Visit 2. Computed tomography (CT) imaging will be acquired at Visit 1 with optional CT imaging at Visit 2, whilst MRI will be acquired on Visits 1 and 2.

Objectives

Primary Objective:	**Outcome Measure:**
To measure the effect of FF/UMEC/VI 200/62.5/25 µg QD on ^{129}Xe MRI VDP after 6 weeks of therapy	^{129}Xe VDP
Secondary Objectives:	**Outcome Measures:**

SO1: To measure the effect of FF/UMEC/VI 200/62.5/25 µg QD on biomarkers of pulmonary function and inflammation	FEV_1, FVC, FEV_1/FVC, FEF_{25-75}, lung volumes, Raw, FOT (R_5, R_{19}, R_{5-19}, X_5, A_X), LCI, S_{con}, S_{acin}, blood eosinophils, FeNO
SO2: To measure the effect of FF/UMEC/VI 200/62.5/25 µg on asthma control and quality-of-life	ACQ-6, AQLQ, SGRQ
Safety Objectives:	**Outcome Measures:**
SO3: To assess the safety and tolerability of FF/UMEC/VI 200/62.5/25 µg	AE/SAE Vital signs
Exploratory Objectives:	**Outcome Measures:**
To evaluate the relationships between MRI VDP and asthma control and quality-of-life	VDP ACQ-6, AQLQ, SGRQ
To evaluate the relationships between MRI VDP and lung function	VDP FEV_1, FVC, FEV_1/FVC, FEF_{25-75}, lung volumes, Raw, FOT (R_5, R_{19}, R_{5-19}, X_5, A_X), LCI, S_{con}, S_{acin}
To evaluate the relationships between MRI VDP, airway and blood eosinophilia	VDP FeNO, Blood eosinophils

Target participants
Thirty participants between 18-75 years of age with a diagnosis of uncontrolled (ACQ-6≥1.5)[4] Global Initiative for Asthma (GINA) 2-4 asthma, according to the treatment step criteria[5] and under the care of a respirologist and prescribed low or medium dose ICS/LABA inhaled therapy.

Duration of treatment
There are no washout or run-in periods for this study. The treatment period will be 6 weeks and the maximum total study time will be 6-8 weeks.

Investigational product, dosage, mode of administration and drug formulation
400mL of hyperpolarized ^{129}Xe is administered via inhalation up to four times per study visit (up to two times pre-bronchodilator and up to two times post-bronchodilator) on Visit 1/Day 0 and Visit 2/Day 42. ^{129}Xe gas polarization will be performed at Robarts Research Institute using a mixture of ^{129}Xe, nitrogen and helium, purified to ≥99.99% purity. 400mL of hyperpolarized ^{129}Xe gas will be mixed with medical grade ^{4}He to a 1L volume and will be used immediately.

Treatment, dosage and mode of administration
FF/UMEC/VI 200/62.5/25 µg used daily at the same time in the morning (±2 hours), starting on Visit 1/Day 0 and completing on Visit 2/Day 42/week 6, at which time a treatment decision will be made in consultation with the respirologist.

Statistical methods
Data will be tested for normality using the Shapiro-Wilk test and when data are not normally distributed, non-parametric tests will be performed. Unpaired t-tests and Mann-Whitney U-tests will be performed to compare participant characteristics, pulmonary function and MRI measurements in participants with eosinophilic asthma. To correct for multiple comparisons, a Holm-Bonferroni correction will be applied. Paired t-tests will be used to analyze the differences between the pre-treatment and post-treatment measurements in each participant. Univariate relationships will be evaluated using Pearson (r) and Spearman (ρ) correlation

coefficients. Statistical analyses will be performed using SPSS (SPSS Statistics 25.0; IBM, Armonk, NJ, USA) and all results will be considered statistically significant when the probability of making a Type I error was less than 5% ($p < 0.05$).

Western

TABLE OF CONTENTS PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or special term	Explanation
^{129}Xe	Xenon-129
ACQ-6	Asthma control questionnaire
AE	Adverse event
AQLQ	Asthma Quality of Life questionnaire
CRF	Case report form (electronic/paper)
CT	Computed tomography
A_X	Area of Reactance which is defined as the area under the curve of reactance between 5Hz and resonant frequency, reflecting a composite index for reactance measured using oscillometry
FeNO	Fractional exhaled nitric oxide
FOT	Forced oscillation technique; oscillometry
GINA	Global Initiative for Asthma
ICH	International Conference on Harmonisation
IP	Investigational product
LCI	Lung clearance index
MBNW	Multiple breath nitrogen washout
MRI	Magnetic resonance imaging
R_{aw}	Airways resistance
R_5	Resistance measured at 5Hz using oscillometry
R_{19}	Resistance measured at 19Hz using oscillometry
R_{5-19}	Resistance difference (5Hz-19Hz) measured using oscillometry
SAE	Serious adverse event
S_{con}	Conducting airways ventilation heterogeneity measured using multiple breath nitrogen washout
S_{acin}	Acinar airways ventilation heterogeneity measured using multiple breath nitrogen washout
VDP	Ventilation defect percent measured using ^{129}Xe MRI
X_5	Reactance measured at 5Hz using oscillometry

1. INTRODUCTION

1.1 Background and rationale for conducting this study

Achieving and maintaining asthma control is a primary goal of asthma treatment. Despite inhaled corticosteroid (ICS)/long-acting β_2-agonist (LABA) therapy, some participants with asthma remain with poorly controlled symptoms.[1]

A recent treatment study in asthma participants of triple therapy [ICS/LABA and a long-acting muscarinic antagonist (LAMA)], demonstrated a statistically significant improvement in the forced expiratory volume in 1 second (FEV_1) after 24 weeks of treatment as compared to dual therapy.[6] Hence, in asthma participants, objective evidence is required to guide personalized treatment decisions directed at the inflammatory and non-inflammatory smooth muscle-mediated components of airways disease.

Biomarkers provided by inhaled ^{3}He and ^{129}Xe magnetic resonance imaging (MRI) have been developed and validated as a way to measure the structural and functional determinants of obstructive lung disease, including in participants with asthma.[7] For example, focal ventilation defects measured using MRI are commonly observed in patients with asthma[8,9] and these were shown to be spatially related to airway abnormalities[10,11] and they also respond to bronchoconstriction[10,12-15] and bronchodilation.[12,16] MRI ventilation defects were shown to be correlated with disease severity[17,18] and to independently predict asthma control in severe asthma.[19]

1.2 Rationale for study design, doses and control groups

1.2.1 Goal and Hypothesis

The goal of this proof-of-principle, investigator-initiated project is to measure the effect of six weeks of triple therapy with fluticasone furoate - an ICS; umeclidinium (UMEC), a LAMA; and vilanterol (VI), a LABA, delivered in a single daily inhalation via an Ellipta inhaler on ^{129}Xe MRI ventilation defect percent (VDP), forced oscillation technique (FOT) and multiple breath nitrogen washout (MBNW) measurements in asthma participants with poor disease control while prescribed low or medium dose ICS/LABA inhaled therapy.

By measuring ^{129}Xe MRI VDP, the function of small and large asthmatic airways can be directly evaluated which may help uncover the mechanism of action by which triple therapy may improve FEV_1 and asthma control.

Our overarching hypothesis is that six weeks of daily therapy with FF/UMEC/VI (200/62.5/25 µg) will result in significantly improved and clinically-relevant changes in ^{129}Xe MRI VDP, as well as MBNW and FOT measurements of airway function in participants with asthma and uncontrolled disease while prescribed low or medium dose ICS/LABA inhaled therapy.

1.2.2 Study design

This is a single-arm open-label study, so no blinding or randomization will be performed. For the evaluation of imaging biomarkers, trained observers will be blinded to time point in order to minimize the potential for bias in image analysis or interpretation. Figure 1 provides a summary of the study which includes two visits.

Figure 1. Study Schematic

Study Duration →

	Visit 1 Week 0 Treatment	Visit 2 Week 6 ± 1
Bloodwork	✓	✓
PFT	✓	✓
MRI	✓	✓
CT	✓	✓
ACQ-6/AQLQ/SGRQ	✓	✓

PFT = pulmonary function test; MRI = magnetic resonance imaging; CT = computed tomography; ACQ-6 = asthma control questionnaire; AQLQ = asthma quality of life questionnaire; SGRQ = St. George's respiratory questionnaire

1.2.3 Primary and secondary outcome measures

Participants with uncontrolled asthma measured using ACQ-6≥1.5 at Visit 1 will be enrolled and receive FF/UMEC/VI 200/62.5/25 µg daily for 6 weeks. The primary outcome measurement is ^{129}Xe MRI VDP at week 6/Day 42. Secondary outcome measurements include: biomarkers of inflammation (FeNO and blood eosinophils), pulmonary function [FEV_1, forced vital capacity (FVC), lung volumes, airway resistance (R_{aw}), FOT, ventilation hetereogeneity of the conducting (S_{con}) and acinar (S_{acin}) airways, lung clearance index (LCI)], asthma control and quality-of-life (ACQ-6, AQLQ, SGRQ).

1.2.4 Dosing and study duration

FF/UMEC/VI 200/62.5/25 µg will be administered via Ellipta inhaler QD for 6 weeks at the same time in the morning (±2 hours). Study visits will be planned for mornings.

1.2.5 Biological samples

Leftover biological samples will be destroyed appropriately. There is no intent to collect additional biological samples or use any leftover samples.

1.3 Benefit/risk and ethical assessment

Study participants with poorly controlled disease may benefit from treatment with FF/UMEC/VI 200/62.5/25 µg, including reduced asthma exacerbations, reduced use of OCS and improved lung function. The safety of FF/UMEC/VI 200/62.5/25 µg has been previously demonstrated[20] and the most common side effects were nasopharyngitis, headache and back pain.[21]

^{129}Xe MRI has well-demonstrated safety and tolerability.[22,23] At our site,[23] we previously observed two mild adverse events after administration of 136 doses of 500 mL Xe gas mixed with ultrapure ^{4}He or N_2 gas (2/136=1%). One of these adverse events was light-headedness that resolved within two minutes without treatment while the other adverse event was determined unrelated to ^{129}Xe gas. Another study in the US, employed a 1L ^{129}Xe gas breathhold inhalation (double the dose used at Robarts) and reported dizziness (59%), paresthesia (34%), euphoria (30%) and hypoesthesia (30%). All these symptoms resolved within three minutes without clinical intervention.[22]

For MRI in general, adverse events do not arise from the MRI scan itself, but from a failure to disclose or detect MRI incompatible objects in or around the body of the participant or in the scanner room. The published reported risk of injury is less than 1 in 100,000 and the risk of

death is less than 1 in 10 million.[24] Other remote risks involve temporary hearing loss from the noise (<95DB) produced by the scanner which can be completely prevented by ensuring the use of ear plug protection that also allows for continuous communication between the participant and research staff during the scan.

There is a possibility that incidental findings may be observed on MRI. If this occurs, participants will be notified by the principal investigator and the qualified clinical investigator will arrange for referral to University Hospital, London Health Sciences Centre, Department of Medical Imaging for follow-up clinical imaging and diagnosis.

1.4 Study objectives

Primary Objective:	**Outcome Measure:**
To measure the effect of FF/UMEC/VI 200/62.5/25 µg QD on ^{129}Xe MRI VDP after 6 weeks of therapy	^{129}Xe VDP
Secondary Objectives:	**Outcome Measures:**
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To evaluate the relationships between MRI VDP, airway and blood eosinophilia	VDP FeNO, blood eosinophils

2. PARTICIPANT SELECTION, ENROLLMENT, RANDOMISATION, RESTRICTIONS, DISCONTINUATION AND WITHDRAWAL

Participants between 18 and 75 years of age with a diagnosis of asthma, according to the Global Initiative of Asthma (GINA) treatment step criteria[5] and under the care of a respirologist, will be recruited. All participants will provide written informed consent to a protocol approved by local research ethics boards at Western University and registered at https://clinicaltrials.gov. Each participant should meet all of the inclusion criteria and none of the exclusion criteria for this study. Under no circumstances will exceptions to this be allowed.

2.1 Inclusion criteria

- Participant understands study procedures and is willing to participate in the study as indicated by the participant's signature
- Provision of written, informed consent prior to any study specific procedures
- Males and females aged 18 to 75 years, inclusively, at the time of Visit 1 (enrollment), under the care of a respirologist
- FEV_1 ≥35 and ≤80% predicted
- Participant is a current non-smoker and non-vaper, having not smoked tobacco or cannabis, pipe or cigar or vaped any product for at least 12 months prior to the study with a tobacco smoking history of no more than 10 pack-years (i.e. 1 pack per day for 10 years)
- Women of childbearing potential (after menarche) must use a highly effective form of birth control (confirmed by the investigator or designee)
- A highly effective form of birth control includes true sexual abstinence, a vasectomized sexual partner, Implanon®, female sterilization by tubal occlusion, any effective intrauterine device/levonorgestrel intrauterine system, Depo-Provera™ injections, oral contraceptive and Erva Patch™ or Nuvaring™
- Women of childbearing potential (after menarche) must agree to use a highly effective form of birth control, as defined above, from enrollment, throughout the study duration, and 8 weeks after last dose of study drug, with negative urine pregnancy test result at Visit 1 and Visit 2
- Male participants who are sexually active must agree to use a double barrier method of contraception (male condom with diaphragm or male condom with cervical cap) from the first dose of the study drug until 8 weeks after last dose
- Participant has documented treatment with a stable dose of low to medium dose inhaled corticosteroids (defined as >250 and ≤500 mcg fluticasone proprionate/day or equivalent or, >400 to ≤800 mcg Budesonide/day for at least 6 months prior to enrollment
- LABA for at least 6 months prior to enrollment
- Participant has ACQ-6 ≥1.5 at Visit 1
- All participants must have received 2 doses of a Health Canada approved COVID-19 vaccine at least 14 days prior to their first visit

2.2 Exclusion criteria

- Participant is, in the opinion of the investigator, mentally or legally incapacitated, preventing informed consent from being obtained, or cannot read or understand written material
- Participant has clinically important pulmonary disease other than asthma (e.g. active lung infection, chronic obstructive pulmonary disease, bronchiectasis, pulmonary fibrosis, cystic fibrosis, hypoventilation syndrome associated with obesity, lung cancer, alpha-1 antitrypsin deficiency and primary ciliary dyskinesia)
- Participant has a medical condition such as narrow-angle glaucoma, urinary retention, prostatic hypertrophy, bladder neck obstruction or any other disorder that may be negatively impacted by antimuscarinic effects and/or could affect the safety of the participant throughout the study, influence the findings of the study or their interpretations, or impede the participant's ability to complete the entire duration of the study, as assessed by the qualified investigator
- Any disorder, including, but not limited to, cardiovascular, gastrointestinal, hepatic, renal, neurological, musculoskeletal, infectious, endocrine, metabolic, haematological, psychiatric, or major physical impairment that is not stable in the opinion of the Qualified Investigator and/or could affect the safety of the participant throughout the study, influence the findings of the study or their interpretations, or impede the

participant's ability to complete the entire duration of the study, as assessed by the qualified investigator
- Known history of allergy or reaction to the study drug formulation
- Acute upper or lower respiratory infections requiring antibiotics or antiviral medication within 30 days prior to the date of informed consent
- Confirmed positive COVID-19 test within the last 30 days (either PCR or rapid antigen test)
- Participant has been prescribed LAMA within the last 6 months
- Clinically significant asthma exacerbation, defined as a change from baseline deemed clinically relevant in the opinion of the qualified investigator, including those requiring the use of OCS, or an increase in maintenance dosage of OCS within 30 days prior to the date of informed consent. Participants with an exacerbation after providing informed consent but prior to treatment start will be excluded from the study
- Receipt of immunoglobulin or blood products within 30 days prior to the date of informed consent
- Receipt of live attenuated vaccines 30 days prior to the date of enrollment
- Previously randomized in any FF/UMEC/VI 200/62.5/25 µg study
- Planned surgical procedure during the conduct of the study
- Concurrent enrollment in another clinical trial
- Participant has history of alcohol or drug abuse within 12 months prior to the date of informed consent
- Participant is a female who is ≤8 weeks post-partum or breast feeding an infant
- Participant is pregnant, or intends to become pregnant during the time course of the study
- Participant is unable to perform MRI breath-hold maneuver
- Participant is unable to perform spirometry maneuver
- Participant is hospitalized or has had a major surgical procedure, major trauma requiring medical attention, or significant illness requiring medical attention within 4 weeks of Visit 1
- Participant has a blood pressure of >150 mmHg systolic or >95 mmHg diastolic on more than 2 measurements done >5 minutes apart at Visit 1
- In the opinion of the investigator, participant suffers from any physical, psychological or other condition(s) that might prevent performance of the MRI, such as severe claustrophobia
- Participant has implanted mechanically, electrically or magnetically activated device or any metal in their body, which cannot be removed, including but not limited to pacemakers, neurostimulators, biostimulators, implanted insulin pumps, aneurysm clips, bioprosthesis, artificial limb, metallic fragment or foreign body, shunt, surgical staples (including clips or metallic sutures and/or ear implants) – at the discretion of the MRI Technologist

2.3 Participant enrollment

Upon study enrollment, all participants will be allocated to receive FF/UMEC/VI 200/62.5/25 µg treatment. This is a single-arm study design therefore there is no randomization or blinding. Participants who discontinue from the study will not be replaced. We expect a 90% retention rate. The principal investigator will keep a record, the participant screening log, of participants who entered pre-study screening and will:

- Obtain signed written informed consent from the potential participant before any study specific procedures are performed
- Assign potential participant a unique enrollment number
- Determine participant eligibility

If a participant withdraws from participation in the study, then his/her enrollment code cannot be reused. Enrollment codes will be assigned strictly sequentially as participants become eligible for the study.

2.4 Procedures for handling incorrectly enrolled participants

Without exception, participants who fail to meet the eligibility criteria should not, under any circumstances, be enrolled or receive study medication. Participants who are enrolled, but subsequently found not to meet all the eligibility criteria must not be initiated on treatment and will be withdrawn from the study.

Where a participant does not meet all the eligibility criteria but is incorrectly started on treatment, the participant will discontinue the treatment. Study personnel at Robarts Research Institute must ensure all decisions are appropriately documented.

2.5 Restrictions

The following restrictions apply once the participant has started the treatment:

- Participants must withhold short-acting beta-agonist (SABA) use 6 hours prior to all study visits
- Participants must withhold ICS/LABA or study drug use 24 hours prior to all study visits
- Participants must withhold leukotriene receptor antagonists (LTRA, i.e. Singulair) use 24 hours prior to all study visits
- Use of immunosuppressive medication (other than prior, stable OCS for the maintenance treatment of asthma) is not permitted

2.6 Discontinuation of treatment or investigational product

Participants may be discontinued from treatment or investigational product (IP) in the following situations:

- Participant decision. The participant is at any time free to discontinue treatment, without prejudice to further treatment
- Adverse event (AE)
- Severe non-compliance with the study protocol
- Participant becomes pregnant
- Any medical condition or personal circumstance which, in the opinion of the investigator, exposes the participant to risk by continuing in the study or does not allow the participant to adhere to the requirements of the protocol

2.6.1 Procedures for discontinuation of a participant from treatment or investigational product

A participant who discontinues the treatment or IP will always be asked about the reason(s) for discontinuation and the presence of any adverse events. The principal investigator will perform the best possible observation(s), test(s) and evaluation(s) as well as give appropriate medication and all possible measures for the safety of the participant. Follow up will be performed for adverse events until resolved or at least 28 days after the end of treatment.

2.7 Criteria for withdrawal

2.7.1 Screen failures

Screening failures are participants who do not fulfil the eligibility criteria for the study, and therefore must not be enrolled. These participants should have the reason for study withdrawal recorded as ‘Incorrect Enrollment’ (i.e. participant does not meet the required inclusion/exclusion criteria).

2.7.2 Withdrawal of the informed consent

Participants are free to withdraw from the study at any time (treatment, investigational product and assessments), without prejudice to further treatment.

A participant who withdraws consent will always be asked about the reason(s) and the presence of any AE. The Investigator will follow up AEs outside of the clinical study.

If a participant withdraws from participation in the study, then his/her enrollment code cannot be reused. Withdrawn participants will not be replaced.

2.8 Discontinuation of the study

The study may be stopped if, in the judgment of the investigator(s), trial participants are placed at undue risk because of clinically significant findings that:

- meet individual stopping criteria or are otherwise considered significant
- are assessed as causally related to study drug
- are not considered to be consistent with continuation of the study

Regardless of the reason for discontinuation all data available for the participant at the time of discontinuation of follow-up must be recorded in the Case Report Form (CRF). All reasons for discontinuation of treatment must be documented. In discontinuing the study, for an individual participant, and for all participants, the investigator(s) will ensure that adequate consideration is given to the protection of the participants' interests.

3. STUDY PLAN AND TIMING OF PROCEDURES

Visit Visit 1 = Day 0 Visit 2 = Day 42 ± 5 days	**1**	**2**
Week	**0**	**6**
Day	**0**	**42**
Month		
Written informed consent	X	
Demographics	X	
Physical examination/ height/ weight	X	
Medical/surgical history	X	
Inclusion/exclusion criteria	X	
Vital signs	X	X
Treatment dispensed/returned	X	X
Concomitant medication	X	
Adverse event review	X	X
Blood samples	X	X
Spirometry	X	X
Plethysmography	X	X
FeNO	X	X
FOT	X	X
MBNW	X	X
ACQ	X	X
AQLQ	X	X
SGRQ	X	X
CT	X	X
MRI	X	X

3.1 Enrollment/screening period

Procedures will be performed according to the Study Plan. Participants will be recruited by respirologist referral. Potential participants will be evaluated to determine whether they fulfil the entry requirements. In addition, the Investigator shall discuss and provide in writing the nature of the study, its requirements and restrictions in the form of a letter of information. Written informed consent will be obtained from participants who elect to participate, prior to any study procedures.

Adherence to the scheduling of study visits and study procedures is of paramount importance; this must be emphasized to participants. Procedures in all treatment visits must be scheduled so that all study procedures are performed at the same time during the day.

Participants will be screened for MR safety using the MRI screening form. This form will be reviewed by the MR technologist, who will compare the identity of the participant to the identity recorded on the form to verify participant safety. The form will then be filed as a part of the study records, separately from the de-identified data associated with that participant. Participants are instructed to restrict their medications prior to study visits.

At the screening/enrollment visit, the participant will provide informed consent, be assigned a baseline number, and review inclusion and exclusion criteria, study procedures, clinical history, medication restrictions, prior therapy, concomitant therapy, adverse experiences, vital signs and temperature. Female participants of childbearing potential will be administered a urine pregnancy test. Participants will undergo pre- and post-bronchodilator spirometry and complete ACQ-6. A blood sample will be acquired pre-bronchodilator.

During the enrollment visit, the participant should demonstrate ACQ-6 $\geq$1.5 to continue in the study. Participants will only be enrolled if all study assessment results during the enrollment visit satisfy inclusion and exclusion criteria. Spirometry and ACQ-6 results may be confirmed immediately during the enrollment visit

The study staff will call the participant prior to each visit to remind the participant of the medication restrictions and the next visit. Study staff will also complete COVID-19 screening by telephone prior to each visit.

3.2 Treatment period

The general study procedures are the same for Visit 1/Day 0 and Visit 2/Day 42.

Any adverse events from the previous visit should be reviewed and assessed at the beginning of each visit.

For Visits 1 and 2, participants are to withhold their medication as previously described.[3] Vital signs will be recorded at the beginning of the visit. FeNO, spirometry, plethysmography, FOT, MBNW, ^{1}H and ^{129}Xe MRI will be performed pre-bronchodilator. FeNO will be performed before all other pulmonary function testing. Following ^{129}Xe MRI, all participants will inhale 4 puffs (100mcg each) of a bronchodilator and quietly rest for 15 minutes. After 15 minutes, participants will undergo post-bronchodilator ^{1}H and ^{129}Xe MRI, and once MRI is complete, participants will undergo post-bronchodilator spirometry, plethysmography, FOT and MBNW. In total, participants will be in the MRI for up to a total of 20 minutes for image acquisition. ACQ-6, AQLQ and SGRQ will be administered after post-bronchodilator assessments are completed. Patients will undergo low dose chest CT after post-bronchodilator MRI. Participants will be accompanied to the CT scanner at Robarts Research Institute. For Visits 1 and 2, bloodwork will be drawn either at University Hospital or Robarts. For blood draws at University Hospital, the patient will be accompanied to the phlebotomy lab, which is connected to Robarts.

4. STUDY ASSESSMENTS

The investigator will ensure that data are recorded on Case Report Forms generated by the local team. The investigator ensures the accuracy, completeness, and timeliness of the data recorded.

4.1 Efficacy assessments

4.1.1 Clinical and Laboratory Measurements for Efficacy

Spirometry, Plethysmography, FOT and Bronchodilator Response

Spirometry and plethysmography will be performed according to American Thoracic Society Guidelines[3] using a *MedGraphics Elite Series* plethysmograph (MedGraphics; St. Paul, MN, USA) pre- and post-bronchodilator. FOT will be performed pre- and post-bronchodilator according to European Respiratory Society Guidelines[25] using a *tremoFlo® C-100* airwave oscillometry system (Thorasys Thoracic Medical Systems Inc.; Montreal, QC, Canada) to measure resistance and reactance at 5 Hz (R_5, X_5), 19 Hz (R_{19}) and the difference between 5 and 19 Hz (R_{5-19}). For post-bronchodilator measurements, four 100 mcg doses of *Novo-Salbutamol® HFA* (Teva Novopharm Ltd.; Toronto, ON, Canada) will be delivered through a pressurized metered dose inhaler using an *AeroChamber Plus* spacer (Trudell Medical International; London, ON, Canada).

FeNO

FeNO will be measured pre-bronchodilator according the American Thoracic Society/European Respiratory Society Guidelines[26] using the NIOX VERO® (Circassia Pharmaceuticals Inc, Morrisville, NC, USA). Participants will be seated upright and given the mouthpiece of the NIOX VERO®. Participants will be instructed to breathe out fully, then bring the mouthpiece to their lips to create a seal around the mouthpiece. Participants are then asked to inhale deeply to total lung capacity, then exhale slowly and completely into the mouthpiece.

MBNW

MBNW will be performed for measurement of LCI pre- and post-bronchodilator using 100% oxygen for nitrogen washout and the *ndd EasyOne Pro LAB* system (ndd Medical Technologies, Zurich, Switzerland) equipped with an ultrasonic flow and molar mass sensor. Participants will be seated upright with the washout phase initiated by switching from room air to 100% oxygen at end expiration. Tidal breathing of 100% oxygen will be performed until the expired nitrogen concentration is <2.5% of the concentration at the start of the test. LCI is the number of functional residual capacity (FRC) lung turnovers and will be calculated as the cumulative expired volume or air during the washout divided by FRC. MBNW will be performed in duplicate and reported as the mean of two maneuvers. S_{con} and S_{acin} will also be estimated.

Image Acquisition and Analysis

Anatomical proton (^{1}H) and hyperpolarized ^{129}Xe MR images will be acquired within 5 minutes of one another using a 3T MR system with broadband imaging capabilities (General Electric Health Care; Milwaukee, WI, USA), as previously described.[16] MRI scanning will take place with the use of a 129Xe Lung coil, which is approved for research use only. Participants will be instructed to inhale 1.0 L of gas (100% N_2 for ^{1}H MRI and a ^{129}Xe/^{4}He mixture for ^{129}Xe MRI) from FRC, and 15 coronal slices will be acquired under breath-hold conditions. ^{129}Xe gas will be polarized to 40-60% (Polarean; XeniSpin, Durham, NC, USA), and diluted with 600 mL of medical grade ^{4}He gas. Quantitative MRI evaluation will be performed by a single trained observer with at least two years' experience using semi-automated segmentation software as previously described.[27] ^{129}Xe MRI VDP will be generated as the ventilation defect volume normalized to the thoracic cavity volume.[28] CT images will be acquired with an Aquilion ONE CT Scanner (Canon Medical Systems USA, INC., Tustin, CA, USA) at Robarts Research Institute. Participants will be scanned in the supine position and during inspiration breath-hold from FRC after inhaling 1.0 L of N_2 gas in order to match the CT and MRI breath-hold volumes

and anatomy. CT images will be acquired with the following parameters: beam collimation of 80 x 0.5 mm, tube voltage of 120 kVp, effective current of 210 mAs, 260 mAs and 390 mAs based on patient body mass index (BMI), 500 ms gantry rotation time and 0.95 pitch. The ImPACT CT patient dosimetry calculator (based on Health Protection Agency [UK] NRBP-SR250) was used to estimate the mean total effective dose as 1.3 mSv or approximately equal to the total of background radiation experienced in one year in London, Ontario. CT images will be quantitatively analyzed using the VIDAvision software (VIDA Diagnostics, Coralville, IA, USA).

Blood
Blood samples will be acquired and analyzed either at Robarts in the phlebotomy lab and analyzed at a commercial lab or at the clinical biochemistry laboratory at University Hospital, London Health Sciences Centre, to measure blood eosinophils. Blood eosinophils will be determined using a complete blood count (CBC) analysis.

4.2 Safety assessments

4.2.1 Laboratory safety assessments

Participants that have provided written informed consent will undergo laboratory testing at Visit 1.

Blood samples for determination of clinical chemistry and haematology will be taken during Visit 1 only; these samples will also be analyzed for blood eosinophils. Blood samples during Visit 2 will be analyzed for blood eosinophils only.

The following laboratory variables will be measured:

Table 1 Laboratory Safety Variables

Haematology/Haemostasis (whole blood)	Clinical Chemistry (serum or plasma)
B-Haemoglobin (Hb)	S/P-Creatinine
B-Leukocyte count	S/P-Bilirubin, total
B-Leukocyte differential count (absolute count)	S/P-Alkaline phosphatise (ALP)
B-Platelet count	S/P-Aspartate transaminase (AST)
	S/P-Alanine transaminase (ALT)

The principal and qualified investigators will evaluate results regarding clinically relevant abnormalities. The laboratory results will be signed and dated and retained as source data for laboratory variables.

4.2.2 Urine Pregnancy Test

On Visits 1 and 2, a urine pregnancy test will be administered to screen female participants for pregnancy.

4.2.3 Physical examination

A complete physical examination will be performed at Visit 1 and include an assessment of : height, weight, general appearance, respiratory, cardiovascular, abdomen, skin, head and neck (including ears, eyes, nose and throat), lymph nodes, thyroid, abdomen, musculo-skeletal (including spine and extremities) and neurological systems.

4.2.4 Vital signs

Vital signs will be taken at all visits prior to all other tests including: sitting blood pressure, respiratory rate, pulse, oxygen saturation and temperature. Blood pressure will be performed on the right arm, pulse, oxygen saturation and blood pressure will be measured using an automated blood pressure cuff and pulse oximeter.

4.2.4.1 Body temperature

Body temperature will be taken at all visits using a laser thermometer.

4.2.4.2 Safety Monitoring and Risk Management of Investigational Product

During the gas administration and breath hold, all participants will be monitored using pulse oximetry for blood oxygenation and heart rate.

Supplemental oxygen will be administered via nasal cannula at a flow rate of 2L/min during the MRI procedure until immediately prior to administration of hyperpolarized ^{129}Xe. Supplemental oxygen will continue after the breath hold is complete. If a participant becomes uncomfortable during the breath hold, they can breathe out the gas and subsequently breathe in room air and/or supplemental oxygen. Furthermore, in the event that oxygen saturation, as measured by pulse oximetry, decreases to less than 88% for a period of >10 seconds, the participant will be administered supplemental oxygen and this will be recorded as an adverse event.

4.3 Other assessments

4.3.1 Participant reported outcomes

All participants will complete the ACQ-6, AQLQ, and SGRQ at Visits 1 and 2.

4.3.1.1 Asthma Control Questionnaire

The ACQ measures the adequacy of asthma control and change in asthma control that may occur as a result of treatment.[29] This study will employ the ACQ-6.[30]

4.3.1.2 Asthma Quality of Life Questionnaire

The AQLQ was developed to evaluate the quality of life in participants suffering from asthma.[31,32] Study personnel will administer AQLQ to participants on paper.

4.3.1.3 St. George's Respiratory Questionnaire

The SGRQ measures impact on overall health, daily life and perceived well-being in patients with obstructive airways disease.[33] Study personnel will administer SGRQ to patients on paper during Visits 1 and 2.

5. SAFETY REPORTING AND MEDICAL MANAGEMENT

The principal investigator is responsible for ensuring that all staff involved in the study are familiar with the content of this section.

5.1 Definition of adverse events

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

5.2 Definitions of serious adverse event (SAE)

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

- Results in death

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

The causality of SAE (their relationship to all study treatment/procedures) will be assessed by the investigator-sponsor. The investigator-sponsor will be responsible for forwarding all serious adverse events regardless of causality to the collaborator, GSK plc within 24 hours of awareness and to the Western Research Ethics Board.
Appendix A: Additional Safety Information provides further guidance on the definition of an SAE.

5.2.1 Management of Acute Adverse Events

The 2nd floor 3T MRI facility, 1st floor 3T/7T MRI facility and room 2252 in Robarts Research Institute are covered by the University Hospital Code Blue Team in case of a Code Blue emergency (cardiac or respiratory arrest). All other areas of Robarts Research Institute utilize paramedic services in case of a medical emergency.

If any life-threatening adverse event occurs at any time during a study visit, the appropriate emergency service (Code Blue team or paramedics) will be mobilized to respond to the event. A Code Blue is initiated by calling 55555 to reach the University Hospital switchboard and reporting the type of emergency and its location, along with directions to the location. Code Blue instructions, including a script of what to say to switchboard, are posted in areas with Code Blue coverage. Each area has a button to unlock the doors between Robarts Research Institute and University Hospital during a Code Blue, which also alerts Robarts Research security and the facility manager of the emergency to activate their support.

There is a Code Blue cart and AED located outside of the MR suite for use during an emergency. This equipment is kept up to date by LHSC staff. All staff involved in study visits are trained in CPR. Mock Code Blues are done periodically with University Hospital staff to ensure all parties are aware of procedures and responsibilities in the event of a Code Blue emergency.

In any event that an acute adverse event does not require immediate response by the Code Blue team or paramedics but requires timely medical intervention, participants will be taken to University Hospital Emergency Department via wheelchair. Robarts Research Institute is connected to University Hospital, providing a direct indoor route to the Emergency Department. The principal investigator and qualified investigator will be alerted of any medical emergency as soon as possible without jeopardizing the immediate care required by the participant.

5.2.2 Time period for collection of adverse events

Adverse Events will be collected from the time of signature of informed consent on Visit 1 throughout the treatment period up to and including Visit 2 and recorded in the CRF.

5.2.3 Follow-up of unresolved adverse events

Any AEs that are unresolved at the participant's last AE assessment or other assessment/visit as appropriate in the study are followed up by the Investigator for as long as medically indicated, but without further recording in the CRF.

5.2.4 Variables

The following variables will be collected for each AE;

- AE (verbatim)
- The date and time when the AE started and stopped
- Maximum intensity and changes in intensity
- Whether the AE is serious or not
- Investigator causality rating against the treatment or investigational product (yes or no)
- Action taken with regard to treatment or investigational product
- AE caused participant's withdrawal from study (yes or no)
- Outcome

In addition, the following variables will be collected for SAEs:

- Date AE met criteria for serious AE
- Date Investigator became aware of serious AE
- AE serious rationale
- Date of hospitalisation
- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to Study procedure(s)
- Causality assessment in relation to other medication
- Causality assessment in relation to Additional Study Drug
- Description of SAE.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 5.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not an SAE unless it meets the criteria shown in Section 5.2. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be an SAE when it satisfies the criteria shown in Section 5.2.

5.2.5 Causality collection

The investigator will assess causal relationship between treatment or investigational product and each Adverse Event, and answer 'yes' or 'no' to the questions 'Do you consider that there is a reasonable possibility that the event may have been caused by the treatment?' and 'Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?'

For SAE, causal relationship will also be assessed for other medication and study procedures. Note that for SAEs that could be associated with any study procedure the causal relationship is implied as 'yes'.

A guide to the interpretation of the causality question is found in Appendix A (Additional Safety Information).

5.2.6 Adverse events based on signs and symptoms

All AEs spontaneously reported by the participant or care provider or reported in response to the open question from the study personnel: 'Have you had any health problems since the previous visit/you were last asked?', or revealed by observation will be collected and recorded in the CRF. When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

5.2.7 Adverse events based on examinations and tests

The results from protocol mandated laboratory tests and vital signs will be summarised in the clinical study report. Deterioration as compared to baseline in protocol-mandated laboratory values and vital signs should therefore only be reported as AEs if they fulfil any of the SAE criteria or are the reason for discontinuation of treatment with the study drug or investigational product.

If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Wherever possible the reporting Investigator uses the clinical, rather than the laboratory term (e.g. anaemia versus low haemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s).

Deterioration of a laboratory value, which is unequivocally due to disease progression, should not be reported as an AE/SAE.

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

5.3 Reporting of serious adverse events to WSREB, Health Canada, GSK Collaborator

All SAEs MUST be reported, whether or not considered causally related to the treatment or investigational product. The investigator is responsible for informing the WSREB and/or the Health Canada as per local requirements. Notification is required within 15 days after becoming aware of the occurrence of the SAE if it is neither fatal nor life threatening, and within seven days of becoming aware of the occurrence of the SAE if it is fatal or life threatening. Within eight days of having informed the SAE, the sponsor will submit a complete report in respect of the information that includes an assessment of the importance and implication of any findings. The investigator-sponsor is also responsible for forwarding all serious adverse events regardless of causality to the collaborator GSK plc immediately and under no circumstances should this exceed 24 hours. The investigator will submit any updated SAE data to GSK Collaborator within 24 hours of it being available.

5.4 Overdose

For overdoses associated with an SAE, the standard reporting timelines apply as above. For other overdoses, reporting must occur within 30 days.

5.5 Pregnancy

All pregnancies and outcomes of pregnancy should be documented appropriately in the CRF.

5.5.1 Maternal exposure

If a participant becomes pregnant during the course of the study, the treatment and investigational product should be discontinued immediately.

Pregnancy itself is not regarded as an adverse event unless there is a suspicion that the treatment or investigational product under study may have interfered with the effectiveness of a contraceptive medication. Congenital abnormalities/birth defects and spontaneous miscarriages

should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth or congenital abnormality) should be followed up and documented even if the participant was discontinued from the study.

5.5.2 Paternal exposure

Male participants should refrain from fathering a child or donating sperm during the study and for 8 weeks following the last dose.

6. TREATMENT, INVESTIGATIONAL PRODUCT AND OTHER TREATMENTS

6.1 Identity of treatment and investigational product(s)

Treatment	Dosage form and strength	Manufacturer
FF/UMEC/VI	200/62.5/25 µg	GlaxoSmithKline plc

Investigational product	Dosage form and strength	Manufacturer
Hyperpolarized ^{129}Xe	400mL per dose, up to four doses per visit administered 15 minutes apart minimum on Visit 1 and 2	Robarts Research Institute

6.2 Dose and treatment regimens

Participants will take the first dose of FF/UMEC/VI 200/62.5/25 µg directly after Visit 1/Day 0 assessments are completed

Participants will receive hyperpolarized ^{129}Xe in doses of 400mL, with up to four doses administered per visit on Visit 1/Day 0 and Visit 2/Day 42. Doses will be administered at least 15 minutes apart.

6.3 Labelling

The FF/UMEC/VI 200/62.5/25 µg label includes the following information:

- Packaging Control #/ Lot Trace ID #
- Allocation #
- Fill Count & Dosage Form
- Interval ID (Day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10)
- Re-evaluation Date
- Dosing Instructions
- Storage conditions
- Compound ID – Protocol #

In addition, the label will indicate ‘for clinical study use only’ and ‘keep out of reach of children’.

6.4 Storage

All study drug is kept in a secure place under appropriate storage conditions. A description of the appropriate storage conditions is specified on the label of the product(s).

6.5 Compliance

Participants will be given instructions on the administration of FF/UMEC/VI 200/62.5/25 µg during Visit 1/Day 0. The administration of all study drugs (including investigational products) will be recorded in the appropriate sections of the CRF.

6.6 Accountability

The study drug provided for this study will be used only as directed in the study protocol. The study personnel will account for all study drugs dispensed and administered to participants. The study drugs remaining after study completion will be destroyed.

6.7 Concomitant and other treatments

Restricted Medication/Class of drug:	**Usage:**
SABA	Withhold use 6 hours before all visits
LTRA	Withhold use 24 hours before all visits

6.7.1 Other concomitant treatment

All medications considered necessary for the participant's safety and well-being, will be recorded in the appropriate sections of the CRF.

The medication described above may be used within the withholding period if medically necessary. In the event that a medication described above is used within the withholding period before a study visit, the study visit may continue as scheduled or be rescheduled at the discretion of the Investigator.

6.8 Device Malfunction

Participants will receive the first dose of study medication under the direction and supervision of study staff. Should the inhaler device malfunction during initial use, it will be replaced with a new one and be set aside. The malfunctioning device will be labelled as such and the study collaborator will be notified within 24 hours. The device will then either be returned to the collaborator or destroyed, according to the collaborator's instructions.

Participants will be instructed to contact research staff if the device malfunctions after they leave. They would be advised to return to Robarts to pick up a replacement device as soon as possible. Details will be documented in the CRF.

Should a device malfunction cause an adverse event, it will be documented as such and replaced as soon as possible. The collaborator will be notified of such an event as soon as possible and within 24 hours. Details will be documented in the CRF.

7. STATISTICAL ANALYSES

7.1 Statistical considerations

Study personnel performing image analysis will be blinded to study visit number.

7.2 Sample size estimate

Our primary hypothesis is that ^{129}Xe VDP in participants with uncontrolled asthma will significantly change after FF/UMEC/VI 200/62.5/25 µg treatment as measured 6 weeks after the first treatment. We expect that the mean change in VDP will be both clinically relevant (> minimal clinically important difference) and statistically significant in a sample size of 30

participants. However, this is a pilot study and we did not power the study to show a statistically significant effect. The study sample size we have in place was chosen for pragmatic reasons and the study results will be used to estimate effect size so that a sample size for a larger multicentre study may be prospectively determined.

7.3 Definitions of analysis sets

7.3.1 Efficacy analysis set

All participants who completed Visits 1 and 2 will be included for the efficacy analysis.

7.3.2 Safety analysis set

All participants who completed Visit 1 will be used for the safety analysis.

7.4 Outcome measures for analyses

Primary Objective:	**Outcome Measure:**
To measure the effect of FF/UMEC/VI 200/62.5/25 µg QD on ^{129}Xe MRI VDP after 6 weeks of therapy	^{129}Xe VDP
Secondary Objectives:	**Outcome Measures:**
SO1: To measure the effect of FF/UMEC/VI 200/62.5/25 µg QD on biomarkers of pulmonary function and inflammation	FEV_1, FVC, FEV_1/FVC, FEF_{25-75}, lung volumes, Raw, FOT, (R_5, R_{19}, R_{5-19}, X_5, X_{19}, A_X), LCI, S_{con}, S_{acin}, blood eosinophils, FeNO
SO2: To measure the effect of FF/UMEC/VI 200/62.5/25 µg on asthma control and quality-of-life	ACQ-6, AQLQ, SGRQ
Safety Objectives:	**Outcome Measures:**
SO3: To assess the safety and tolerability of FF/UMEC/VI 200/62.5/25 µg	AE/SAE Vital signs
Exploratory Objectives:	**Outcome Measures:**
To evaluate the relationships between MRI VDP and asthma control and quality-of-life	VDP ACQ-6, AQLQ, SGRQ
To evaluate the relationships between MRI VDP and lung function	VDP FEV_1, FVC, FEV_1/FVC, FEF_{25-75}, lung volumes, Raw, FOT, (R_5, R_{19}, R_{5-19}, X_5, A_X), LCI, S_{con}, S_{acin},
To evaluate the relationships between MRI VDP, airway and blood eosinophilia	VDP FeNO, blood eosinophils

7.5 Methods for statistical analyses

Data will be tested for normality using the Shapiro-Wilk tests and when data are not normally distributed, non-parametric tests will be performed. Unpaired t-tests and Mann-Whitney U-tests will be performed to compare participant characteristics, pulmonary function and MRI measurements in participants with eosinophilic asthma. To correct for multiple comparisons, a Holm-Bonferroni correction will be applied. Paired t-tests will be used to analyze the differences between the pre-treatment and post-treatment measurements in each participant.

Univariate relationships will be evaluated using Pearson (r) and Spearman (ρ) correlation coefficients. Statistical analyses will be performed using SPSS (SPSS Statistics 25.0; IBM, Armonk, NJ, USA) and all results will be considered statistically significant when the probability of making a Type I error was less than 5% ($p < 0.05$).

8. STUDY AND DATA MANAGEMENT

8.1 Training of study site personnel

The principal investigator will ensure that appropriate training relevant to the study is provided to all of study staff, and as well she will insure that staff are appropriately qualified for their role and that any new information relevant to the performance of this study is forwarded to the staff involved.

The principal investigator will maintain a record of all individuals involved in the study (medical, nursing and other staff).

8.2 Study timetable and end of study

Planned duration of the study: 12 months
Study period: June 1 2022 – April 30 2023

8.3 Data management

Data management will be performed by study personnel at Robarts Research Institute. Adverse events and medical/surgical history will be classified according to the terminology of the latest version the Medical Dictionary for Regulatory Activities (MedDRA). Data reporting will be provided annually by the sponsor investigator to all collaborators and stakeholders. There will be no interim analysis.

8.3.1 Database Setup

A password protected Microsoft Access database will be created for this study. De-identified data will be stored in this database and a unique database ID will be assigned to each participant. In a second database the unique database ID will link each participant to personal identifiers such as address, phone number, email address, full date of birth and family physician. The two databases will be stored separately to minimize the breach of identity.

8.3.2 Data Access

Only the clinical research coordinator and delegated personnel with proper privacy and confidentiality training will have access to the databases described in section 10.3.1. This will ensure that personal information is kept secure. The clinical coordinator will ensure that individuals with access to research data maintain competence in proper data handling by ensuring that privacy training and recertification is kept up to date.

8.3.3 Data Security

Paper copies will be stored in a locked filing cabinet, in a locked office in a security monitored building at Robarts Research Institute. Electronic records will be stored on an internal firewall protected server that is located in a secured server room at Robarts Research Institute. Furthermore, only de-identified data may also be saved on local hard drives at Robarts Research Institute for data analysis purposes. Robarts Research Institute is a controlled access building with security on site.

8.3.4 Data Lock

Only the clinical research coordinator and individuals delegated to perform data entry with proper training will have write access to the databases described in section 10.3.1. Read only access will be granted to other trained personnel involved in data analysis.

8.3.5 Data Archiving

All records pertaining to this study will be retained for a period of 25 years, at which time all such records will be destroyed. Paper documents with collected study participant information will be stored in a locked file cabinet in the clinical coordinator's secured office. Electronic

records will be stored on an internal firewall protected server that is located in a secured server room at Robarts Research Institute. Password protection will be used on any document containing participant information. De-identified data may be saved on local hard drives at Robarts Research Institute for data analysis purposes. Robarts Research Institute is a controlled access building with security on site.

9. ETHICAL AND REGULATORY REQUIREMENTS

9.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonisation /Good Clinical Practice, applicable local regulatory requirement, for example 'Good Clinical Practice for Trials on Drugs (MHLW Ordinance No. 28, 27 March 1997, partially revised by MHLW Ordinance and their related notifications).

9.2 Participant data protection

The Master Informed Consent Form will explain that:

- Study data will be stored in a computer database, maintaining confidentiality
- Participant data will be stored confidentially
- For data verification purposes, the Western Research Ethics Board may require direct access to the records relevant to the study, including participants' medical history

The Western Research Ethics Board (WREB) will be required to approve the final study protocol, including the final version of the Informed Consent Form and any other written information and/or materials to be provided to the participants.

The opinion of the WREB should be given in writing. The WREB should approve all advertising used to recruit participants for the study.

9.3 Informed consent

The principal investigator will:

- Ensure each participant is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study
- Ensure each participant is notified that they are free to discontinue from the study at any time
- Ensure that each participant is given the opportunity to ask questions and allowed time to consider the information provided
- Ensure each participant provides signed and dated informed consent before conducting any procedure specifically for the study
- Ensure the original, signed Informed Consent Form(s) is/are stored in the Investigator's Study File
- Ensure a copy of the signed Informed Consent Form is given to the participant
- Ensure that any incentives for participants who participate in the study as well as any provisions for participants harmed as a consequence of study participation are described in the informed consent form that is approved by WREB

If any new information on the study medication becomes available which may influence the decision of the participant to continue the study, the Investigator(s) MUST inform the participant of such information immediately, record this in a written form, and confirm with the participant if he or she wishes to continue the participation in the study. In addition, if the Investigator(s) deem it necessary to revise the Informed Consent Form, they should revise it

immediately (Refer to Section 9.4). The investigator(s) should re-explain the participants using updated Informed Consent Form even if the participants have already been informed of the new information verbally. Written informed consent to continue participation in the study should be provided separately.

9.4 Changes to the protocol and informed consent form

In principal, study procedures will not be changed. If it is necessary for the study protocol to be amended, the amendment will first be approved by Western's REB. Approval of the revised Informed Consent Form by WREB is required before the revised form is used. If an administrative change is required, such a change should be notified to or approved by each IRB according to local requirements.

9.5 Audits and inspections

The WSREB or Health Canada may perform audits or inspections at the centre, including source data verification.

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Appendix A Additional Safety Information

Further Guidance on the Definition of a Serious Adverse Event (SAE)

Life threatening
'Life-threatening' means that the participant was at immediate risk of death from the AE as it occurred, or it is suspected that use or continued use of the product would result in the participant's death. 'Life-threatening' does not mean that had an AE occurred in a more severe form it might have caused death (e.g. hepatitis that resolved without hepatic failure).

Hospitalisation
Treatment in an emergency room is not a serious AE, although the reasons for it may be (e.g. bronchospasm, laryngeal oedema). Hospital admissions and/or surgical operations planned before or during a study are not considered AE if the illness or disease existed before the participant was enrolled in the study, provided that it did not deteriorate in an unexpected way on study.

Important medical event or medical intervention
Medical judgement will be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life threatening or result in death, hospitalisation, disability or incapacity but may jeopardize the participant or may require intervention to prevent one or more outcomes listed in the definition of serious. These should usually be considered as serious.

A Guide to Interpreting the Causality Question
The following factors will be considered:

- Time Course
- Consistency with known drug profile
- De-challenge experience. Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?
- No alternative cause. The AE cannot be reasonably explained by another aetiology such as the underlying disease, other drugs, other host or environmental factors
- Re-challenge experience
- Laboratory tests. A specific laboratory investigation (if performed) has confirmed the relationship

In difficult cases, other factors could be considered such as:

- Is this a recognized feature of overdose of the drug?
- Is there a known mechanism?

Causality of 'related' is made if following a review of the relevant data, there is evidence for a 'reasonable possibility' of a causal relationship for the individual case. The expression 'reasonable possibility' of a causal relationship is meant to convey, in general, that there are facts (evidence) or arguments to suggest a causal relationship.

The causality assessment is performed based on the available data including enough information to make an informed judgment. With limited or insufficient information in the case, it is likely that the event(s) will be assessed as 'not related'.

Causal relationship in cases where the disease under study has deteriorated due to lack of effect should be classified as no reasonable possibility.

Appendix B International Airline Transportation Association (IATA) 6.2 Guidance Document

Labelling and shipment of biohazard samples
International Airline Transportation Association (IATA) classifies biohazardous agents into 3 categories. For transport purposes the classification of infectious substances according to risk groups was removed from the Dangerous Goods Regulations (DGR) in the 46th edition (2005). Infectious substances are now classified either as Category A, Category B or Exempt. There is no direct relationship between Risk Groups and categories A and B.

Category A Infectious Substances are infectious substances in a form that, when exposure to it occurs, can cause permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. Category A pathogens are eg, Ebola, Lassa fever virus:

- are to be packed and shipped in accordance with IATA Instruction 602.

Category B Infectious Substances are infectious Substances that do not meet the criteria for inclusion in Category A. Category B pathogens are eg, Hepatitis A, B, C, D, and E viruses, Human immunodeficiency virus (HIV) types 1 and 2. They are assigned the following UN number and proper shipping name:

- UN 3373 – Biological Substance, Category B
- are to be packed in accordance with UN3373 and IATA 650

Exempt - all other materials with minimal risk of containing pathogens

- Clinical trial samples will fall into Category B or exempt under IATA regulations
- Clinical trial samples will routinely be packed and transported at ambient temperature in IATA 650 compliant packaging
- Biological samples transported in dry ice require additional dangerous goods specification for the dry-ice content
- IATA compliant courier and packaging materials should be used for packing and transportation and packing should be done by an IATA certified person, as applicable
- Samples routinely transported by road or rail are participant to local regulations which require that they are always packed and transported in a safe and appropriate way to contain any risk of infection or contamination by using approved couriers and packaging / containment materials. The IATA 650 biological sample containment standards are encouraged wherever possible when road or rail transport is used