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Effects of glycopyrrolate/formoterol (Bevespi) on ventilation and gas exchange abnormalities in COPD assessed by 129Xe MRI

Sponsor: AstraZeneca/MedImmune

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this Clinical Study Protocol.

Abbreviation or special term	Explanation
COPD	Chronic obstructive pulmonary disease
LAMA	Long-acting muscarinic receptor antagonist
LABA	Long-acting beta-receptor agonist
SABA	Short-acting beta-receptor agonist
ADC	Apparent diffusion coefficient
VDP	Ventilation defect percentage
TEAE	Treatment-emergent adverse event
GFF	Glycopyrronium-formoterol fumarate
6MWT	6-minute walk test
SGRQ	St. George's respiratory questionnaire
DLCO	Carbon monoxide diffusing capacity of the lung

1. INTRODUCTION

The Centers for Disease Control (CDC) updated projections that chronic obstructive pulmonary disease (COPD) is now the 3rd leading cause of death in the US (1, 2). Although there have been significant advances in care, the COPD epidemic persists, leading to greater than 130,000 deaths/yr in the US alone (2). COPD represents the only disease in the top ten causes of death that has consistently increased in frequency over the past 4 decades only showing a slight decrease (~4%) in the preliminary data for deaths in 2011 recently released (2). The economic burden of COPD in the US in 2005 was estimated at 38.8 billion dollars in total costs (direct plus indirect) (3). Population based studies have suggested that as many as 24 million people in the US have airflow limitation consistent with COPD (4, 5). In addition, in the U.S., there are approximately 90 million current or former smokers who are at risk of developing COPD.

1.1 Background

Glycopyrronium (the active moiety of glycopyrronium bromide, also referred to as glycopyrrolate) is a LAMA which exerts its bronchodilatory effect via muscarinic receptors located on smooth muscle cells within the trachea and bronchi. Glycopyrronium is approved in many countries in multiple formulations for different indications, including COPD. There is also a large body of published data evaluating the safety and efficacy of inhaled glycopyrronium in healthy volunteers, patients with COPD and patients with asthma. Glycopyrronium is also approved in many countries worldwide as an intravenous/intramuscular injection or as an oral tablet and is indicated for systemic administration in adults for use as a preoperative antimuscarinic to reduce salivary, tracheobronchial, and pharyngeal secretions; to reduce the volume and free acidity of gastric secretions; and to block cardiac vagal inhibitory reflexes during induction of anesthesia and intubation. Glycopyrronium is also approved in the United States (US) as an oral solution which is indicated to reduce chronic severe drooling in patients aged 3-16 with neurologic conditions associated with problem drooling (e.g., cerebral palsy).

Formoterol fumarate is a potent and selective LABA approved in the US and worldwide for use in asthma and COPD. Formoterol fumarate is also approved in the US and worldwide in combination with budesonide for use in patients with asthma and COPD. When inhaled, formoterol fumarate acts locally in the lung as a bronchodilator. Formoterol fumarate stimulates β2 adrenoreceptors in the airways, inducing airway smooth muscle relaxation and reducing or preventing bronchoconstriction.

Although formoterol fumarate is classified as a LABA, it has a rapid onset of action similar to short-acting β2-agonists (SABAs). Formoterol fumarate is highly potent, displays high intrinsic activity, and can result in greater than 80% relaxation even under induced tone. Studies in patients with COPD have demonstrated that the onset of action with formoterol fumarate is faster than with anticholinergic agents or salmeterol and similar to that of SABAs, such as albuterol, and that the duration of action is ≥12 hours. Large, placebo-controlled clinical studies of up to 12 months in duration in nearly 2,500 patients demonstrated that formoterol fumarate is effective and well tolerated in patients with COPD.

1.2 Research hypothesis

Glycopyrrolate/formoterol improves regional ventilation and gas transfer assessed by 129Xe MRI in GOLD II or III COPD patients.

1.3 Rationale for conducting this study

COPD like most common chronic diseases has a complex etiology involving interactions between environmental exposures and genetic risk factors. While airflow obstruction is the defining phenotype of COPD, the abnormality is not uniform in all parts of the lung. The current literature and clinical experience clearly indicate *non-homogeneity* for this disease, even in patients who are within the same GOLD category. The non-homogeneity (i.e. clinical phenotype) includes the substantial variability in an individual's susceptibility to smoking and other insults, in disease progression and tempo (e.g. exacerbation frequency), and clinical manifestations, and in response to bronchodilator and anti-inflammatory therapies. These clinical phenotypes in large part are related to the insults that cause variable structural damages to different lung units resulting in an array of impairment in ventilation and gas exchange. Currently there is no single assessment modality that provides a comprehensive evaluation of regional lung function. The most recent revision of the GOLD criteria (Global Initiative for Chronic Obstructive Lung Disease) has expanded beyond spirometric measures to include the patient level of symptoms, risk of exacerbations, and evaluation of comorbidities (6), but no regional lung function parameters were included. A recent CT based study in the COPDGene project suggests that imaging parameters (% emphysema and mean segmental wall thickness) may be used to model progression/prognosis of exacerbations in COPD (7), but the CT imaging is static and cannot reflect dynamic lung function. Other currently available imaging techniques such as scintigraphy, single photon computed tomography (SPECT) or positron emission tomography (PET), don't provide enough resolution for the lung. Recent work with stable Krypton gas as a contrast agent with dual energy CT adds another possible strategy (8). All of these above imaging modalities, however, deliver ionizing radiation, and thus serial use in patients is dose limited and they are largely unsuitable for use in clinical trials beyond inclusion criteria.

This gap is well addressed by MR imaging that allows regional ventilation to be directly "visualized" and quantified. These techniques employ inhalation of gases, such as oxygen (9-11), perfluorinated gases (12, 13), and hyperpolarized (HP) ^{3}He (14-16). Ventilation defects visualized by hyperpolarized ^{3}He have been shown to correlate with airway tone (16-19) and quantitatively and spatially correlated to abnormal airways on CT scan (20). Hyperpolarized ^{3}He imaging has shown in patients with COPD. showed multiple ventilation defects, with irregular and delayed patterns of redistribution and air trapping (21). The apparent diffusion coefficient (ADC) correlates with the severity of emphysema (22-24). In many patients with asthma whose spirometric indices were normal, there was an increased number of ventilation defects (15). In asymptomatic active and passive smokers, the ^{3}He MRI technology was able to shown a higher ADC compared with never-smokers, indicating the presence of emphysema (25). In COPD patients, treatment with inhaled beta-agonist (albutamol), there was significant decrease in ADC in the most anterior and most posterior image slices, suggesting a reduction in regional gas trapping (26). These changes in regional lung function occurred despite minimal improvement in FEV1 (26). In a COPD ex-smoker who underwent pulmonary

function tests and hyperpolarized ^{3}He MRI serially over 4 years, ventilation defect percent (VDP) and ADC were worse without worsening FEV1 six months prior to acute exacerbation. After hospitalization and AE treatment, VDP decreased, whereas FEV1 did not improve (27). The above results support the importance of measuring regional lung function in patients with obstructive lung diseases. Unfortunately, the limited supply of the ^{3}He isotope makes the ^{3}He MRI technology unsustainable (see report to Congress) (28).

More recently, ^{129}Xe has emerged as the most prominent alternative to ^{3}He (29-31). ^{129}Xe MRI appears to more readily detect ventilation defects than ^{3}He MRI, perhaps due to its higher density and lower diffusivity in the distal airways (19, 32). ^{129}Xe MRI has been shown by Svenningsen et al to readily visualize the elimination of ventilation defects after bronchodilator administration (33). Our group has shown different ventilation histograms in COPD patients with similar airway obstruction by spirometry (30). In addition to ventilation, ^{129}Xe also has a unique property that, *d*uring the transfer *of* ^{129}Xe from the alveolar space to RBC, it generates barrier and RBC signals distinct from the gas phase signals. These additional signals correspond to diffusion across the lung parenchyma and plasma and perfusion respectively. The capability of ^{129}Xe MRI to evaluate both ventilation and gas transfer functions of the lung non-invasively and the high sensitivity to detect abnormalities make this technique ideal for assessment of mild changes in the disease lung and small improvement after treatment.

Pharmacologic therapy in COPD is used to reduce symptoms, reduce the frequency and severity of exacerbations and improve health status and exercise tolerance (34). Bronchodilator medications are central to the symptomatic management of COPD. The principal bronchodilator treatments are β2-agonists, anticholinergics, and methylxanthines used as monotherapy or in combination. Treatment with long-acting bronchodilators is more convenient and more effective at producing symptom relief than treatment with short-acting bronchodilators. Combining bronchodilators from different pharmacological classes may improve efficacy and decrease the risk of side effects compared to increasing the dose of a single bronchodilator (34). LAMAs and LABAs reduce bronchoconstriction through different mechanisms, and there is a long history of combination therapy for COPD with short-acting agents in these classes.

In clinical trials, LAMAs and LABAs have been shown to improve FEV1 in patients with COPD (35). The increment in FEV1, however, was relatively small and is insufficient to explain clinical improvement in exercise tolerance and reduction in acute exacerbations. In addition, the individual response to LAMAs/LABAs is variable and it is possible that the degree of ventilation heterogeneity at baseline could determine the magnitude of the improvement. 129Xe MRI is a unique tool that may be used to address these questions and provide a mechanistic link between physiological improvement and the patient-centered outcome measures.

1.3 Benefit/risk and ethical assessment

In the three clinical trials conducted by Pearl Therapeutics (PT003006, PT003007, PT003008), 1,329 patients with moderate to severe COPD were treated with glycopyrronium-

formoterol fumarate (GFF). GFF consistently produced a maximal 150-175 ml increase in FEV1 at 2 weeks better than placebo, LAMA or LABA alone across the studies. The benefitial effects on FEV1 persisted for up to 52 weeks (PT003008).

The most commonly reported TEAEs in patients taking GFF that occurred at higher incidences (at least 1 percentage point higher) than the Placebo group were urinary tract infection (3.3% versus 2.2%, respectively), cough (3.3% versus 2.2%, respectively), and oropharyngeal pain (1.6% versus 0%, respectively), although the differences in incidence were small (<2%) across treatment groups (Study PT003007). Serious TEAEs were most frequently reported in the SOCs of Respiratory, thoracic, and mediastinal disorders, and Infections and infestations. The most frequently reported serious TEAEs by preferred term were COPD and pneumonia. In general, most other serious TEAEs were reported for fewer than 2 subjects (Study 003006). The incidence of drug-related serious TEAEs was low and similar between GFF group (0.6%) and Placebo (0.5%). None of the drug-related serious TEAEs were reported by >1 subject in any treatment group. (Study 003006). A total of 4 treatment-emergent deaths in the GFF group were reported in Study PT003006; 1 of the subject deaths in the GFF group occurred within 14 days post-treatment. Two subjects (0.4%) in the GFF group died of acute myocardial infarction and cardiac arrest respectively. Two subjects (0.4%) died due to a cause other than a probable cardiovascular or respiratory cause: metastatic neoplasm and gunshot wound. There were no treatment-emergent deaths due to a probable respiratory cause. In Study 003006, the only TEAE leading to permanent discontinuation of study drug in the GFF group that had a higher incidence (at least 1 percentage point higher) than the Placebo group was pneumonia (1.0% versus 0%, respectively). The incidences of the other TEAEs leading to permanent discontinuation of study drug in the GFF group were similar to those in the individual component groups.

Overall, the benefit-risk ratio is quite favorable for GFF in the management of moderate to severe COPD patients. In this study, a 2-week treatment protocol will be used. GFF achieved the maximal improvement on FEV1 at 2 weeks, the first time point after the treatment was started in all three studies (PT003006, PT003007, PT003008). The shorter protocol decreases the probability of adverse events, and further increases the benefit-risk ratio.

2. STUDY OBJECTIVES

2.1 Primary objective

1) Characterize ventilation and gas transfer distributions in GOLD II and III COPD patients and assess the potential for these physiological parameters as a novel phenotyping method.

2) Quantify regional ventilation and gas transfer response to glycopyrrolate/formoterol in GOLD II and III COPD patients.

2.2 Secondary objectives

- Correlate changes in ventilation and gas transfer parameters with other endpoints, including spirometry, lung volumes, DLCO, 6MWD, dyspnea, and SGRQ.

2.3 Safety objective

N/A

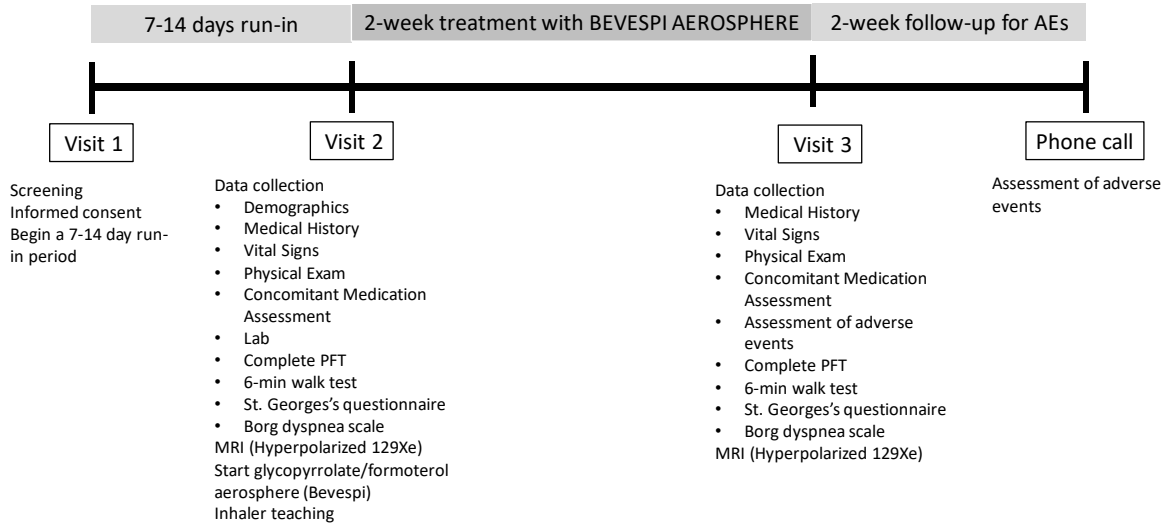
2.4 Exploratory objectives

N/A

3. STUDY PLAN AND PROCEDURES

3.1 Overall study design and flow chart

The study uses a pre-post treatment design.



3.2 Rationale for study design, doses and control groups

The study uses a pre-post design. The pre-post design is preferred for several reasons. 1) Since each patient will have a difference ventilation and gas exchange distribution pattern before the treatment, the paired design increases the sensitivity to detect regional changes using each patient as his or her own control. This eliminates the need for a separate control with no or placebo treatment. 2) The pre-post design decreases the number of patients needed to detect changes produced by the treatment.

Subject Selection Criteria

3.3 Inclusion criteria

For inclusion in the study subjects should fulfill the following criteria:

- Outpatients of either gender, age ≥ 40.
- Clinical diagnosis of COPD confirmed by post-bronchodilator spirometry demonstrating FEV1/FVC < 0.70 and FEV1 in GOLD 2 or 3 stage (30%≤ FEV1 < 80%) (http://www.goldcopd.org/).
- Willing and able to give informed consent and adhere to visit/protocol schedules. (Consent must be given before any study procedures are performed.)
- Women of childbearing potential must have a negative serum pregnancy test. This will be confirmed before participation in this investigational protocol.

3.4 Exclusion criteria

Subjects should not enter the study if any of the following exclusion criteria are fulfilled:

- Upper respiratory tract infection within 6 weeks (in this case, we will rescreen the patient after 6 weeks)
- Chronic systemic corticosteroid use > 10 mg/day of prednisone
- Chronic oxygen use (intermittent or continuous)
- Previous lung resection surgery or decortication
- Previous history of pneumothorax
- Evidence of interstitial, occupational or chronic infectious lung disease by imaging studies
- History of exposure to occupational or environmental hazards that are known to cause lung diseases
- For women of child bearing potential, positive pregnancy test
- Major chronic illnesses which in the judgement of the study physician would interfere with participation in the study
- Patients who are not willing to withhold COPD inhalers for the run-in period.
- For MRI:
    - MRI is contraindicated based on responses to MRI screening questionnaire
    - Subject is pregnant or lactating
    - Respiratory illness of a bacterial or viral etiology within 30 days of MRI
    - Subject has any form of known cardiac arrhythmia
    - Subject does not fit into 129Xe vest coil used for MRI
    - Subject cannot hold his/her breath for 15 seconds
    - Subject deemed unlikely to be able to comply with instructions during imaging

4. STUDY CONDUCT

4.1 Restrictions during the study

- Do not use albuterol HFA or nebulization treatment for at least 6 hours before Visit 2 and Visit 3

4.2 Subject enrollment <<and randomization>> <<and initiation of investigational product>>

4.2.1 Procedures for randomization

N/A

4.3 Procedures for handling subjects incorrectly enrolled<< or randomized>> <<or initiated on investigational product>>

N/A

4.4 Blinding and procedures for unblinding the study

4.4.1 Methods for ensuring blinding

N/A

4.4.2 Methods for unblinding the study

N/A

4.5 Treatments

4.5.1 Identity of investigational product(s)

Investigational product	Dosage form and strength	Manufacturer
glycopyrrolate/formoterol (Bevespi)	7.2 µg glycopyrronium and 4.8 µg of formoterol fumarate ex-actuator/per actuation.	AstraZeneca

Bevespi will be provided to the patient with a voucher BEVESPI AEROSPHERE that can be filled at a pharmacy.

4.5.2 Doses and treatment regimens

2 puffs BID

4.5.3 Additional study drug

N/A

4.5.4 Labeling

The label will include the following information:

INDICATIONS AND USAGE

BEVESPI AEROSPHERE is a combination of glycopyrrolate, an anticholinergic, and formoterol fumarate, a long-acting beta2-adrenergic agonist (LABA) indicated for the long-term, maintenance treatment of airflow obstruction in patients with chronic obstructive ulmonary disease (COPD).

Limitation of Use: Not indicated for the relief of acute bronchospasm or for the treatment of asthma.

DOSAGE AND ADMINISTRATION

- For oral inhalation only.
- Maintenance treatment of COPD: 2 inhalations of BEVESPI AEROSPHERE twice daily.

DOSAGE FORMS AND STRENGTHS

Inhalation aerosol: Pressurized metered dose inhaler containing a combination of glycopyrrolate (9 mcg) and formoterol fumarate (4.8 mcg) as an inhalation aerosol. Two inhalations equal one dose.

CONTRAINDICATIONS

- All LABAs are contraindicated in patients with asthma without use of a long-term asthma controller medication. BEVESPI AEROSPHERE is not indicated for the treatment of asthma.
- Hypersensitivity to glycopyrrolate, formoterol fumarate, or to any component of this product.

WARNINGS AND PRECAUTIONS

- Do not initiate in acutely deteriorating COPD or to treat acute symptoms.
- Do not use in combination with an additional medicine containing a LABA because of risk of overdose.
- If paradoxical bronchospasm occurs, discontinue BEVESPI AEROSPHERE and institute alternative therapy.
- Use with caution in patients with cardiovascular disorders.
- Use with caution in patients with convulsive disorders, thyrotoxicosis, diabetes mellitus, and koacidosis.

- Be alert to hypokalemia and hyperglycemia.

- Worsening of narrow-angle glaucoma may occur. Use with caution in patients with narrow-angle glaucoma and instruct patients to contact a physician immediately if symptoms occur.

- Worsening urinary retention may occur. Use with caution in patients with prostatic hyperplasia or bladder-neck obstruction and instruct patients to contact a physician immediately if symptoms occur.

ADVERSE REACTIONS

Most common adverse reactions (incidence ≥2 % and more common than with placebo) include: urinary tract infection and cough

To report SUSPECTED ADVERSE REACTIONS, contact AstraZeneca at 1-800-236-9933 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch

DRUG INTERACTIONS

Other adrenergic drugs may potentiate effect: Use with caution

Xanthine derivatives, steroids, diuretics or non-potassium sparing diuretics may potentiate hypokalemia or ECG changes. Use with caution.

Diuretics: Use with caution. Electrocardiographic changes and/or hypokalemia associated with non-potassium sparing diuretics may worsen with concomitant beta 2-agonists.

Monoamine oxidase inhibitors and tricyclic antidepressants: Use with extreme caution. May potentiate effect of formoterol fumarate on cardiovascular system.

Beta-blockers: Use with caution and only when medically necessary.

Anticholinergics: May interact additively with concomitantly used anticholinergic medications. Avoid administrations of BEVESPI AEROSPHERE with other anticholinergic-containing drugs.

4.5.5 Storage

Each canister of glycopyrrolate/formoterol aerosphere (Bevespi) is foil overwrapped with desicant and is formulated with sufficient suspension to ensure delivery of 120 inhalations from the nominal 50 µL valve over the product shelf life. Glycopyrrolate/formoterol aerosphere (Bevespi) should be stored below 25°C (77°F). Do not freeze. Excursions permitted up to 30°C (86°F).

4.6 Concomitant and post-study treatment(s)

All medications that the patient is taking prior to the enrollment will continue, except for long-acting inhalers that the patient takes for COPD, including LABA, LAMA and inhaled steroids. These medications will be held for 7-14 days of the run-in period. BEVESPI AEROSPHERE will be given at the end of the run-in period and continue for the study period (2 weeks). At the end of the study period, the patient can go back to their original COPD medications, or continue BEVESPI AEROSPHERE, depending on the patient's insurance coverage. The patient can continuealbuterol HFA and nebulization treatments, but will be asked not use it at least 6 hours before Visit 2 and Visit 3.

4.7 Treatment compliance

4.7.1 Accountability

Each subject will be provided with a voucher of BEVESPI AEROSPHERE that can be filled at a pharmacy. We will monitor treatment compliance by checking the dose indicator on the inhaler, which moves after tenth actuation.

4.8 Discontinuation of investigational product

If the patient calls complaining of any new symptoms after the use of BEVESPI AEROSPHERE, including but not limited to adverse events described above, he or she will enter the following procedures for discontinuation from investigational product.

4.8.1 Procedures for discontinuation of a subject from investigational product

1. The patient will be asked to stop taking BEVESPI AEROSPHERE

2. A visit with PI or co-PI will be scheduled. During the visit, PI will evaluate the patient and restart the patient's COPD medications that were taken by the patients before the study. The patient will also return the unused BEVESPI AEROSPHERE

3. If the patient was considered having an acute exacerbation or infection, appropriate treatments, including prednisone taper and antibiotics, will be prescribed. A follow-up appointment with the patient's primary pulmonary physician will be scheduled.

4.9 Withdrawal from study

The patient can withdraw from the study at any time for any reason.

5. COLLECTION OF STUDY VARIABLES

5.1 Recording of data

Data will be recorded in Excel spreadsheets stored in a specific folder (e.g. Bevespi MRI COPD) of a common drive of the Duke Network.

5.2 Data collection at enrollment and follow-up

Visit 1 (Screening Visit)

- Outpatients of either gender, age ≥ 40.
- Clinical diagnosis of COPD confirmed by post bronchodilator spirometry demonstrating FEV1/FVC < 0.70 and FEV1 in GOLD 2 or 3 stage (30%≤ FEV1 < 80% of predicted normal value) (http://www.goldcopd.org/). The PFT should be within 6 months of the screening visit.
- Current or former cigarette smokers with 10 or more pack-years cigarette smoking history
- Begin a run-in period (7-14 days) for eligible patients
- Sign informed consent
- MRI suitability using MRI questionnaire

Visit 2 (7-14 days after Visit 1)

- Demographics
- Medical History (including AEs)
- Vital Signs
- Physical Exam
- Concomitant Medication Assessment
- Lab (including existing lab in medical records and serum pregnancy test, if needed)
- MRI (Hyperpolarized 129Xe)
- Complete pulmonary function test (including DLCO)
- 6-min walk test (6MWT)
- St. Georges's respiratory questionnaire (SGRQ)
- Borg's dyspnea scale
- COPD assessment test (CAT)
- Start glycopyrrolate/formoterol aerosphere (Bevespi)
- Teaching the subject how to use the inhalers (glycopyrrolate/formoterol aerosphere and albuterol)

Visit 3 (2 weeks after Visit 2)

- Medical History (including AEs)
- Vital Signs
- Physical Exam
- Concomitant Medication Assessment
- Assessment of Adverse Events
- MRI (Hyperpolarized 129Xe)
- Complete pulmonary function test (including DLCO)

- 6MWT
- SGRQ
- Borg's dyspnea scale
- CAT

Follow-up telephone call (2 weeks after Visit 3)

- Assessment of Adverse Events

5.2.1 Enrollment procedures

The study coordinator will screen and recruit patients from Duke Asthma, Allergy and Airway Center (AAAC). All clinical data, including PFT, 6MWT, SGRQ and Borg's dyspnea scale, will be collected at AAAC. After clinical data collection is finished, the patient will be referred for 129Xe MRI scanning, which is located at Duke North Hospital. We expect that the clinical assessment and data collection can be finished in the morning of the visit. 129MRI scan can be performed in the afternoon.

5.2.2 Follow-up procedures

A telephone call will be placed at the end of the first week to discuss with the subject regarding his or her COPD status and Bevespi use. It also serves to remind the subject of the follow-up visit (Visit 3) of th study.

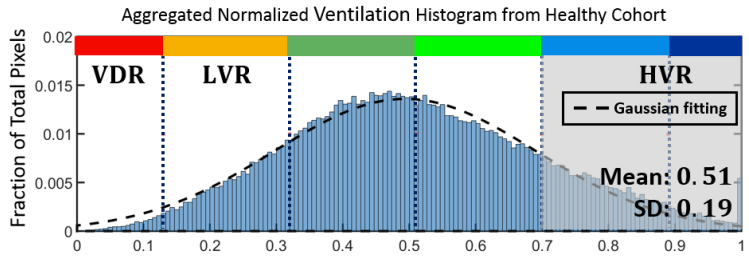
5.3 Efficacy

5.3.1 Efficacy variables: the following variables will be measured and compared before and 2 weeks after glycopyrrolate/formoterol aerosphere (Bevespi) treatment. We will allow a 1-week window for all visits.

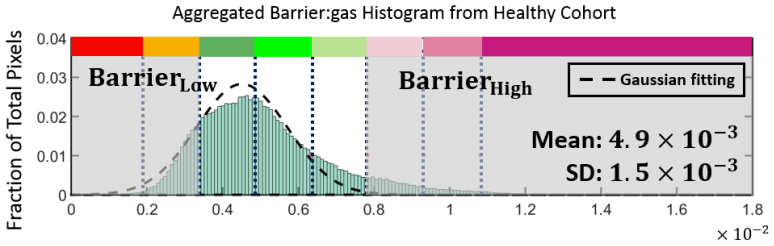
- MRI (Hyperpolarized 129Xe)
  - Assessment of ventilation distribution (Figure 1A)
    - Bin 1 (red): ventilation defect region (VDR, 0 to mean-2SD)
    - Bin 2 (orange): low ventilation region (LVR, mean-2SD to mean-1SD])
    - Bins 3 and 4 (greens): regions with normal ventilation (mean-1SD to mean+1SD)
    - Bins 5 and 6 (blues): high ventilation region (HVR, > mean+1SD).
  - Assessment of dissuion distribution (Figure 1B)
    - Bins 1 and 2 (red and orange)($Barrier_{Low}$): high diffusion regions (0 to mean-1SD)
    - Bins 3 and 4 (greens)($Barrier_{Normal}$): normal diffusion regions (mean-1SD to mean+1SD)
    - Bins 5 to 8 (pinks)($Barrier_{High}$): low diffusion regions (> mean +1SD).
  - Assessment of perfusion distribution (Figure 1C)
    - Bins 1 and 2 (red and orange) (RBC_{Low}): low perfusion regions (0 to mean-1SD)
    - Bins 3 and 4 (greens) (RBC_{Normal}): regions with normal perfusion (mean-1SD to mean+1SD)
    - Bins 5 and 6 (blues) (RBC_{High}): high perfusion regions (> mean+1SD).

Figure 1. The nearly Gaussian distributions of ventilation (A), Barrier:gas (B) and RBC:gas (C) in the reference population of 10 healthy subjects (n=10, age 29±8 yrs). Their mean and standard deviations were used to define the thresholds of intensity bins used to display the maps, with colors depicted above the histogram. The color coding for ventilation and RBC maps used the same scheme as previously introduced (36): red for defects; orange for low intensity; greens for the two bins around either side of the mean of the reference population; and blues for higher intensities. For the barrier maps, the first 4 colors were the same as for ventilation and RBC. However, to allow for better differentiation of the high barrier signals anticipated in patients with severe interstitial lung diseases, the highest bins were assigned pink and purple colors.

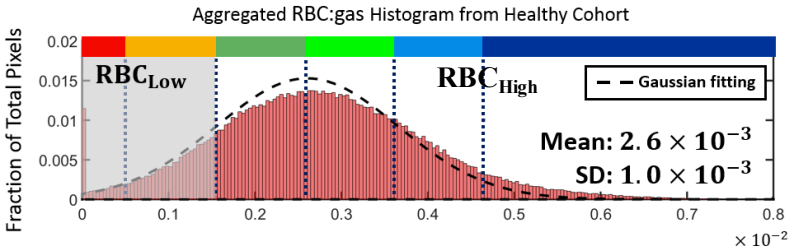
A



B



C



- Complete pulmonary function test (including DLCO)
- 6-min walk test
- St. Georges's questionnaire
- Borg dyspnea scale

- CAT

5.4 Safety

The Principal Investigator is responsible for ensuring that all staff involved in the study is familiar with the content of this section.

5.4.1 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 (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

The term AE is used to include both serious and non-serious AEs.

5.4.2 Definitions of serious adverse event

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

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

The causality of SAEs (their relationship to all study treatment/procedures) will be assessed by the investigator(s) and communicated to AstraZeneca.

5.4.3 Recording of adverse events

To the ISSROT:

The Patient Safety CDP Technical Planning Document (TPD), or other appropriate project document, provides recommendation on recording of adverse events. The text in this guideline should be modified to comply with the Patient Safety TPD/ other appropriate project/TA documents if not otherwise agreed with Patient Safety.

Insert un numbered subheadings as appropriate.

Consider:

- How AEs related to specific study procedures (eg, biopsy, endoscopy) will be handled
- How AEs related to abnormal ECGs will be handled
- How symptoms of the Disease Under Study and/or symptoms of expected disease progression and/or lack of effect would be handled. There may be circumstances when it is justifiable not to record these symptoms as adverse events. Refer to the Technical Planning Document for how symptoms of the disease under study/disease progression and/or adverse event variables should be collected and analyzed.
- How SAEs that are also efficacy endpoints should be handled/whether there are waivers for endpoints

Time period for collection of adverse events

During the 2-week study period and 2 weeks after the study ends.

Follow-up of unresolved adverse events

The PI will follow up with the patients who develop adverse events. These patients will be scheduled to be seen in the clinic if needed.

The following variables will be collected for each AE:

- AE (verbatim)
- The date when the AE started and stopped
- Whether the AE is serious or not
- Investigator causality rating against the Investigational Product (yes or no)
- Action taken with regard to investigational product
- AE caused subject'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
- Causes for serious AE
- Date of hospitalization
- 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
- Description of AE.

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 5.4.2. 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 a SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE.

Adverse Events based on signs and symptoms

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.

Adverse Events based on examinations and tests

Deterioration as compared to baseline in physical exams and lab tests will therefore only be reported as AEs if they fulfill any of the SAE criteria or are the reason for discontinuation of treatment with the 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 (eg, anemia versus low hemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s 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.

Pregnancy

All pregnancies and outcomes of pregnancy should be reported to AstraZeneca.

There are currently no adequate data of on the use of this study medication, or its individual components in pregnant women. Animal reproduction studies in rats and rabbits showed no teratogenic effects of glycopyrrolate individually, when dosed at approximately 18000 and 270 times the maximum recommended human daily inhalation dose in adults. However, single-dose studies in humans have found that very small amounts of glycopyrrolate pass through the placental barrier. Formoterol fumarate individually has been shown to have teratogenic effects, embryocidal effects, lead to increased pup loss at birth and during lactation, and lead to decreased pup weight in rats and rabbits when dosed at approximately 1500 and 61000 times, respectively, the maximum recommended human daily inhalation dose in adults. Additional complications such as umbilical hernia, prolonged pregnancy, and fetal brachygnathia were observed in rats as well when dosed at 1500 and 7600 times the maximum

recommended human daily inhalation dose. No teratogenic effects were seen when dosed at approximately 600 times the maximum recommended dose, however (BEVESPI Aerosphere® PACKAGE INSERT 2016).

Therefore the study medication and its individual components, glycopyrrolate and formoterol fumarate, should only be used during pregnancy if the expected benefits outweigh the potential risks.

Pregnancy itself is not regarded as an AE unless there is a suspicion that the study medication 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 SAEs. 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 patient was discontinued from the study.

If any pregnancy occurs, the Investigator or other site personnel will inform AstraZeneca the appropriate AstraZeneca representatives within 1 day, ie, immediately but no later than 24 hours of when he or she becomes aware of it.

The designated AstraZeneca (?? should it not read Quintiles??) representative will work with tThe Investigator should to ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site within 1 or 5 calendar days for SAEs (see Section 6.4 please check if this is the right section) and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

Disease progression

Disease progression can be considered as a worsening of a subject's condition attributable to the disease for which the investigational product is being studied. It may be an increase in the severity of the disease under study and/or increases in the symptoms of the disease. In this proposal, the development of progressive dyspnea on exertion would be considered as disease progression and not an AE. Events, which are unequivocally due to disease progression, should not be reported as an AE during the study.

5.4.4 Reporting of serious adverse events

Investigators and other site personnel must inform the FDA, via a MedWatch/AdEERs form, of any serious or unexpected adverse events that occur in accordance with the reporting obligations of 21 CFR 312.32, and will concurrently forward all such reports to AZ. A copy of the MedWatch/AdEERs report must be faxed to AstraZeneca at the time the event is reported to the FDA. It is the responsibility of the investigator to compile all necessary information and ensure that the FDA receives a report according to the FDA reporting requirement timelines and to ensure that these reports are also submitted to AstraZeneca at the same time.

When reporting to AstraZeneca, a cover page should accompany the MedWatch/AdEERs form indicating the following:

- Investigator Sponsored Study (ISS)
- The investigator IND number assigned by the FDA
- The investigator's name and address
- The trial name/title and AstraZeneca ISS reference number

Investigative site must also indicate, either in the SAE report or the cover page, the causality of events in relation to all study medications and if the SAE is related to disease progression, as determined by the principal investigator.

Send SAE report and accompanying cover page by way of fax to AstraZeneca's designated fax line: 1-866-984-7229

Serious adverse events that do not require expedited reporting to the FDA need to be reported to AstraZeneca preferably using the MedDRA coding language for serious adverse events.

In the case of blinded trials, AstraZeneca will request that the Sponsor either provide a copy of the randomization code/ code break information or unblind those SAEs which require expedited reporting.

All SAEs have to be reported to AstraZeneca, whether or not considered causally related to the investigational product. All SAEs will be documented. The investigator is responsible for informing the IRB and/or the Regulatory Authority of the SAE as per local requirements.

5.4.5 Laboratory safety assessment

Minimum. No blood samples are obtained, except for pregnancy test for females of child bearing age.

For blood volume see Section 6.1.

5.4.6 Physical examination

Minimum risk

5.4.7 ECG

N/A

5.4.7.1 Resting 12-lead ECG

N/A

5.4.7.2 Real time display (telemetry)

N/A

5.4.8 Vital signs

Minimum risk

5.4.8.1 Pulse and blood pressure

Minimum risk

5.4.8.2 Body temperature

Minimum risk

5.4.9 Other safety assessments

N/A

5.5 Patient reported outcomes (PRO)

- St. Georges's questionnaire -- minimumrisk
- Borg dyspnea scale – minimum risk

5.6 Pharmacokinetics

N/A

5.6.1 Collection of samples

N/A

5.6.2 Determination of drug concentration

N/A

5.7 Pharmacodynamics

N/A

5.7.1 Collection of pharmacodynamic markers

N/A

5.8 Pharmacogenetics

N/A

5.8.1 Collection of pharmacogenetic samples

5.9 Health economics

N/A

6. BIOLOGICAL SAMPLING PROCEDURES

6.1 Volume of blood

N/A

6.2 Handling, storage and destruction of biological samples

6.2.1 Pharmacokinetic and/or pharmacodynamic samples

N/A

6.2.2 Pharmacogenetic samples

6.3 Labeling and shipment of biohazard samples

N/A

6.4 Chain of custody of biological samples

N/A

6.5 Withdrawal of informed consent for donated biological samples

N/A

7. ETHICAL AND REGULATORY REQUIREMENTS

7.1 Ethical conduct of the study

LABA/LAMA combination is the mainstay therapy for COPD patients. Glycopyrrolate/formoterol aerosphere belongs to this medication category. The 7-14 day run-in period has been used routinely in all randomized controlled trials that compared the efficacy of a specific inhaler with placebo or another inhaler in GOLD 2 and 3 COPD patients. The risk for worsening symptoms during the short run-in period should be minimum.

7.2 Ethics and regulatory review

The protocol will be submitted to Duke IRB for approval prior to the start of the study. IRB will be informed of all AEs and SAEs.

7.3 Informed consent

Eligible patient will sign the informed consent during Visit 1

7.4 Changes to the protocol and informed consent form

Any changes in the prototol and informed consent will be submited to IRB for approval.

7.5 Audits and inspections

AstraZeneca does not conduct ESR onsite audits, however, the AstraZeneca ESR ESSROs portal containing study information and site updates are internally audited

8. STUDY MANAGEMENT

8.1 Training of study site personnel

All study personnel will keep human study training up to date. The CRC will all be trained to be familiar with the protocol and procedures involved in the study. These procedures include pulmonary function test, 6 minute walk test, St. George's questionnaire and Borg's dyspnea scale. 129Xe MRI is a core facility at Duke and all personnel in this core has been well trained to perform the scan.

8.2 Monitoring of the study

8.2.1 Source data

The raw data will be entered into Excel. Each patient will have a spreadsheet. This will be the source data.

8.3 Study timetable and end of study

After the protocol approval by AstraZeneca, the study will be submitted to Duke IRB. It usually takes 6-8 months for approval. Once the protocol is approved by IRB, the study is expected to be completed in 18-24 months.

9. DATA MANAGEMENT

All data will be entered into Excel spreadsheets via a Duke computer and stored in a specific folder (e.g., Bevespi MRI COPD) in a common drive. The drive can be accessed by the study team. The spreadsheets will be passwork protected. Only the study team can access the data. The data can be shared with AstraZeneca upon request. When sharing data with AstraZeneca, the study team will follow the Duke IT security guidelines. These guidelines discourage sending data outside of Duke network by E-mail. A more secure mechanism to share the data will be used, including face-to-face meetings,

10. EVALUATION AND CALCULATION OF VARIABLES

10.1 Calculation or derivation of efficacy variable(s)

- 129Xe MRI ventilation and gas exchange parameters, described above.
- Pulmonary function test
- 6 minute walk test

10.2 Calculation or derivation of safety variable(s)

N/A

10.2.1 Other significant adverse events (OAE)

N/A

10.3 Calculation or derivation of patient reported outcome variables

- St. Georg's questionnaire
- Borg's dyspnea scale

10.4 Calculation or derivation of pharmacokinetic variables

N/A

10.5 Calculation or derivation of pharmacodynamic variable(s)

N/A

10.5.1 Calculation or derivation of the relationship between pharmacokinetic and pharmacodynamic variables

N/A

10.5.2 Population analysis of pharmacokinetic/pharmacodynamic variables

N/A

10.6 Calculation or derivation of pharmacogenetic variables

N/A

10.7 Calculation or derivation of health economic variables

N/A

11. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION

11.1 Description of analysis sets

11.1.1 Efficacy analysis set

- 129Xe MRI parameters
- Complete pulmonary function test (including FVC, FEV1, FEF25-75, TLC, RV, RV/TLC and DLCO)
- 6-min walk test
- St. Georges's questionnaire

- Borg dyspnea scale

11.1.2 Safety analysis set

Descriptive analysis. Patients who only have visit 2 will be included (for Aim 1).

11.2 Methods of statistical analyses

Paired t-test. Only patients who have both visits 2 and 3 will be included (for Aim 2). Significance level is at 0.05 for a 2-taliled test. We plan to use line graph or box plots to show pre- an dpost-changes.

MRI endpints will be correlated with other parameters using linear regression analyses. We plan to use scattered plots to demonstrate the correlation.

11.2.1 Interim analyses

None

11.3 Determination of sample size

In this study, the sample size was estimated using the paired t-test based on the following parameters: alpha 0.05, beta 0.20, effect size of 20% (based on ventilation defect percentage) and standard deviation of 30% (based on MRI ventilation distribution). At least 18 patients will be needed. The effect size of 20% was derived from the preliminary data in asthma patients before and after albuterol. The ventilation defect percentage in older asthma patients decreased by approximately 40% after albuterol treatment. If we assume 40% as the upper limit of airway reversibility in older asthma patients, we could take half of that size (i.e. 20%) for COPD patients since airway obstruction in COPD patients is only partially reversible. The standard deviation of 30% is derived from previous studies that assessed ventilation distribution in pulmonary patients.

11.4 Data monitoring committee

N/A

12. IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

N/A

12.1 Overdose

If an overdose on an AstraZeneca study drug occurs in the course of the study, then investigators or other site personnel inform appropriate AstraZeneca representatives **within one day**, ie, immediately but no later than **the end of the next business day** of when he or she becomes aware of it.

The designated AstraZeneca representative works with the investigator to ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site.

The reporting process will be the same as that described in section 5.4.4 above.

12.2 Pregnancy

All outcomes of pregnancy should be reported to AstraZeneca.

12.2.1 Maternal exposure

N/A

12.2.2 Paternal exposure

N/A

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