University of New Mexico

Clinical Study Protocol

Study Title: A Dose-escalation Study to Detect Urease-producing Bacteria in Lungs of Healthy Volunteers and Subjects with Cystic Fibrosis Using Aerosolized ^{13}C-urea

Test Product: ^{13}C-urea Breath Test Kit (IDE# G120190)

HRPO Number: 12-521

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STUDY SYNOPSIS

Location of study: University of New Mexico Hospital, Pediatric/Adult Pulmonary Clinic, Ambulatory Care Center, Albuquerque, NM		**Tabulated Study Protocol**	
Finished product: ^{13}C-urea Breath Test Kit			
Active ingredient: ^{13}C-urea			
Protocol date 29 November 2012	**HRPO#** 12-521	**Planned study period** January1, 2013-March 31,2013	

Title of Study:

A Dose-escalation Study to Detect Urease-producing Bacteria in Lungs of Healthy Volunteers and Subjects with Cystic Fibrosis Using Aerosolized ^{13}C-urea

Indication: Use of a ^{13}C-urea breath test in the qualitative detection of urease-containing bacteria in the respiratory tract and as an aid in the initial and post-treatment detection of respiratory tract infections

Study Design: This is a single-center, single-administration, dose-escalation study designed to determine the safety and dose response of aerosolized ^{13}C-urea 20 mg and 50 mg in detecting urease-producing bacteria in the lungs of healthy volunteers (those without and those diagnosed with *Helicobacter pylori* infection) and in subjects with cystic fibrosis (CF). Subjects will be contacted by telephone the next day and asked a non-leading question about adverse events.

The dose will not be escalated until at least 24 hours after all subjects in the cohort have been tested at the current dose. If a subject has an adverse event or new symptom that remains unresolved at 48 hours, no new subjects will be enrolled until the relationship between the AE and symptom can be evaluated. If any subject experiences an adverse event considered by the data safety monitor to be dose limiting, then three additional subjects will be enrolled at that current dose. Dose escalation will stop if more than one additional subject experiences dose-limiting toxicity.

After the safety of the maximum dose in the cohort of healthy volunteers without *H. pylori* infection has been reviewed by a data safety monitor, healthy volunteers diagnosed with *H. pylori* infection and subjects with CF will be enrolled. Three subjects diagnosed with *H. pylori* infection will be tested at the maximum dose to assess potential interference of urease producing bacteria in the stomach; subjects infected with *H. pylori* can be enrolled at any time after the safety of the maximum dose in healthy volunteers without *H. pylori* infection has been evaluated, but they will meet the entrance criteria for healthy volunteers without *H. pylori* infection.

Sputum will be collected from each subject with CF before administering study drug in order to identify bacterial flora using two standard microbiologic methods. The first is a quantitative method in which colonies are stained and counted, the second is a colorimetric method in which a sample is plated in a medium that changes to a blue color as its pH is increased by the production of ammonia, a by-product of the urease-catalyzed degradation of urea.

Study Duration: The maximum duration of study participation for each subject is approximately 9 days: up to 7 days between the screening visit and the study visit plus a follow-up visit 24 hours after study drug administration.

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Study Objectives: The primary objective is to assess the safety of ^{13}C-urea administered by inhalation. The secondary objective is to assess the kinetics of ^{13}C-carbon dioxide production by measuring the isotopic ratio of ^{13}C to ^{12}C in exhaled carbon dioxide up to 15 minutes after administration and to assess potential interference of urease-producing bacteria in the stomach.

Study Endpoints: The primary endpoint is the safety of inhaled ^{13}C-urea assessed by adverse events including pulmonary findings and changes from baseline in physical examination findings, pulse oximetry results, pulmonary function test results, and vital signs. Secondary endpoints are the isotopic ratios of ^{13}C to ^{12}C in exhaled carbon dioxide measured with the POCone detector (Meretek Diagnostics Group of Otsuka America Pharmaceutical, Rockville, MD) at 5, 10, and 15 minutes after study drug administration.

Study Population:

Subjects who meet all the following inclusion and exclusion criteria are eligible to enroll in the study.

Inclusion Criteria for Healthy Volunteers Without *H. pylori* infection:

Healthy volunteers who meet all the following inclusion criteria are eligible to enroll.

- At least 18 years old at the time of providing informed consent in a manner approved by the University of New Mexico, Institutional Review Board and willing to comply with the requirements of the study
- In generally good health as determined by the investigator, with no respiratory illness within 2 weeks before the screening visit and no pulmonary history
- No history of allergy or asthma
- Has not smoked tobacco within 6 months before the screening visit and agrees not to smoke until after the follow up visit
- Forced expiratory volume in 1 second (FEV_1) at least 80% at the time of providing informed consent

Inclusion Criteria for Healthy Volunteers Diagnosed With *H. pylori* infection:

Healthy volunteers diagnosed with *H. pylori* infection who meet all the inclusion criteria for healthy volunteers and additionally who have been diagnosed with *H. pylori* infection are eligible to enroll.

Inclusion Criteria for Subjects With Cystic Fibrosis:

Subjects with CF who meet all the following inclusion criteria are eligible to enroll.

- At least 18 years old at the time of providing informed consent in a manner approved by the University of New Mexico, Institutional Review Board and willing to comply with the requirements of the study
- Diagnosed with CF at least 24 months before the screening visit

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- Documented presence of *Pseudomonas* organisms in at least three sputum cultures within 2 years before the screening visit, one of which within 6 months before the screening visit
- Is able to produce sputum at the time of the screening visit
- FEV_1>60% or more than 1.5 L at the time of providing informed consent
- Has not smoked tobacco within 6 months before the screening visit and agrees not to smoke until after the follow up visit

Exclusion Criteria

Subjects who meet any of the following exclusion criteria are not eligible to enroll.

- Positive *H. pylori* serology, except for subjects in the *H. pylori* cohort
- Subjects intolerant of albuterol
- Subjects diagnosed with asthma
- Subjects with evidence of upper respiratory tract infection within 14 days before the screening visit
- Use of inhaled antibiotics within 12 hours before the administration of the breath test on Day 1 or required use of inhaled antibiotics during the study
- Has smoked tobacco within 6 months before the screening visit
- Females who are pregnant or nursing or of child-bearing potential who are not using a medically acceptable form of contraception (acceptable forms of contraception are hormonal birth control, intrauterine device, or barrier method plus a spermicidal agent) or abstinence
- Any condition or history that, in the judgment of the investigator, would compromise the ability of the subject to comply with the study protocol or to complete the study
- Subjects who have used an investigational agent within 28 days before Day 1

Study Product, Dose, and Mode of Administration:

^{13}C-urea: 20 mg and 50 mg administered by inhalation

Reference Product, Dose, and Mode of Administration: Not applicable

Efficacy Variables: Breath test results

Safety Variables: Adverse events (frequency, intensity, seriousness, and relationship to study drug), spirometry test results (FEV_1 and FVC), and respiratory examinations (respiratory rate, auscultation [presence of wheezing and crackles], pulse oximetry, and related symptoms, such as cough)

Pharmacokinetics: Production of carbon dioxide

Sample Size and Statistical Rationale: Approximately 15 subjects will be enrolled, 9 healthy volunteers and 6 subjects with CF. Three healthy volunteers without *H. pylori* infection will each receive

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a single 20 mg dose and 3 will each receive a single 50 mg dose. After the safety of the 50 mg dose has been evaluated, 3 subjects with CF will each receive a single 20 mg dose and 3 subjects with CF will each receive a single 50 mg dose. Three healthy volunteers diagnosed with *H. pylori* infection will also each receive a 50 mg dose to assess potential interference of urease producing bacteria in the stomach. Three additional subjects will be added to every dose level at which a dose-limiting adverse event occurs; no more than 30 subjects will be enrolled. This sample size is based upon a dose-escalation model, not a calculation of statistical power.

Study Sites and Location: The study will be conducted at a single center at the University of New Mexico Hospital, Pediatric/Adult Pulmonary Clinic, Ambulatory Care Center, Albuquerque, NM.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Term
AE	adverse event
CF	cystic fibrosis
CFR	Code of Federal Regulations
COPD	chronic obstructive pulmonary disease
CRF	case report form
DLT	dose-limiting toxicity
FDA	Food and Drug Administration
FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
MedDRA	Medical Dictionary for Regulatory Activities
SAE	serious adverse event
SAR	suspected adverse reaction
SSAR	serious suspected adverse reaction
TEAE	treatment-emergent adverse event
WFI	water for injection

1. INTRODUCTION

1.1 CYSTIC FIBROSIS

Cystic fibrosis (CF) is a genetic disease which affects approximately 30,000 people in the United States. Prior to the development of therapies, most people with CF died in childhood. New advances in therapies have increased the average lifespan to about 35 years with respiratory failure continuing to be the primary cause of death.

The CF gene that codes the CF protein (cystic fibrosis transmembrane conductance regulator [CFTR]) was discovered in 1989. CFTR is a protein that regulates the flows of ions, including chloride and bicarbonate, across membranes.

One consequence of abnormal CFTR is that glandular fluids thicken, which leads to obstruction of the ducts. In the pancreas, this obstruction blocks the release of digestive enzymes into the gastrointestinal tract, resulting in poor absorption of nutrients. Patients with CF take replacement pancreatic enzymes before every meal to aid digestion and absorption.

In the lungs of patients with CF, thickened layers of fluid lining the airways obstruct the removal of bacteria. Bacteria that are normally present in healthy lungs, and that are normally washed out by movement of thin layers of surface fluid, are not cleared easily from lungs of patients with CF. Urease-producing bacteria *Staphylococcus aureus* and *pseudomonas* are the most prominent bacteria found in increased numbers in lungs of patients with CF. As bacterial loads increase, patients with CF start to develop mildly increased symptoms such as cough, but they generally do not develop the dramatic signs of lung infection seen in healthy patients who have sudden increases in bacteria that develop into pneumonia.

Patients with CF are followed in CF centers where the insidious nature of increasing bacterial loads is followed by routine sputum or throat cultures at least every three months. The bacterial profile of each patient is known and used to guide aggressive antibiotic therapy for exacerbations. All bacteria are not cleared with antibiotic therapy, but it is believed that antibiotic therapy decreases bacterial burden. It is further believed that patients with CF would experience fewer or less severe exacerbations if more timely antibiotic therapy was administered in advance of exacerbations. To date, however, the timing of these exacerbations cannot be predicted.

1.2 ^{13}C-UREA BREATH TEST

The ^{13}C-urea breath test is based upon a modification of existing breath testing technology that detects and monitors a biomarker associated with peptic ulcers. The mechanism of action for detecting urease-containing bacteria in the lungs is the same as for detecting another urease-containing bacterium, *Helicobacter pylori*, in the stomachs of patients with peptic ulcers.

For the intended indication, inhaled ^{13}C-urea is catalytically hydrolyzed into ^{13}C-carbon dioxide by urease, an enzyme associated with certain bacterial pathogens common to lung infections in patients who develop CF (**I**).

$$^{13}CO(NH_2)_2 + 3H_2O \xrightarrow{\text{urease}} {}^{13}CO_2 + 2NH_4OH \qquad \textbf{(I)}$$

By measuring the ratio of two stable and naturally occurring isotopes of carbon in exhaled carbon dioxide before and after inhalation of ^{13}C-urea, the presence of urease-containing bacteria can be confirmed. In the absence of urease-containing bacteria in the lungs, the isotopic ratio of ^{13}C to ^{12}C in the baseline and test breath samples will be approximately the same. If urease-containing bacteria are present within the lungs, the concentration of exhaled ^{13}C will be increased measurably in the test breath sample relative to baseline. It is not anticipated that the identity or numbers of organisms in the lungs can be deduced from the numerical δ value reported by the breath test, but it is anticipated that the magnitude of the numerical δ value will reflect the underlying bacterial load. It is anticipated that urease-producing bacteria can be detected within 15 minutes of initiating the breath test.

1.3 UREA

Urea has been approved by the Food and Drug Administration (FDA) for oral administration (75 mg when labeled with ^{13}C) in the diagnosis of *H. pylori* infection in the stomach and for intravenous administration in high doses (500 to 1500 mg/kg/day, up to 120 g/day) as an osmotic diuretic to treat patients with cerebral edema and glaucoma. There is a significant amount of safety and toxicology data of urea administration via these routes (Crews and Davidson 1961, Crews and Davidson 1962, Gisbert and Pajares 2005, Pathak et al 2004, Pathak et al 2010, Soupart and Decaux 1996).

Urea liquefies viscid mucus when injected into the auditory canal (Bauer 1970) and is capable of reducing mucus viscosity and increasing mucus expectoration in lung diseases such as chronic obstructive pulmonary disease (COPD), bronchiectasis, CF, and asthma when administered by inhalation.

No adverse effects on lung function were reported when 2 to 14 g of urea was administered daily by inhalation with an intermittent positive-pressure ventilator over “short intervals” for 14 to 30 days to 32 patients with chronic bronchitis, cystic fibrosis, asthma, bronchiectasis, and emphysema; and no adverse effects were reported when approximately 7.3 g of urea was administered by inhalation over 10 minutes to 6 patients with CF using a DeVilbis nebulizer (assuming 6 mL 2M urea/min) (Waldron-Edward and Skoryna 1966).

The mucolytic properties of urea have not been pursued, however, because hypertonic solutions were subsequently shown to be a broncho-constrictor in patients with asthma (Cade and Pain 1972, Pain and Denborough 1967). In one such study, mean forced expiratory volume in 1 second (FEV_1) decreased 12% ± 20% in 56 adults who inhaled urea aerosolized using a Wright nebulizer (Cade and Pain 1972). It is estimated that approximately 32 mg of urea was inhaled by each patient in this study assuming typical values of parameters of operation (output,

0.13 mL/min; airflow, 9 L/min) and reported experimental detail (urea concentration, 4M; inhalation time, 10 min).

It is now known that inhalation of osmotic agents such as urea, mannitol, and hypertonic saline cause the release of mast cell mediators (histamine and leukotrienes) in patients with asthma, resulting in bronchospasm (Anderson 2010, Brannan et al 2003, Brannan et al 2009, Sverrild et al 2009). Hypertonic saline has been used as a test for bronchial hyper-responsiveness in patients with asthma, and is widely used, nonetheless, with preventive bronchodilator administration to treat hyper-responsive patients with CF.

Overall, evidence points to the safety of inhaled urea. As with all inhaled solutions, it is possible that it will trigger some cough and bronchospasm; this does not occur in all subjects, however, and when it does occur, it is mild and transient. Further, pretreatment with a bronchodilator (albuterol) has been shown to prevent the response in most subjects. When bronchospasm does occur, it also can be rapidly reversed with albuterol therapy.

1.4 RATIONALE

It is anticipated that rapid detection and monitoring of lung pathogens will provide an early warning signal of the onset of bacterial load and early confirmation of the therapeutic effect of the treatment regimen. In laboratory experiments, urease-producing bacteria were detected within approximately 15 to 20 minutes after exposure to urea.

1.5 BENEFIT-TO-RISK ASSESSMENT

The safety profile of ^{13}C-urea 20 mg and 50 mg administered by inhalation will be assessed in a dose escalation study conducted in two parts at a single center located at

University of New Mexico Hospital
Pediatric/Adult Pulmonary Clinic
Ambulatory Care Center
2211 Lomas Blvd NE
Albuquerque, NM 87106

This facility has the required equipment/protocol on site to respond to an emergent adverse effect of treatment. Co-investigators Dr. Harkins or Davies will be available on site should an emergent adverse event (such as severe bronchospasm or cardiopulmonary arrest) occur during study administration.

This is the first study in humans of this ^{13}C-urea breath test. In part 1, a conventional dose-escalation study design will assess the safety of inhaled ^{13}C-urea in healthy volunteers. The maximum dose administered will not exceed 50 mg, which approximates the lowest estimated dose (32 mg) studied in humans (Cade and Pain 1972). In part 2, the same dose-escalation study design will assess the safety of inhaled ^{13}C-urea in subjects with CF.

Thus, the risk to subjects enrolled in this study is small; there is no direct benefit to subjects who enrolled. The potential benefit of an early warning of accelerated colonization of bacteria that compromises or destroys lung function that may extend or save lives is significant.

2. OBJECTIVES

2.1 PRIMARY OBJECTIVE

The primary objective is to assess the safety of ^{13}C-urea administered to healthy volunteers and subjects with CF by inhalation.

2.2 SECONDARY OBJECTIVES

The secondary objective is to assess the kinetics of ^{13}C-carbon dioxide production by measuring the isotopic ratio of ^{13}C to ^{12}C in exhaled carbon dioxide up to 15 minutes after administration.

3. INVESTIGATIONAL PLAN

3.1 OVERALL DESCRIPTION OF STUDY DESIGN AND PLAN

3.1.1 Primary Endpoint

The primary endpoint is the safety of inhaled ^{13}C-urea in healthy volunteers and in subjects with CF assessed by adverse events, spirometry test results (FEV_1 and forced vital capacity [FVC]), and respiratory examinations (respiratory rate, auscultation [presence of wheezing and crackles], pulse oximetry, and related symptoms, such as cough).

3.1.2 Secondary Endpoint

The secondary endpoint is the isotopic ratio of ^{13}C to ^{12}C in exhaled carbon dioxide of healthy volunteers and in subjects with CF measured with the POCone detector (Meretek Diagnostics Group of Otsuka America Pharmaceutical, Rockville, MD) before study drug administration and at 5, 10, and 15 minutes after study drug administration.

3.1.3 Study Design

This is a single-center, single-administration, dose-escalation study designed to determine the safety and dose response of aerosolized ^{13}C-urea 20 mg and 50 mg in detecting urease-producing bacteria in the lungs of healthy volunteers (those without and those diagnosed with *H. pylori* infection) and in subjects with CF. Subjects will be contacted by telephone the next day and asked a non-leading question about adverse events.

The dose will not be escalated until at least 24 hours after all subjects in the cohort have been tested at the current dose. If a subject has an adverse event or new symptom that remains unresolved at 48 hours, no new subjects will be enrolled until the relationship between the AE and symptom can be evaluated. If any subject experiences an adverse event considered by the data safety monitor to be dose limiting, then three additional subjects will be enrolled at that

current dose. Dose escalation will stop as described in Table 1 if more than one additional subject experiences dose-limiting toxicity (DLT) (Section 3.1.3.1).

After the safety of the maximum dose in the cohort of healthy volunteers without *H. pylori* infection has been reviewed by a data safety monitor, healthy volunteers diagnosed with *H. pylori* infection and subjects with CF will be enrolled. Three subjects diagnosed with *H. pylori* infection will be tested at the maximum dose to assess potential interference of urease producing bacteria in the stomach; subjects infected with *H. pylori* can be enrolled at any time after the safety of the maximum dose in healthy volunteers without *H. pylori* infection has been evaluated, but they will meet the entrance criteria for healthy volunteers without *H pylori* infection. Subjects will be discharged from the study site after 30 minutes or thereafter when oxygen saturation levels and FEV_1 values return to baseline and coughing symptoms are resolved. Supportive treatment for symptom resolution, if needed, will be recorded on the case report form (CRF).

Table 1 Dose Escalation Rules

Condition within 24 hours after administration of study drug	Number of DLTs/ Subjects in Cohort	Action
If no subject experiences DLT	0/3	Enroll three subjects at the 50 mg dose level
If one subject experiences DLT	1/3	Enroll three additional subjects at the 20 mg dose
If no additional subject experiences DLT	1/6	Enroll three subjects at the 50 mg dose level
If more than one subject experiences DLT	>1/6	Stop enrollment

Subjects will be receive ^{13}C-urea 20 mg and 50 mg unless enrollment stops because of DLT.
DLT Dose-limiting toxicity.

Sputum will be collected from each subject with CF before administering study drug in order to identify bacterial flora using two standard microbiologic methods. The first is a quantitative method in which colonies are stained and counted, the second is a colorimetric method in which a sample is plated in a medium that changes to a blue color as its pH is increased by the production of ammonia, a by-product of the urease-catalyzed degradation of urea.

The pharmacokinetics of the production of ^{13}C-carbon dioxide will be characterized by determining the isotopic ratio of ^{13}C to ^{12}C in exhaled carbon dioxide of subjects in each part before study drug administration and at 5, 10, and 15 minutes.

3.1.3.1 Dose-limiting Toxicity

The following significant respiratory events will be considered DLTs (Rosenfeld et al 2011):

- an absolute decrease from baseline FEV_1 of 20% of baseline or greater
- an absolute decrease from baseline oxygen saturation of at least 10%
- an increase from baseline respiratory rate of 10 breaths per minute for more than 2 minutes during quiet breathing
- new wheezes or rales on examination.

In addition to these events that are mandated DLT, the data safety monitor may determine any serious adverse event attributed to study drug to be dose limiting.

3.1.3.2 Maximum Tolerated Dose

The maximum tolerated dose is either the dose level administered before enrollment was stopped because of DLT or a lower dose if, in the data safety monitor's opinion, significant evidence exists to reasonably predict an adverse safety profile at that dose for the study drug.

3.2 DISCUSSION OF STUDY DESIGN

Aerosolized urea has been studied in doses ranging from approximately 32 mg (Cade and Pain 1972) to approximately over 7000 mg (Waldron-Edward and Skoryna 1966). The present study investigates doses up to 50 mg, which, based upon laboratory studies discussed below, is anticipated to evidence early indication of changes in the number of urease-containing bacteria in the lungs.

Subjects with CF are selected because they have large amounts of urease-producing bacteria in their lungs, are not acutely ill, and are familiar with procedures used in this study. Subjects with CF who are known to be colonized with *Pseudomonas* will be selected because *pseudomonas* has well documented urease activity. It is likely that subjects with CF will have additional lung pathogens that may also contribute to the signal; the breath test is intended to be specific for urease-producing bacteria. Pathogens in sputum cultures from subjects with CF will also be identified and tested for urease activity.

Healthy volunteers diagnosed with *H. pylori* infection are enrolled to determine whether a urease-producing pathogen in the stomach can produce ^{13}C-carbon dioxide in exhaled breath within 15 minutes after administering study drug by inhalation.

The dose-escalation model minimizes subject risk by incrementally increasing the dose of study drug administered to healthy volunteers, beginning at a dose that approximates 0.1% of one that produced no adverse effects in patients with CF and ending with a dose that approximates the minimum amount administered to patients with asthma.

A maximum dose of 50 mg of ^{13}C-urea was selected because it can be reconstituted to provide an isotonic solution in a volume that can be inhaled in a sufficiently short time window using a standard nebulizer. The urease reaction is rapid, and the direct delivery of ^{13}C-urea to the lung

combined with immediate release of ^{13}C-carbon dioxide enables the production of a signal. Because a 75 mg oral dose of ^{13}C-urea is sufficient to reliably produce a measurable amount of exhaled ^{13}C-carbon dioxide in patients infected with stomach *H. pylori*, which requires the carbon dioxide produced by the catalyzed hydrolysis of urea in the stomach to be absorbed into the blood stream and transported to the lung, it is likely that a 50 mg dose administered to the lung directly will produce a reliably measurable signal. The suitability of a lower dose, 20 mg dose, ^{13}C-urea will also be tested.

In addition to mandatory rules that stop dose escalation in the event of DLT, dose escalation may also be stopped at the discretion of the data safety monitor.

Albuterol is standard therapy for relief of bronchospasm (Eng et al 1996) and also for the prevention of bronchospasm in diseases such as exercise-induced asthma (Raissy et al 2008). Patients with CF are frequently pretreated with albuterol if a therapy may produce bronchospasm, e.g., inhalation of 7% saline. Although nebulized isotonic solutions are not expected to produce bronchospasm, subjects with CF will be pretreated with albuterol because of the potential of mild bronchospasm induced from irritation of lung pathogens at the time of study drug administration.

The characterization of the pharmacokinetics of ^{13}C-carbon dioxide will inform the useful time interval during which the isotopic ratio of ^{13}C to ^{12}C can be measured after administration of study drug in patients with lung infections (Otsuka America Pharmaceutical, Rockville, Maryland). Multiple breath specimens will be obtained at 15 minutes to estimate intra-patient variability.

3.2.1 Number of Subjects

Approximately 15 subjects will be enrolled, 9 healthy volunteers and 6 subjects with CF. Three healthy volunteers without *H. pylori* infection will each receive a single 20 mg dose and 3 will each receive a single 50 mg dose. After the safety of the 50 mg dose has been evaluated, 3 subjects with CF will each receive a single 20 mg dose and 3 subjects with CF will each receive a single 50 mg dose. Three subjects diagnosed with *H. pylori* infection will also each receive a 50 mg dose to assess potential interference of urease-producing bacteria in the stomach. Three additional subjects will be added to every dose level at which a dose-limiting adverse event occurs. If one subject at each dose level experiences DLT, then a maximum of 30 subjects will enroll.

3.2.2 Duration of Study

Subjects will participate in the study for approximately 9 days, up to 7 days between the screening visit and the study visit plus a follow-up visit 24 hours after study drug administration. Subjects will receive a single dose of study drug and remain in the clinic for at 30 minutes for evaluation and observation. Subjects will be discharged from the study site after 30 minutes or thereafter when oxygen saturation levels and FEV_1 values return to baseline and coughing symptoms are resolved. Twenty-four hours after administration of study drug, subjects will be contacted by telephone for a safety evaluation by study personnel. If an adverse event is

reported at the telephone contact, the subject will be asked to return to the clinic for an evaluation.

A 24-hour period will separate dose escalations. Thus, the study is anticipated to be completed within approximately 4 to 8 weeks after the first subject enrolls.

3.2.3 Premature Termination of Study

Dose escalation will be stopped if more than one subject experiences DLT at any dose level. The data safety monitor may terminate the study early by stopping enrollment based upon safety concerns.

4. SELECTION OF STUDY POPULATION

4.1 INCLUSION CRITERIA

4.1.1 Healthy Volunteers Without *Helicobacter pylori* infection

Healthy volunteers who meet all the following inclusion criteria are eligible to enroll:
1. At least 18 years old at the time of providing informed consent in a manner approved by the University of New Mexico, Institutional Review Board and willing to comply with the requirements of the study
2. In generally good health as determined by the investigator, with no respiratory illness within 2 weeks before the screening visit and no pulmonary history
3. No history of allergy or asthma
4. Has not smoked tobacco within 6 months before the screening visit and agrees not to smoke until after the follow up visit
5. FEV_1 at least 80% at the time of providing informed consent

4.1.2 Healthy Volunteers Diagnosed With *Helicobacter pylori* infection

Healthy volunteers diagnosed with *H. pylori* infection who meet all the inclusion criteria for healthy volunteers in Section 4.1.1 and additionally who have diagnosed with *H. pylori* infection are eligible to enroll.

4.1.3 Inclusion Criteria for Subjects With Cystic Fibrosis

Subjects who meet all the following inclusion criteria are eligible to enroll.
1. At least 18 years old at the time of providing informed consent in a manner approved by the University of New Mexico, Institutional Review Board and willing to comply with the requirements of the study
2. Diagnosed with CF at least 24 months before the screening visit
3. Documented presence of *Pseudomonas aeruginosa* in at least three sputum cultures within 2 years before the screening visit, one of which within 6 months before the screening visit
4. Is able to produce sputum at the time of the screening visit
5. FEV_1>60% or 1.5 L at the time of providing informed consent

6. Has not smoked tobacco within 6 months before the screening visit and agrees not to smoke until after the follow up visit

4.2 EXCLUSION CRITERIA

Subjects who meet any of the following exclusion criteria are not eligible to enroll.

1. Positive *H. pylori* serology, except for subjects in the *H. pylori* cohort
2. Subjects intolerant of albuterol
3. Subjects diagnosed with asthma
4. Subjects with evidence of upper respiratory tract infection within 14 days before the screening visit
5. Use of inhaled antibiotics within 12 hours before the administration of the breath test on Day 1 or required use of inhaled antibiotics during the study
6. Has smoked tobacco within 6 months before the screening visit
7. Females who are pregnant or nursing or of child-bearing potential who are not using a medically acceptable form of contraception (acceptable forms of contraception are hormonal birth control, intrauterine device, or barrier method plus a spermicidal agent) or abstinence
8. Any condition or history that, in the judgment of the investigator, would compromise the ability of the subject to comply with the study protocol or to complete the study
9. Subjects who have used an investigational agent within 28 days before Day 1

4.3 REMOVAL OF SUBJECTS

Subjects may be removed from the study by the investigator at any time for any of the following reasons:

1. Adverse event
2. Non-compliance with study protocol
3. Any condition that, in the judgment of the investigator, would compromise the ability of the subject to comply with the study protocol or to complete the study

In addition, subjects may remove themselves from the study at any time for any reason without consequence to further treatment by the investigator.

All subjects who are removed prematurely from the study should be assessed according to the procedures and assessments scheduled for the end-of-study visit in Section 6.3.

4.4 REPLACEMENT OF SUBJECTS

Subjects who are removed from the study prematurely will be replaced in order to maintain the required number of subjects in each cohort.

5. PROCEDURES FOR ADMINISTRATION OF TREATMENTS

All investigational medications will be prepared and dispensed by the investigational pharmacist or delegate at the University of New Mexico Hospital in accordance with procedures in this protocol.

5.1 TREATMENTS TO BE ADMINISTERED

5.1.1 Identification of Investigational Product

The active pharmaceutical ingredient is lyophilized ^{13}C-urea; the carbon content is enriched in ^{13}C (at least 99%), a naturally-occurring, non-radioactive isotope of carbon (Table 2).

Table 2 Identification of Investigational Product

	Study Product
Active Substance (INN)	^{13}C-urea
Trade Name (if applicable)	Not applicable
Formulation (including dosage form and strength)	Lyophilisate to be reconstituted with 3 mL of sterile water for injection to form isotonic solutions of pH 7.2 containing 20 mg or 50 mg of active ingredient
Route/Mode of Administration	Inhalation
Manufacturer	Coldstream Laboratories, Lexington, Kentucky USA

5.1.2 ^{13}C-urea Breath Test Kit

The ^{13}C-urea breath test kit in this study contains ^{13}C-urea lyophilized in a 10 mL glass vial with an aluminum crimp closure, 3 mL of sterile water for injection (WFI), a nebulizer cup (LC Sprint Star reusable nebulizer cup, PARI, Midlothian, Virginia, part number 023F35), 12 300-mL mylar breath collection bags (Meretek, Rockville, Maryland), and plastic tubing to connect the nebulizer cup to the compressor (Vios™ Adult Aerosol Delivery System, PARI, Midlothian, Virginia, part number 310F35-LCS). Additionally, each kit will contain 24 self-adhesive bar-code stickers used to identify collection bags and to maintain integrity of data collection. The lyophilisate will be reconstituted by transferring WFI using a 3 mL syringe (Becton Dickinson, Franklin Lakes, New Jersey, part number 309595). All components will be supplied sterile; all are intended for single use.

5.1.3 Administration of Study Drug

The lyophilisate will be reconstituted in WFI (3 mL) supplied with the breath test kit and administered by inhalation.). If all the reconstituted study drug does not go into solution, or if

the reconstituted solution is cloudy or contains particulate material, the vial will be discarded. The nebulizer cup is connected to the compressor with the supplied tubing, and the entire clear, reconstituted solution of ^{13}C-urea is transferred into the nebulizer cup After turning on the compressor, the subject will inhale the study drug by breathing through the mouthpiece of the nebulizer cup until all the solution has been aerosolized and inhaled (Appendix A).

5.1.4 Pre-treatment

Subjects with CF will receive two puffs of albuterol (90 μg albuterol base per puff) from a standard holding chamber (Aerochamber, Lupin Pharmaceuticals, Baltimore, Maryland) 10 minutes before the administration of study drug (Table 3).

Table 3 Identification of Pre-treatment

	Study Product
Active Substance (INN)	Albuterol
Trade Name (if applicable)	Ventolin HFA
Formulation (including dosage form and strength)	Aerosol (108 μg albuterol sulfate, 90 μg albuterol base from mouthpiece per actuation)
Route/Mode of Administration	Inhalation
Source	GlaxoSmithKline

5.1.5 Rescue Medication

In the event of wheezing or bronchospasm following administration of study drug, albuterol may be administered as rescue medication.

5.1.6 Methods of Assigning Subjects to Treatment Group

Subjects without *H. pylori* infection will be assigned to a single, open-label treatment, beginning with the lower dose of study drug, in the order in which they enter the study according to the dose escalation rules in Table 1 (Section 3.1.3). Healthy volunteers diagnosed with *H. pylori* infection will received the MTD.

5.1.7 Selection of Doses in the Study

Enrolled subjects will receive single doses of ^{13}C-urea 20 mg or 50 mg.

5.1.8 Selection and Timing of Doses for Each Subject

Subjects will receive single doses of ^{13}C-urea in increasing amounts, 20 mg in the first cohort and 50 mg in the second cohort as detailed in Table 1. Study drug may be administered in the fasted or as-fed state, but subjects should not ingest food until after the last breath sample is

collected; subjects may receive water *ad libitum*. Subjects may be administered study drug at any time of day with the provision they are required to be available 24 hours after the study drug is administered for a follow-up evaluation.

5.1.9 Blinding

This is an open-label study.

5.1.10 Packaging and Labeling

Lyophilized ^{13}C-urea 20 mg and 50 mg will be supplied in 10 mL stoppered vials sealed with aluminum closures and labeled for investigational use only. Study drug will be reconstituted in WFI (3 mL) supplied with the breath test kit and administered by inhalation (see Table 2 for the identification of the investigational product).

5.1.11 Storage

^{13}C-urea should be stored at ambient conditions (from 15º to 30º C [59º to 86º F]) in an area restricted to investigational staff or the pharmacy at the study center.

5.1.12 Accountability and Destruction of Study Drug

All investigational medications will be signed for by the investigational pharmacist at the University of New Mexico (or designee) when they are received. The study drug must be handled and stored as described and dispensed only to those subjects formally entered into the study.

At the completion of the study, and after reconciliation of all delivery and usage records, any unused study drug will be destroyed.

5.2 CONCOMITANT THERAPY

With the exception of inhaled antibiotics, subjects are allowed concomitant medications for pre-existing conditions as deemed necessary by the investigator from the time of study drug inhalation until the last breath sample is collected. Drugs taken from the beginning of inhalation of study drug until the follow-up visit will be recorded on the CRF. The generic name of the drug, the dose, and the time and reason for administration will be recorded on the CRF.

Inhaled antibiotics are disallowed from the beginning of the inhalation of study medication until the collection of the final breath sample at 2 hours.

5.3 TREATMENT COMPLIANCE

As all subjects will receive study drug in the clinic under supervision of study personnel, each subject will be considered treatment compliant who inhales the full volume of reconstituted study drug.

6. VISIT SCHEDULE

The schedule of assessments and procedures are summarized in Table 4.

Table 4 Schedule of Procedures and Assessments for Dose-ranging Study

Assessment/Procedure	Screening (Day –7 to Day –1)	Day 1	Follow-up[a]
Informed consent	X		
Inclusion/exclusion criteria	X	X	
Medical history	X		
Concomitant medications, including all medications taken within the last 48 hours	X	X	X
Twelve-lead electrocardiogram	X		
Helicobacter pylori serology	X		
Urine pregnancy test (child-bearing females)		X	
Five-minute vital signs measurements[c]		X	
Respiratory examination (respiration rate, auscultation, pulse oximetry, and related symptoms, such as cough)		X	
Perform spirometry	X[b]	X	
Administer albuterol (subjects with cystic fibrosis only)		X	
Perform spirometry[d]		X	
Obtain sputum specimen		X[e]	
Perform breath test[f]		X	
Assess adverse events		X	X[g]

[a] Subjects will be contacted by telephone 24 hours after administration of study drug for a follow-up assessment.
[b] Spirometry does not need to be performed at the screening visit if performed within the past 14 days.
[c] Heart rate, systolic and diastolic blood pressure, and body temperature will be recorded before study drug administration to assist in the determination that enrolled subjects are in generally good health.
[d] Spirometry will be performed before and at 10 and 30 min after the end of study drug administration. In addition, only for subjects with cystic fibrosis, spirometry will be performed before and 10 ± 2 minutes after pre-treatment with albuterol.
[e] Sputum specimens will be collected immediately after performing baseline spirometry only from subjects with cystic fibrosis. Sputum specimens will be cultured to determine the identity and number of pathogens, and will also be qualitatively analyzed nonselectively for the presence of urease-producing bacteria using a colorimetric assay.
[f] Study drug will be administered immediately following spirometry, or for subjects with cystic fibrosis, the collection of sputum. Five baseline exhaled breath specimens will be obtained immediately before administration of ^{13}C-urea. Exhaled breath specimens will be collected at 5 ± 1, 10 ± 2, 15 ± 2 (in triplicate at this timepoint only) after administration of ^{13}C-urea and sent within 24 h after collection to the central laboratory for analysis.
[g] Subjects who report adverse events at the follow-up assessment will be asked to return to the clinic for evaluation.

6.1 SCREENING (DAY –7 TO DAY –1)

The following procedures and assessments will be performed at the screening visit (Day –7 to Day –1).

1. Acquire written informed consent.
2. Review inclusion and exclusion criteria.
3. Document medical history, including all medications taken within 48 hours before the administration of study drug.
4. Obtain a 12-lead electrocardiogram (ECG).
5. Collect a blood specimen (5 mL) to assess *H. pylori* infection.
6. Perform spirometry, if not performed within the previous 14 days (Section 7.2.1).

6.2 ACTIVE TREATMENT (DAY 1)

1. Review inclusion and exclusion criteria.
2. Document all medications taken within the last 48 hours.
3. Perform a urine pregnancy test on women of child-bearing potential.
4. Record 5-minute sitting vital signs measurements (heart rate, systolic and diastolic blood pressure, and body temperature).
5. Perform respiratory examination (respiration rate, auscultation [presence of wheezing and crackles], pulse oximetry, and related symptoms, such as cough) (Section 7.2.1).
6. Perform spirometry (Section 7.2.1).
7. Administer albuterol, only to subjects with CF (Section 5.1.4).
8. Perform baseline spirometry 10 ± 2 minutes after administering albuterol to subjects with CF only (Section 7.2.1).
9. For subjects with CF only, collect sputum specimen.
10. Collect five breath specimens. Record the clock time at which the sample was collected on two self-adhesive stickers. Place one sticker on the collection bag and the other on the CRF (Section 7.1.2.1).
11. Administer study drug by inhalation (Section 5.1.3). Record the clock time at the beginning and end of study drug administration.
12. Measuring time from the end of the inhalation of study drug, collect breath specimens at 5 ± 1, 10 ± 2, 15 ± 2 minutes (three specimens at this time point only) after the inhalation of study drug is completed. Record the clock time of each breath collection on two self-adhesive stickers. Place one sticker on the collection bag and the other on the CRF (Section 7.1.2.1).
13. Perform spirometry after collecting the breath specimen at 10 and 30 minutes after the end of study drug administration (Section 7.2.1).
14. Assess adverse events (Section 8).

6.3 FOLLOW-UP

Twenty-four hours after administration of study drug, subjects will be contacted by telephone and asked a non-leading question to assess adverse events such as “How do you feel after participating in the study?” Additionally, subjects will be asked if they have taken any

medications since receiving study drug. All medications taken from the time of study drug administration until the follow-up assessment will be recorded on the CRF. Subjects who have adverse events at the follow-up visit will be asked to return to the clinic for evaluation.

6.4 DISCONTINUATION

Subjects who discontinue from the study prematurely will receive a follow-up assessment (Section 6.3).

7. STUDY VARIABLES AND METHODS OF ASSESSMENT

7.1 EFFICACY VARIABLE

7.1.1 Overview of Variable

The ^{13}C-urea breath test will be administered to all subjects after performing baseline spirometry.

7.1.2 Methods of Assessment

7.1.2.1 Collection of Breath Specimen

Samples of exhaled breath will be collected immediately before and at 5 ± 1, 10 ± 2, 15 ± 2 minutes after the inhalation of study drug is completed. Five breath specimens will be obtained before the inhalation of study drug; one specimen will be obtained at each subsequent assessment except for the 15-minute assessment at which time three exhaled breath specimens will be obtained. Step-wise instructions for performing the breath test are provided in Appendix A.

1. Record the clock time, intended sample time (e.g., baseline, 5-minute), and subject demographic information on two self-adhesive stickers in the ^{13}C-urea breath test kit. Place one of the stickers on the breath collection bag and the other sticker on the CRF.
2. Remove the pull-off cap from the mouthpiece of the breath collection bag.
3. Instruct the subject to breath normally, then take a deep breath and pause momentarily before exhaling into the mouthpiece of the bag.
4. Place the cap on the mouthpiece of the bag until it clicks into place.
5. Store the breath specimen at 15º to 30º C (59º to 86º F) until analyzed.

7.1.2.2 Analysis of Breath Specimen

After collection, all breath test specimens will be picked up within 24 hours by the central laboratory to be analyzed within 7 days of collection. The isotopic ratio of ^{13}C to ^{12}C is calculated from breath samples collected before and after administration of ^{13}C-urea using a POCone® Infrared Spectrophotometer (Otsuka America Pharmaceutical, Rockville, Maryland). The relative increase in isotopic ratio, δ, is determined automatically by the spectrophotometer (**I**).

$$\delta = \left(\frac{R_x - R_{std}}{R_{std}} \right) \times 1000, \quad \textbf{(I)}$$

where R_x is the ratio of the abundance of ^{13}C to ^{12}C in the post-administration sample and R_{std} is the corresponding ratio at baseline.

7.1.3 Microbial Analysis of Sputum

7.1.3.1 Overview of Variables

Sputum specimens will be obtained from subjects with CF after the performance of baseline spirometry and before administration of study drug. Specimens will be obtained using standard practices at the study site, and in all cases will be expectorated into a sterile sputum cup, labeled with the time of day, date, and unique study identifier for that patient.

7.1.3.2 Methods of Assessment

The presence of urease-containing bacteria will be determined locally using a colorimetric method in which a sample is plated in a medium that changes to a blue color as its pH is increased by the production of ammonia, a by-product of the urease-catalyzed degradation of urea (Cox et al 1977, Damato et al 1982, Isenberg 2004).

Additionally, bacterial flora in collected sputum will be assessed using standard quantitative microbiologic methods employed at the study site in which colony forming units (CFU) are identified and counted, and in all cases organisms in numbers greater than 10^4 and appearing in more than one CFU will be identified and counted.

7.2 SAFETY VARIABLES

The safety of ^{13}C-urea will be assessed from adverse events (Section 8), spirometry test results (FEV_1 and FVC), and respiratory examinations (respiratory rate, auscultation [presence of wheezing and crackles], pulse oximetry, and related symptoms, such as cough).

7.2.1 Spirometry

7.2.1.1 Overview of Variables

Spirometry will be performed at the initial respiratory examination (baseline, Day 1) and after collecting breath specimens at 10 and 30 minutes. If the last FEV_1 measurement is less than 10% of baseline, 4 puffs of albuterol will be administered and spirometry will be repeated in 15 minutes. Patients will be discharged from the clinic when FEV_1 is within 10% of baseline measurements and patients are symptom free. For subjects with CF, spirometry will be performed before and 10 minutes after administration of albuterol on Day 1.

7.2.1.2 Methods of Assessment

Spirometry will be performed using standard techniques specified by the American Thoracic Society and the European Respiratory Society (Miller et al 2005).

7.2.2 Respiratory Examination

7.2.2.1 Overview of Variables

Respiratory rate, pulse oximetry data, and related symptoms, such as cough, will be recorded before performing each spirometry (at baseline and after collecting the breath specimen at 10 and 30 minutes). Auscultation, noting the presence of wheezing and crackles, will be performed at baseline and at any assessment at which FEV_1 decreases at least 20% from baseline.

7.2.2.2 Methods of Assessment

Respiratory rate will be determined by counting the number of breaths in one minute. Arterial blood saturation will be determined by oximetry; the same location will be used for each individual subject. The number of coughs in the 90-second interval following the end of the administration of study drug will be recorded in the CRF (Koskela et al 2005). Wheezing and crackles will be noted when auscultation is performed.

7.3 PHARMACOKINETICS

The pharmacokinetics of ^{13}C-carbon dioxide production will be determined from isotopic ratios of ^{13}C to ^{12}C as described in Section 7.1.2.2.

7.4 APPROPRIATENESS OF MEASUREMENTS

Data will be collected using standard methods, widely used and generally regarded as reliable, accurate, and relevant.

8. ADVERSE EVENTS, SERIOUS ADVERSE EVENTS, AND SUSPECTED ADVERSE REACTIONS

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an adverse event (AE) or serious adverse event (SAE) as provided in this protocol. During the study, when there is a safety evaluation, the investigator or site staff will be responsible for detecting AEs and SAEs as detailed in this section of the protocol. All AEs will be reviewed by a data safety monitor.

8.1 DEFINITION OF AN ADVERSE EVENT

An AE is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An AE may be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a study medication, whether or not considered causally associated with the use of the study medication. Any abnormal laboratory value deemed clinically significant by the investigator, regardless of causal relationship, must be reported as an AE.

Examples of an AE include the following:

- significant or unexpected worsening or exacerbation of the condition or indication under study
- exacerbation of a chronic or intermittent preexisting condition, including either an increase in frequency or intensity of the condition (e.g., abnormal physical examination finding)
- signs, symptoms, or clinical sequelae of a suspected interaction
- signs, symptoms, or clinical sequelae of a suspected overdose of the study medication or a concurrent medication (overdose per se should not be reported as an AE or SAE, unless nonserious or serious sequelae occur)

The following examples are not considered AEs:

- medical or surgical procedure (e.g., endoscopy, appendectomy), although the condition that leads to the procedure is an AE
- anticipated day to day fluctuations of preexisting disease(s) or condition(s) (including laboratory values) present or detected at the start of the study that do not worsen
- the disease or disorder being studied, or expected progression, signs, or symptoms of the disease or disorder being studied, unless they become more severe or occur with a greater frequency than expected for the subject's condition

All AEs, whether volunteered, elicited, or noted on physical examination, and regardless of causality or seriousness, will be assessed and recorded on the CRF beginning after administration of study medication through the final follow-up assessment. SAEs will be assessed and recorded after administration of study medication through 30 days after study drug administration (Sections 8.2 and 8.4).

8.2 DEFINITION OF A SUSPECTED ADVERSE REACTION

A suspected adverse reaction (SAR) is defined as any adverse event for which there is a reasonable possibility that the adverse event was caused by the study drug. 'Reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

8.3 DEFINITION OF A SERIOUS ADVERSE EVENT OR SUSPECTED ADVERSE REACTION

An SAE or serious suspected adverse reaction (SSAR) is defined as any event that meets the following criteria:

- It results in death or is life-threatening (i.e., presents an immediate risk of death from the event as it occurred). (This criterion is not intended to include an AE that, had it occurred in a more severe form, might have caused death.)
- It results in persistent or substantial disability or incapacitation. (This criterion is not intended to include experiences of relatively minor medical significance, such as uncomplicated headache, diarrhea, or sprained ankle.)
- It results in hospitalization.
- It results in prolongation of an existing hospitalization.
- It is a congenital anomaly or birth defect.
- It requires medical or surgical intervention to prevent any of the above outcomes.

Medical and scientific judgment should be exercised in determining whether an AE is serious when considering important medical events that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject or may require intervention to prevent any of the other outcomes listed. Examples of such medical events that may also be considered serious include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization.

Hospitalization for elective treatment of a preexisting condition that did not worsen from baseline does not meet the definition of an SAE.

Social or convenience admission to a hospital or prolongation of a hospitalization for social or convenience reasons not associated with the occurrence of an AE does not meet the definition of an SAE.

8.3.1 Serious Adverse Events That Occur Before Administration of Study Medication

Before administration of study medication, only SAEs assessed by the investigator as related to study participation (e.g., related to study procedures or a change in existing therapy) will be transcribed onto the SAE reporting form and reported to the data safety monitor.

8.3.2 Serious Adverse Events That Occur After Last Administration of Study Drug

If an investigator becomes aware of an SAE or death occurring within 30 days after a subject receives the last administration of study medication, the investigator should report this to the data safety monitor.

If an investigator becomes aware of an SAE or death occurring more than 30 days after a subject receives study medication and the investigator considers the event to be related to the study medication, the investigator is obligated to report the SAE to the data safety monitor.

8.4 RECORDING AND EVALUATING ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

The investigator will attempt to establish a diagnosis of the event on the basis of signs, symptoms, or other clinical information. In such cases, the diagnosis, not the individual signs or symptoms, should be documented as the AE or SAE.

8.4.1 Assessment of Intensity

The investigator will assess the intensity of each AE and SAE reported during the study. The intensity of each AE and SAE recorded on the CRF should be assigned to one of the following categories:

- mild: an event that is easily tolerated by the subject, causes minimal discomfort, and does not interfere with everyday activities
- moderate: an event that is sufficiently discomforting to interfere with normal everyday activities
- severe: an event that prevents normal everyday activities

An AE that is assessed as severe should not be confused with an SAE. Severity is a term used to describe the intensity of a specific event, and both AEs and SAEs can be assessed as severe. The event itself, however, may be of relatively minor medical significance (such as a severe headache). This is not the same as serious, which is based on the subject's or event's outcome or on action criteria usually associated with events that pose a threat to a subject's life or functioning (Section 8.2).

8.4.2 Assessment of Causality

The investigator is obligated to use his or her clinical judgment to assess the relationship between the study medication and the occurrence of each AE or SAE. The investigator will assess the relationship to the study medication by using the following criteria:

- Definitely Related: An AE has a strong temporal relationship to the study drug. The AE is most likely explained by study drug. Dechallenge and rechallenge (if possible) are positive. The AE is consistent with a known response to the study drug. Another etiology is unlikely or significantly less likely.
- Probably Related: An AE has a strong temporal relationship to the study drug. The AE is more likely explained by study drug than by another cause. Dechallenge (if performed) is positive.
- Possibly Related: An AE has a reasonable temporal relationship to study drug. The AE could have been due to another equally likely cause. Dechallenge is positive.
- Not Related: The subject did not receive the study drug OR the AE has no temporal relationship to study drug OR the AE has a much more likely alternate etiology OR the AE is due to an underlying or concurrent illness or effect of another drug.

Even in situations in which minimal information is available for the initial SAE report, it is important that the investigator always make an assessment of causality for every event before

transmitting the SAE reporting form and AE CRF page(s) to the data safety monitor. The causality assessment is one of the criteria used when determining regulatory reporting requirements. The investigator may change his or her opinion of causality in light of follow-up information and amend the SAE reporting form and AE CRF page(s) accordingly.

8.4.3 Assessment of Outcome

The investigator will assess the outcome of the event by using the following terms:

- Resolved: The event resolved or the subject recovered without sequelae. An event (either serious or nonserious) occurred and had an endpoint, and the subject experienced no restrictions. For example, if an AE led to surgery that resulted in a postoperative wound infection, the infection would not be considered a sequela.
- Resolved with sequelae: The event has at least one secondary outcome that may result in permanent disability, functional limitation, or both. Such sequelae are usually limited to SAEs. For example, if an AE of stroke resulted in paralysis, or emboli formation after an AE of bacterial infection resulted in a renal infarct and loss of renal function, then paralysis and loss of renal function would be considered sequelae.
- Not resolved: At the end of the study, a nonserious event either has not changed in intensity or may not have recovered to baseline values. Examples include headache, low-grade fever, or nausea.
- Unknown: The status of the event is unknown.
- Death

8.5 DATA SAFETY MONITOR

Clinical data obtained from each subject will be presented daily to the data safety monitor, who will adjudicate AEs and determine when dose levels may be increased and when subjects may be enrolled into the study. All the adverse events will be reported to IRB as soon as possible but no later than 5 days from the event date or the reported date by the patients or as specified in Section 8.7, whichever is earlier.

8.6 FOLLOW-UP OF ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

After the occurrence of an AE or SAE, the investigator is required to follow each subject proactively and provide further information on the subject's condition. All AEs and SAEs documented at a previous visit or contact are designated as ongoing and will be reviewed at subsequent visits or contacts.

Nonserious AEs will be followed after the last scheduled study visit, until an appropriate resolution can be documented.

SAEs will be followed until the event resolves, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up. The investigator will ensure that follow-up information provided to the data safety monitor includes results of any additional laboratory tests

or investigations, histopathologic examinations, or consultations with other healthcare professionals that serve to clarify the nature of the event, the cause of the event, or both.

For SAEs only, new or updated information will be recorded on the originally completed SAE reporting form and CRF pages, with all changes signed and dated by the investigator. The updated SAE reporting form and CRF pages should be resubmitted to the data safety monitor within the time frames outlined in Section 8.7.

8.7 PROMPT REPORTING OF SERIOUS ADVERSE EVENTS TO THE DATA SAFETY MONITOR

Once the investigator determines that an event meets the protocol definition of an SAE, the investigator must notify the data safety monitor within 24 hours.

Complete the SAE details reporting form and forward by e-mail to the following contact:

Mark Schuyler, MD
mschulyer@salud.unm.edu

In the initial e-mail, the investigator must provide to the data safety monitor the following CRF pages, completed to the greatest extent possible:

- AE record
- medical history
- prior and concomitant medications

Also, the following documents are to be forwarded: any laboratory results, diagnostic test results, and medical reports relevant to the SAE.

E-mail transmission is the preferred method to transmit SAE information. In rare circumstances and in the absence of e-mail capacity, notification by fax (505-272-8700) or telephone (505-272-4751) is acceptable, with a copy of the SAE reporting form and CRF pages sent by overnight mail to:

Mark Schuyler, MD
Data Safety Monitor
Professor of Medicine
The University of New Mexico
Department of Internal Medicine, Pulmonary Division, MSC10-5590
1 University of New Mexico
Albuquerque, NM 87131

Initial notification via telephone does not replace the need for the investigator to complete the SAE reporting form and CRF pages within the time frames outlined.

The investigator must not wait to have all information regarding an SAE before notifying the data safety monitor of the event. The SAE details reporting form must be updated when

additional information is received. Follow-up information received on all SAEs must be forwarded to the data safety monitor using the same procedure and in the same timeframe as for an initial report.

8.8 REGULATORY REPORTING REQUIREMENTS

The investigator must promptly report all SAEs to the data safety monitor in accordance with the procedures detailed in Section 8.7, "Prompt Reporting of Serious Adverse Events to the Data safety monitor." The data safety monitor has a legal responsibility to notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation.

Prompt notification of SAEs by the investigator to the appropriate project contact for SAE receipt is essential so that SSARs that are either unexpected or observed with increasing occurrence, be reported and legal obligations and ethical responsibilities regarding the safety of other subjects are met.

The investigator, or responsible person according to local requirements, must comply with requirements related to the reporting of SAEs to the Institutional Review Board (IRB) or Independent Ethics Committee (IEC).

8.9 PRECAUTIONS

Any subject who becomes pregnant during the study must be discontinued immediately, but should be followed through delivery or termination of the pregnancy. A subject should also notify the investigator if she becomes pregnant within 30 days after receiving study medication. The data safety monitor must be notified of all pregnancies reported to the investigator (see Section 8.7 for contact information).

9. STATISTICS

9.1 DETERMINATION OF SAMPLE SIZE

The sample size is not based upon inferential analyses, but is deemed appropriate based on standard designs of Phase 1 dose-escalation studies in which increasing doses of study drug are administered to cohorts of three subjects. Approximately 15 subjects will be enrolled, 9 healthy volunteers and 6 subjects with CF. Three healthy volunteers without *H. pylori* infection will each receive a single 20 mg dose and 3 will each receive a single 50 mg dose. After the safety of the 50 mg dose has been evaluated, 3 subjects with CF will each receive a single 20 mg dose and 3 subjects with CF will each receive a single 50 mg dose. Three subjects diagnosed with *H. pylori* infection will also each receive a 50 mg dose to assess potential interference of urease-producing bacteria in the stomach. Three additional subjects will be added to every dose level at which a dose-limiting adverse event occurs. If one subject at each dose level experiences DLT, then a maximum of 30 subjects will enroll.

9.2 RANDOMIZATION AND BLINDING

This is an open-label study. Subjects are assigned to treatment based upon their sequential order of enrollment.

9.3 ANALYSIS POPULATIONS

Three populations will be analyzed:

- The intention-to-treat (ITT) population is those subjects who enroll in the study.
- The safety population is the subset of the ITT population who are exposed to study drug and receive at least one safety evaluation.
- The pharmacodynamics population is the subset of the safety population who contribute at least one post-administration evaluable breath specimen.

9.4 STATISTICAL ANALYSES AND METHODS

This section outlines the nature of and rationale for the statistical methods to be used for the analysis of data from the study. A separate Statistical Analysis Plan will describe the data handling and statistical techniques in full detail.

All subjects who meet the inclusion criteria for the study, sign the informed consent form, and are enrolled and exposed to the study medication will be accounted for in the analyses. Subjects who are deemed screen failures will not be accounted for in the data presentation of response or safety.

Demographic variables and subject characteristics will be summarized descriptively by cohort and overall. Demographic variables will include age, weight, height, and race/ethnicity. Continuous demographic parameters such as the subject's age at the time of enrollment will be summarized for the ITT population using descriptive statistics (N, mean, median, standard deviation, minimum and maximum value, and 95% two-sided confidence limits). Categorical demographic parameters, such as sex, will be summarized as a proportion of the ITT population. Other important subject characteristics for the ITT population will also be summarized using counts and percentages and presented in a listing format. These tabular summaries will be prepared by cohort and overall.

Heart rate, systolic and diastolic blood pressure, and body temperature recorded before study drug administration will be summarized descriptively by cohort and overall for the ITT population. Spirometry measurements will summarized descriptively by cohort at each time point, including the change from baseline, for the ITT population. The number of coughs in the 90-second interval following the end of the administration of study drug will summarized descriptively by cohort.

The incidence of all reported adverse events and treatment-emergent adverse events (TEAE) will be tabulated. A TEAE is defined as an event that first occurs or worsens in intensity after the administration of study drug. Adverse events will be classified by system organ class and

preferred term using the Medical Dictionary for Regulatory Activities (MedDRA). For incidence reporting, if a subject reports more than one adverse event that is coded to the same system organ class or preferred term, the subject will be counted only once for that specific system organ class or preferred term.

An overview of adverse events, which includes incidence of TEAEs, treatment-related adverse events, adverse events related to study procedures, SAEs, deaths, and adverse events leading to discontinuation, will be presented. For adverse events presented by severity, the worst severity during the study will be presented for each subject. The subject incidence of TEAEs will be summarized by system organ class and preferred term. The subject incidence of treatment-related adverse events will be summarized by preferred term.

Missing values will not be substituted using estimated values, but treated as missing in the statistical evaluation. All data from all subjects who receive ^{13}C-urea in the study will be included in all listings, plots, summary tables, and statistical analyses. All analyses will be performed using SAS (version 9.2).

10. STUDY ADMINISTRATION

10.1 REGULATORY AND ETHICAL CONSIDERATIONS

10.1.1 Regulatory Authority Approval

The investigator will obtain approval to conduct the study from the appropriate regulatory agency in accordance with any applicable country-specific regulatory requirements before any site may initiate the study in that country.

10.1.2 Ethical Conduct of the Study and Ethics Approval

This study will be conducted according to GCP; US 21 Code of Federal Regulations (CFR) Part 50 (Protection of Human Subjects); US 21 CFR Part 56 (IRBs); US 21 CFR Part 54 (Financial Disclosure); International Conference on Harmonization (ICH) Guidance for Industry, E6 GCP: Consolidated Guidance; the Nuremberg Code; and, where applicable, the principles of the Declaration of Helsinki (Recommendations guiding Medical Doctors in Biomedical Research Involving Human Subjects).

10.1.2.1 Ethics Committees

The investigator is responsible for ensuring that this protocol, the site's informed consent form, and any other information that will be presented to potential subjects (e.g., advertisements or information that supports or supplements the informed consent form) are reviewed and approved by the appropriate IRB or IEC. The investigator agrees to allow the IRB or IEC direct access to all relevant documents. The IRB or IEC must be constituted in accordance with all applicable regulatory requirements. The investigator will provide relevant documents or data needed for IRB or IEC review and approval of the study. Before investigational products and CRFs can be shipped to the site, the investigator must receive copies of the IRB or IEC approval, the approved

informed consent form, and any other information that the IRB or IEC has approved for presentation to potential subjects.

If the protocol, the informed consent form, or any other information that the IRB or IEC has approved for presentation to potential subjects is amended during the study, the investigator is responsible for ensuring that the IRB or IEC reviews and approves, where applicable, these amended documents. The investigator must follow all applicable regulatory requirements pertaining to the use of an amended informed consent form, including obtaining IRB or IEC approval of the amended form, before new subjects consent to take part in the study using the new version of the form. IRB or IEC approval of the consent forms must be obtained in addition to the approval given for the clinical study. Regulatory review and approval may be required in some countries before IRB or IEC approval can be sought.

10.1.2.2 General Considerations

The ethical standards defined within GCP are intended to ensure the following:

- Human subjects are provided with an adequate understanding of the possible risks of their participation in the study, and they have a free choice to participate or not.
- The study is conducted with diligence and in conformance with the protocol in such a way as to ensure the integrity of the findings.
- The potential benefits of the research justify the risks.

This is an investigator-sponsored study. The investigator is responsible for all of the following:

- selecting qualified co-investigators
- providing co-investigators with the information they need to conduct the investigation properly
- ensuring proper monitoring of the investigation
- ensuring that appropriate regulatory agencies and all participating co-investigators are properly informed of significant new information regarding AEs or risks associated with ^{13}C-urea.

10.1.3 Informed Consent

The investigator will provide an informed consent form for this study to suit the needs of the institution (although it must reflect the required elements of informed consent specified in 21 CFR Part 50.25). The final informed consent form must be approved by the IRB or IEC. If any new information becomes available that might affect subjects' willingness to participate in the study, or if any amendments to the protocol require changes to the informed consent form, the investigator will revise the informed consent form. The revised informed consent form must be approved by the IRB or IEC in advance of its use.

Investigators must provide subjects with all the information necessary to make an informed decision about their participation in the study, including the nature and intended purpose of the study, possible benefits, and possible risks.

All information in the informed consent form should be provided in a language (whether written or spoken) that is as nontechnical as practical and that is understandable to the subjects.

Before written informed consent is obtained, the subject should be given ample time and opportunity to inquire about the details of the study. All questions must be answered to the satisfaction of the subject or the subject's legally authorized representative.

Before a subject undergoes procedures specific to the protocol, the informed consent form must be signed and dated by the subject or the subject's legally authorized representative and any other signatories as required by the IRB or IEC in accordance with 21 CFR 50.27 (b2).

After all required signatures have been obtained, a copy of the informed consent form should be provided to the subject, and the original must be kept on file at the site and made available for review by the IRB or IEC. Documentation of the informed consent discussion must be noted in the subject's CRF.

10.1.4 Investigator Reporting Requirements

The investigator is responsible for completing and maintaining adequate and accurate CRFs and source documentation. Source documentation constitutes original records (first point of entry, either hard copy or electronic), which may include progress notes, medication administration records, operation reports, laboratory reports, discharge summaries, and so on. All CRFs should be completed contemporaneously in their entirety and stored in a confidential and locked location.

10.2 STUDY MONITORING

The investigator is responsible for ensuring the proper conduct of the study with regard to subject protection, ethics, protocol adherence, site procedures, and integrity of the data. The IRB or IEC will review study progress and CRF completion and address any concerns or questions regarding the study conduct. The IRB or IEC may monitor documentation and procedures including, but not limited to, subjects' informed consent documents, subject recruitment procedures, subjects' compliance with the study procedures, source-data verification, drug accountability, use of concomitant therapy by subjects, AE and SAE documentation and reporting, and quality of data.

10.3 QUALITY ASSURANCE

A regulatory authority or an IRB representative may visit the study site at any time during the study or after completion of the study to perform audits or inspections. The purpose of an audit or regulatory inspection is to examine systematically and independently all study-related activities and documents to determine whether these activities were conducted according to the protocol, GCP, ICH guidelines, and any other applicable regulatory requirements. Investigators should contact the IRB or IEC immediately if contacted by a regulatory agency about an inspection at their site.

10.4 STUDY AND SITE CLOSURE

If the investigator, IRB or IEC, or officials from regulatory agencies discover conditions arising during the study that indicate that the study should be halted or that the study site should be closed, this action may be taken after appropriate consultation between the IRB or IEC and investigator. Conditions that may warrant termination of the study include, but are not limited to, the following:

- discovery of an unexpected, serious, or unacceptable risk to the subjects enrolled in the study
- submission of knowingly false information from the research facility to the data safety monitor, IRB or IEC or regulatory agencies
- failure of the investigator to comply with GCP (e.g., ICH guidelines, regulatory agency guidelines)
- insufficient adherence to protocol requirements or an unacceptably high rate of missing, erroneous, or improperly collected data
- evidence from the blinded data of sufficient technical problems with the study that one could believe with a high degree of certainty that subjects are being exposed to the investigational drug without a realistic expectation of evaluable data
- a decision on the part of the data safety monitor to suspend or discontinue testing evaluation or development of the product
- failure of the investigator to enroll subjects into the study at an acceptable rate

10.5 RECORDS RETENTION

10.5.1 Health Insurance Portability and Accountability Act of 1996

The investigator agrees to comply with all applicable federal, state, and local laws and regulations relating to the privacy of subjects' health information, including, but not limited to, the Standards for Individually Identifiable Health Information, 45 CFR Parts 160 and 164 (the Health Insurance Portability and Accountability Act of 1996 privacy regulation). The investigator shall ensure that study subjects authorize the use and disclosure of protected health information in accordance with the privacy regulations of the Health Insurance Portability and Accountability Act and in a form satisfactory to the University of New Mexico.

10.5.2 Financial Disclosure

Financial disclosure is not required for this study.

10.5.3 Access to Original Records

Regulatory authorities expect that monitors, auditors, and representatives of national and international government regulatory agency bodies have access to original source documentation (see examples in Section 10.1.4) to ensure data integrity. "Original" in this context is defined as the first documentation of an observation and does not differentiate between hard copy and electronic records.

10.5.4 Archiving of Study-Related Documents

Records related to this clinical study must be retained either for at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. The University of New Mexico will notify the investigator as to when these documents no longer need to be retained for this use.

10.6 SUBJECT TRACKING

Drug accountability logs, a subject identification log (to be retained by the investigator only), and a subject enrollment log will be used to track subject participation in the study.

11. REFERENCES

Anderson SD. Indirect challenge tests: Airway hyperresponsiveness in asthma: its measurement and clinical significance. Chest. 2010 Aug;138(2 Suppl):25S-30S.

Bauer F. The use of urea in exudative otitis media. Otolaryngologic clinics of North America. 1970 Feb;3(1):67-78.

Brannan JD, Gulliksson M, Anderson SD, Chew N, Kumlin M. Evidence of mast cell activation and leukotriene release after mannitol inhalation. Eur Respir J. 2003 Sep;22(3):491-6.

Brannan JD, Porsbjerg C, Anderson SD. Inhaled mannitol as a test for bronchial hyper-responsiveness. Expert Rev Respir Med. 2009 Oct;3(5):457-68.

Cade JF, Pain MC. Lung function in provoked asthma: responses to inhaled urea, methacholine and isoprenaline. Clin Sci. 1972 Dec;43(6):759-69.

Cox FR, Cox ME, Martin JR. Rapid urease test for mycobacteria: preliminary observations. J Clin Microbiol. 1977 Jun;5(6):656-7.

Crews SJ, Davidson SI. Intravenous urea therapy in glaucoma. The British journal of ophthalmology. 1961 Dec;45(12):769-77.

Crews SJ, Davidson SI. Biochemical observations during intravenous urea therapy. The British journal of ophthalmology. 1962 Oct;46(10):597-600.

Damato JJ, Collins MT, McClatchy JK. Urease testing of mycobacteria with BACTEC radiometric instrumentation. J Clin Microbiol. 1982 Mar;15(3):478-80.

Eng PA, Morton J, Douglass JA, Riedler J, Wilson J, Robertson CF. Short-term efficacy of ultrasonically nebulized hypertonic saline in cystic fibrosis. Pediatr Pulmonol. 1996 Feb;21(2):77-83.

Gisbert JP, Pajares JM. 13C-urea breath test in the management of Helicobacterpylori infection. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2005 Dec;37(12):899-906.

Isenberg HD. Clinical Microbiology Procedures Handbook. 2004 [November 8, 2011]; Second edition:[Available from: http://vanguardia.udea.edu.co/cursos/Bacteriologia/Hand%20Book/sections/03.17.48.pdf.

Koskela HO, Kontra KM, Purokivi MK, Randell JT. Interpretation of cough provoked by airway challenges. Chest. 2005 Nov;128(5):3329-35.

Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005 Aug;26(2):319-38.

Pain M, Denborough M. The acute action of urea on ventilatory function in patients with asthma. Med J Aust. 1967;2(2):68-9.

Pathak CM, Bhasin DK, Khanduja KL. Urea breath test for Helicobacter pylori detection: present status. Tropical gastroenterology : official journal of the Digestive Diseases Foundation. 2004 Oct-Dec;25(4):156-61.

Pathak CM, Kaur B, Khanduja KL. 14C-urea breath test is safe for pediatric patients. Nuclear medicine communications. 2010 Sep;31(9):830-5.

Raissy HH, Harkins M, Kelly F, Kelly HW. Pretreatment with albuterol versus montelukast for exercise-induced bronchospasm in children. Pharmacotherapy. 2008 Mar;28(3):287-94.

Rosenfeld M, Davis S, Brumback L, Daniel S, Rowbotham R, Johnson R, et al. Inhaled hypertonic saline in infants and toddlers with cystic fibrosis: short-term tolerability, adherence, and safety. Pediatr Pulmonol. 2011 Jul;46(7):666-71.

Soupart A, Decaux G. Therapeutic recommendations for management of severe hyponatremia: current concepts on pathogenesis and prevention of neurologic complications. Clinical nephrology. 1996 Sep;46(3):149-69.

Sverrild A, Porsbjerg C, Thomsen S, Backer V. Diagnostic properties of inhaled mannitol in the diagnosis of asthma: a population study. J Allergy Clin Immunol. 2009;124(5):928-32.

Waldron-Edward D, Skoryna S. The Mucolytic Activity of Amides: A New Approach to Mucus Dispersion. Can Med Assoc J. 1966;94(24):1249-56.

Appendix A ^{13}C-urea Breath Test Kit Instructions for Use

Warnings and Precautions:

1. The use of this kit is restricted by federal law to investigational use only. The ^{13}C-urea breath test should be administered only by trained personnel to patients who have enrolled in clinical studies approved by a local institutional review board.
2. Reconstituted ^{13}C-urea should not be used if the resulting solution is cloudy.
3. No information is available on the use of ^{13}C-urea in pregnant or nursing women.
4. The performance characteristics for persons under the age of 18 have not been established for this test.
5. The specimen integrity of breath samples and reference gases stored in breath bags under ambient conditions has not been determined beyond 7 days.
6. A correlation between the number of organisms in the lungs and the ^{13}C-urea breath test result has not been established.

Preparation of ^{13}C-urea:

1. Remove the overseals on the vial of ^{13}C-urea and WFI to expose the septa.
2. Using the supplied 3 mL syringe, withdraw 3 mL of WFI and inject it into the vial of ^{13}C-urea.
3. While the syringe needle remains in the vial of ^{13}C-urea, gently swirl the 10 mL vial in order to ensure complete dissolution of ^{13}C-urea. Do not use the solution if it is cloudy or undissolved material is present.
4. Draw the reconstituted ^{13}C-urea into the syringe for subsequent charging of the nebulizer cup.

Preparation of the nebulizer:

1. Ensure availability of PARI compressor for the nebulizer.
2. Connect the tubing from the air source to the nebulizer cup.
3. Express the ^{13}C-urea solution into the nebulizer cup.

Collection of baseline sample:

1. Place a self-adhesive bar-code sticker supplied with the kit onto the blue Mylar collection bag and the case report form.
2. On the blue Mylar collection bag, record the subject ID, initials, sex, and age, collection date, and clock time at which the baseline sample is being taken.
3. Remove the pull-off cap from the breath collection bag.
4. Instruct the subject to breath normally, to take a deep breath and pause momentarily, and then to exhale into the mouthpiece of the collection bag.

5. Immediately place the cap over the inlet port of the bag to prevent sample loss.
6. Store the breath sample at 15º to 30º C (59º to 86º F) until analyzed.

Delivery of ^{13}C-urea:

1. Attach the mouthpiece to the nebulizer outlet with the expiratory valve facing up.
2. Seat the subject in a relaxed, upright position.
3. Ensure that the ^{13}C-urea solution has been expressed into the nebulizer cup.
4. Turn on power to the compressor.
5. Instruct the subject to place the mouthpiece on top of the tongue, closing the lips around the mouthpiece. The subject should take slow, deep breaths, inhaling and exhaling though the mouth. The valve on the mouthpiece will open during exhalation to allow exhaled mist to escape.
6. The subject should continue to breath through the mouthpiece of the nebulizer cup until the study drug is emptied from the nebulizer cup or until a slight sputtering sound is heard.
7. Turn power off to the compressor.
8. Dispose of nebulizer cup.

Collection of test sample:

1. Place a self-adhesive bar-code sticker supplied with the kit onto the pink Mylar collection bag.
2. On a pink Mylar collection bag, record the subject ID, initials, sex, and age, collection date, and clock time at which the test sample is being taken.
3. At the specified times after stopping inhalation of ^{13}C-urea, remove the pull-off cap.
4. Instruct the subject to breath normally, to take a deep breath and pause momentarily, and then to exhale into the mouthpiece of the collection bag.
5. Immediately place the cap over the inlet port of the bag to prevent sample loss.
6. Store the sample at 15º to 30º C (59º to 86º F) until analyzed.