# Effect of Bronchitol on Mucociliary Clearance in CFTR-Modulator Treated Patients with Cystic Fibrosis with Moderate to Severe Lung Disease

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## CLINICAL STUDY PROTOCOL

# Effect of Bronchitol on Mucociliary Clearance in CFTR-Modulator Treated Patients with Cystic Fibrosis with Moderate to Severe Lung Disease

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**Confidentiality Statement:**

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# Synopsis

**Primary Objective**

The primary objective of this study is to measure the sustained effect of dry powder inhaled mannitol (Bronchitol) on whole lung mucociliary clearance (MCC) in persons with cystic fibrosis (PwCF) with moderate to severe lung disease who are using elexacaftor/tezacaftor/ivacaftor (E/T/I).

**Secondary Objectives (if applicable)**

Secondary objectives include determining whether Bronchitol leads to improvement in other measures of MCC, spirometry, respiratory symptoms, and treatment satisfaction in PwCF with moderate to severe lung disease who are using E/T/I.

**Study Duration**

Each subject will participate in 2-3 study visits, including a screening visit, over a 2-4 week period.

The study is anticipated to begin enrollment in March 2022 with completion of study procedures by March 2023.

**Study Design**

This is an open-label, interventional study to assess the effect of Bronchitol on MCC in PwCF with moderate to severe lung disease who have been using E/T/I for more than 3 months. Adult PwCF with an $FEV_1$ of 30%-70% who are not using hypertonic saline (HS) or who are willing to withhold HS for 2 weeks prior to visit 1, will be approached for enrollment.

Screening visit: After obtaining informed consent, eligibility will be confirmed at this visit, including a urine pregnancy test for women of childbearing potential. Patients using hypertonic saline will be asked to discontinue this treatment for the remainder of the study period.

Visit 1: For patients who are currently not using HS, this can be done immediately following the screening visit; for patients using HS, this will be done 14 days after the screening visit to allow for complete washout of HS. Subjects will be asked to hold dornase alfa and bronchodilators on the morning of Visit 1. Baseline measures of MCC, spirometry, and patient reported outcomes (PROs) will be obtained. Following this, a Bronchitol tolerability test will be performed and if tolerated, subjects will be instructed on home use.

Visit 2: This visit will be scheduled 14 +/- 2 days after Visit 1. Subjects will be asked to withhold their Bronchitol, dornase alfa and bronchodilators on the morning of the visit. MCC, spirometry, and PROs will be obtained.

**Study Population**

| |
|---|
| The study population is adult PwCF with moderate to severe lung disease who are currently treated with E/T/I.<br><br>Inclusion Criteria:<br><br>1. Able to provide informed consent<br><br>2. Age ≥ 18 at the time of screening<br><br>3. Diagnosis of CF<br><br>4. Regularly using E/T/I for ≥ 90 days<br><br>5. $FEV_1$ between 30% and 70%, inclusive, at time of screening<br><br>6. Denies active smoking or vaping<br><br>7. Clinically stable with no significant changes in health status within the 28 days prior to and including the screening visit<br><br>8. Has no other conditions that, in the opinion of the Site Investigator/Designee, would preclude informed consent, make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives<br><br>Exclusion Criteria:<br><br>1. Use of an investigational drug within 28 days prior to and including the screening visit<br><br>2. Unable or unwilling to withhold HS for 4 weeks (2 weeks prior to Visit 1 and 2 weeks between Visit 1 and Visit 2)<br><br>3. Unable or unwilling to withhold dornase alfa and bronchodilators on the morning of Visit 1 and Visit 2, until completion of study procedures<br><br>4. Initiation of new chronic CF pulmonary therapy (e.g. dornase alfa, azithromycin, inhaled antibiotic) within 28 days prior to and including the screening visit<br><br>5. Acute use of antibiotics (oral, inhaled, or IV) or acute use of systemic corticosteroids for respiratory tract symptoms within 28 days prior to and including the screening visit.<br><br>6. Chronic use of oral corticosteroids > 10 mg of prednisone daily or equivalent<br><br>7. Unable to tolerate albuterol or any other bronchodilator<br><br>8. History of intolerance to HS or inhaled mannitol<br><br>9. Pregnancy or breast feeding<br><br>10. Have had more than 2 chest CTs in the past year or a combination of procedures that are believed to have exposed the subject's lungs to >150 mSv<br><br>11. History of significant hemoptysis (≥ 60 mL) in the last three months |
| **Number of Participants** |

The estimated number of participants in this study is 22 subjects, enrolled and completed. We anticipate enrolling up to 25 subjects to account for drop-out.

**Number of Study Sites**

This study will be conducted at up to 4 sites that are part of the CF Foundation MCC National Resource Center. The central coordinating site and MCC scan reading site will be at the University of North Carolina at Chapel Hill.

**Primary Outcome Variables**

The primary outcome will be whole lung MCC from 0 to 60 minutes ($MCC_{60}$).

**Secondary Outcome Variables**

1. Other MCC endpoints (e.g., central and peripheral lung clearance; cough clearance (CC); aerosol deposition homogeneity indices)

2. Forced expiratory volume in one second ($FEV_1$) % predicted

3. Respiratory symptoms as measured by the respiratory domain of the Cystic Fibrosis Respiratory Questionnaire Revised (CFQR-R) and the Cystic Fibrosis Respiratory Symptom Diary and Chronic Respiratory Infection Symptom Score (CFRD-CRISS)

4. Treatment satisfaction as measured by the Treatment Satisfaction Questionnaire (TSQ)

**Visit Schedule Table (Optional)**

| Procedures | Screening visit | Visit 1 | Visit 2 |
|---|---|---|---|
| Informed Consent | X | | |
| Medical History | X | | |
| Vital signs | X | X* | X |
| Physical exam | X | X* | X |
| Urine pregnancy test (if applicable) | X | X | X |
| Spirometry | | X | X |
| Questionnaire administration | | X | X |
| MCC measurement | | X | X |
| Bronchitol tolerance test | | X | |
| Adverse event assessment | | | X |

*Not required if visit 1 is done on the same day as screening visit

# Abbreviations

| Abbreviation | Explanation |
|---|---|
| BMI | Body mass index |
| BTT | Bronchitol tolerance test |
| CC | Cough clearance |
| CFQ-R | Cystic Fibrosis Respiratory Questionnaire Revised |
| CFRSD-CRISS | Cystic Fibrosis Respiratory Symptom Diary and Chronic Respiratory Infection Symptom Score |
| CFTR | Cystic Fibrosis Transmembrane Conductance Regulator |
| CRF | Case report form |
| CTCAE v3. | Common Terminology Criteria for Adverse Events Version 3.0 |
| E/T/I | Elexacaftor/Tezacaftor/Ivacaftor |
| $FEV_1$ | Forced expiratory volume in one second |
| HS | Hypertonic saline |
| ICF | Informed consent form |
| MCC | Mucociliary Clearance |

| | |
|---|---|
| $MCC_{60}$ | Average whole lung clearance through 60 minutes |
| PRO | Patient reported outcome |
| PwCF | Persons with cystic fibrosis |
| $SpO_2$ | Oxygen saturation |
| TSQ | Treatment Satisfaction Questionnaire |

# Table of Contents

# 1 - Introduction

## 1.1 Introductory Statement

This study will provide important mechanistic information regarding the effect of inhaled mannitol (Bronchitol) in PwCF with moderate to severe disease who are already using elexacaftor/tezacaftor/ivacaftor (E/T/I). Many patients have already discontinued hypertonic saline and other pulmonary therapies because of the profound effect of E/T/I of their symptoms and lung function. Further, because both inhaled osmotic agents (i.e., Bronchitol, HS) and E/T/I are believed to exert their beneficial effects through improvements in mucociliary clearance (MCC), it is unknown if the combination of these therapies might be additive or are redundant in a population with moderate to severe disease where bronchiectasis and chronic infection persists, and where eventual decline in lung function is expected over time. This study, therefore, will be the first to determine whether “add on” therapy with inhaled Bronchitol is able to further accelerate MCC in E/T/I patients. These data would provide some guidance regarding the use of these approved therapies in PwCF.

# 2 - Background

### 2.1.1 Preclinical Experience

Mechanism of Action: Bronchitol improves the hydration of airway secretions by osmotically drawing water across the airway epithelium into the lumen. This, in turn, has been shown to acutely accelerate the rate of mucociliary clearance[1].

Pharmacodynamics: The pharmacodynamics of mannitol are unknown[2].

### 2.1.2 Clinical Experience[2]

The efficacy of Bronchitol for the treatment of cystic fibrosis (CF) was evaluated in 3 randomized, double-blind, controlled trials (Trials 1, 2, and 3). All three trials were 26-week, randomized, double-blind, controlled studies in patients with CF. Trial 1 (NCT02134353) evaluated patients 18 years of age or older with baseline $FEV_1$ >40% to <90% of predicted. Trial 2 (NCT00446680) evaluated patients 6 years of age or older with baseline $FEV_1$ >30% to <90% of predicted. Trial 3 (NCT00630812) evaluated patients 6 years of age or older with baseline $FEV_1$ >40% to <90% of predicted. All three trials excluded CF patients with an episode of hemoptysis (>60 mL) in the 3 months prior to enrollment. The use of inhaled hypertonic saline was not permitted in any of the three trials but continued use of their other standard of care CF therapies were allowed (e.g., bronchodilators, inhaled antibiotics, and dornase alfa). While CF patients aged 6 to 17 years were included in Trials 2 and 3, Bronchitol is not approved for use in this age group. Patients were randomized to receive either Bronchitol 400 mg or control (50 mg inhaled mannitol) twice daily. Each dose of Bronchitol was preceded by use of an inhaled short-acting bronchodilator (albuterol or equivalent) taken 5 to 15 minutes prior to initiation of Bronchitol dosing. The primary efficacy endpoint in all three studies was improvement in lung function as determined by the mean change from baseline in pre-dose $FEV_1$ (mL) over 26 weeks of treatment and was analyzed using the pattern mixture model with multiple imputation. Trial 1 evaluated 423 adult patients with a mean age of 28 years and a mean FEV1 63.9% predicted (range: 40.3% = minimum, 89.6% = maximum). Treatment with Bronchitol resulted in a statistically significant improvement in $FEV_1$. In Trial 1, the treatment difference between Bronchitol and control for the adjusted mean change in $FEV_1$ from baseline over 26 weeks was 51 mL (95% CI 6 to 97 mL) shown in the table below.

| | Control (N=214) | Bronchitol (N=209) |
|---|---|---|
| **Adjusted mean change from baseline** | 12 mL | 63 mL |
| **Adjusted mean difference (95% CI), p-value** | 51 mL (6 to 97 mL), p=0.028 | |

Trials 2 and 3 evaluated 295 and 305 patients, respectively. For the adjusted mean difference in the change from baseline in $FEV_1$ over 26 weeks in the intention-to-treat population in Trials 2 and 3, the treatment difference between Bronchitol and control was 68 mL (95% CI: 24 to 113 mL) and 52 mL (95% CI: -3 to 107 mL), respectively. Post-hoc descriptive analyses of the adult subgroups from Trials 2 and 3 were performed. The adult subgroup analyses in Trial 2 and 3 evaluated 209 and 157 adult patients, respectively. In

Trial 2, there was an adjusted mean difference in the change from baseline in FEV1 over 26 weeks in the intention-to-treat population of adults of 78 mL (95% CI: 21 to 135 mL). In Trial 3, there was an adjusted mean difference in the change from baseline in FEV1 over 26 weeks in the intention-to-treat population of adults of 78 mL (95% CI: 2 to 153 mL).

### 2.2 Relevance of Bronchitol in Cystic Fibrosis

Cystic Fibrosis (CF) lung disease is caused by dehydration of airway secretions that leads to mucus adhesion, infection and airways inflammation. A simple means to restore hydration of CF airway surfaces is to inhale an osmotic agent, such as hypertonic (3-7%) saline (HS) or Bronchitol. These osmotic agents draws water across the airway mucosa into the airway lumen. Rehydration of airway mucus and the airway surface liquid layer facilitates mucociliary clearance (MCC). Inhaled hypertonic saline twice daily has been shown to have both short-term benefits on pulmonary function, MCC and quality of life and long-term benefits on lung function and reduction in exacerbations[3,4]. As a result, hypertonic saline, delivered by nebulization twice daily, has long been standard of care for PwCF though considered burdensome by patients.

In October 2019, the FDA approved the first triple combination therapy, elexacaftor/tezacaftor/ivacaftor (E/T/I), for patients 12 and older with cystic fibrosis who have at least one F508del mutation in the cystic fibrosis transmembrane conductance regular (CFTR) gene. Clinical trials demonstrated a substantial improvement in lung function in patients treated with E/T/I compared to placebo, as well as fewer pulmonary exacerbations and improved body mass index (BMI) and quality of life[5,6]. Given these potent effects of modulator therapies, the question of how to simplify the treatment burden of PwCF including reducing the need for laborious nebulizer therapies is now a research priority[7]. A large study of stopping chronic therapies in PwCF with mild-moderate lung function reduction ($FEV_1$>60%) is currently underway[8]. However, this will not provide evidence to support reducing therapeutic burden in patients with more severe lung disease who may be more reliant on continued treatment with an inhaled osmotic agent.

Mannitol, an osmotic agent delivered via dry powdered inhaler (Bronchitol), may provide a viable alternative to HS in PwCF with moderate to severe lung function reduction ($FEV_1$ 30%-70%) currently taking E/T/I who still require treatment with an inhaled osmotic agent. In a randomized, double-blind, placebo-controlled trial conducted prior to E/T/I approval, PwCF treated with Bronchitol demonstrated a significant improvement in $FEV_1$ and a reduction in exacerbations over one year[9]. Importantly, Bronchitol is delivered via a simple, hand-held inhaler, providing benefits of shorter delivery time, increased portability and no need for large, specialized equipment for delivery over nebulizer hypertonic saline.

This study will provide foundational information regarding the effect of Bronchitol in a unique population of PwCF who would benefit from decreased treatment burden but should continue to use an inhaled osmotic agent for airway clearance due to their moderate to severe lung disease despite treatment with a CFTR modulator.

# 3 - Rationale/Significance

### 3.1 Problem Statement

In the era of highly effective CFTR modulator therapy, there is a renewed focus on reducing the treatment burden for PwCF while maintaining effective airway clearance to optimize lung function and reduce pulmonary exacerbation frequency. This is particularly important for patients who have moderate to severe lung disease despite treatment with E/T/I and may be particularly reliant on therapies that improve mucus clearance from airways.

This study will provide foundational information regarding the effect of Bronchitol on MCC and treatment satisfaction in PwCF with moderate to severe lung disease who are already being treated with E/T/i.

### 3.2 Purpose of Study/Potential Impact

This trial is designed to test the hypothesis that Bronchitol leads to a sustained improvement in MCC in adults with CF who are receiving treatment with E/T/I. Secondary objectives include determining whether Bronchitol leads to other improvements in other MCC measures, $FEV_{1,}$ respiratory symptoms and treatment satisfaction.

#### 3.3.1 Potential Risks

i. Related to Bronchitol: Inhaled mannitol can cause transient cough, bronchospasm, and hemoptysis[2]. Based on published reports, the overall rate of intolerability is after bronchodilator pretreatment appears to be ~6%[10]. To minimize risks, patients with a history of intolerance to hypertonic saline or mannitol and patients with significant hemoptysis (≥60 mL) in the previous three months will be excluded. The ability to tolerate inhaled mannitol will be tested at Visit 1 (after baseline MCC measured), via a Bronchitol Tolerance Test (BTT). A 20% drop in $FEV_1$ or 10% drop in baseline $SpO_2$ after serial increases in Bronchitol dose will preclude further study participation. A bronchodilator (albuterol or levalbuterol) via metered dose inhaler will be administered prior to each dose of the study medication. The multiple precautionary measures should substantially limit any risk associated with the use of this therapy.

ii. Related to radiation exposure: The amount of radiation dose received during a given study (2 MCC scans) is approximately 0.9 mSV. The risk from the radiation dose received from this procedure is too small to be detected but is significantly less than the radiation dose received from the natural environment over the course of 1 year (3 mSv). Since radiation risk is a cumulative risk concern, an eligibility criterion has been added to exclude patients whose recent radiation exposure is greater than typical. Typically, significant additional radiation exposure in CF patients (above environmental exposure) would come from chest computed tomography (CT) procedures. Patients who have had more than 2 chest CTs in the past year or a combination of procedures that are believed to have exposed the subject's

lungs to >150 mSv would be excluded. In addition, a potential risk of radiation exposure to an unborn fetus exists among pregnant females. All those able to become pregnant will be required to undergo a pregnancy test at screening and before each MCC scan.

### 3.3.2 Potential Benefits

Potential benefits include improvement in transiently promoting the clearance of secretions and inhaled infectious agents, thereby improving respiratory symptoms, lung function, and exacerbation frequency in PwCF.

# 4 - Study Objectives

### 4.1 Hypothesis

This trial is designed to test the hypothesis that Bronchitol leads to a sustained improvement in MCC in adults with moderate to severe cystic fibrosis lung disease ($FEV_1$ 30% to 70%) who are being treated with E/T/I.

### 4.2 Primary Objective

The primary objective of this study is to measure the sustained effect of Bronchitol on whole lung mucociliary clearance from 0-60 minutes ($MCC_{60}$) in PwCF with moderate to severe lung disease who are using E/T/I.

### 4.3 Secondary Objectives (if applicable)

Secondary objectives include determining whether Bronchitol leads to improvement in other measures of MCC, $FEV_1$, respiratory symptoms, and treatment satisfaction in PwCF with moderate to severe lung disease who are using E/T/I.

# 5 - Study Design

### 5.1 General Design Description

The proposed study is an open-label, interventional study. Patients meeting inclusion criteria and having no exclusion criteria will be enrolled into the study, which will be conducted at up to four sites. Study treatment will be Bronchitol (dry powder mannitol) 400 mg twice a day by oral inhalation (the contents of 10 capsules administered individually) for 14 days +/- 2 days.

The study will consist of a screening visit and two study visits separated by 14 +/- 2 days. Screening procedures may be completed on the same day as Visit 1 if a subject has not used nebulized hypertonic saline in the preceding 14 days. No study procedures will be completed prior to obtaining subject's informed consent. A medical history will be conducted at the screening visit, followed by physical exam. At Visit 1, PRO administration will be followed by a physical exam, spirometry, and MCC. Prior to completion of Visit 1, a BTT will be conducted followed by instruction on home use of Bronchitol. Subjects who do not pass the BTT will be excluded from the study and data analysis and will be replaced. Subjects who pass the BTT will be instructed to self-administer Bronchitol 400 mg twice a day via oral inhalation for the next 14 +/- 2 days until the day prior to Visit 2. At Visit 2, PRO administration, physical exam, spirometry, adverse event review and MCC will be performed. Subjects will be instructed to hold the study drug, home bronchodilators and dornase alfa on the day of Visits 1 and 2.

#### 5.1.1 Study Date Range and Duration

The study is expected to take place over approximately 1 year. The estimated start date is March 1, 2022.

#### 5.1.2 Number of Study Sites

This study will be conducted at up to 4 sites that are part of the CF Foundation MCC National Resource Center. The central coordinating site and MCC scan reading site will be at the University of North Carolina at Chapel Hill.

### 5.2 Outcome Variables

#### 5.2.1 Primary Outcome Variables

The primary outcome will be the average rate of MCC measured in the whole right lung compartment over 60 minutes ($MCC_{60}$), calculated using point estimates collected every 10 minutes.

#### 5.2.2 Secondary Outcome Variables

Secondary MCC outcomes will include changes in the rate of cough clearance (CC) from 60-90 minutes, MCC rates measured in other lung compartments (central, peripheral lung) and over other time domains (90 minutes, 6 hours). We will include an analysis of lung function (change in post-bronchodilator $FEV_1$) as a secondary outcome.

To assess the effect of Bronchitol on respiratory symptoms, we will use the validated respiratory symptom domain of the CFQ-R questionnaire and the CFRSD-CRISS[11,12]. As treatment burden is also a potential benefit to inhaler delivery of an osmotic agent for airway clearance, the score on the TSQ will be a secondary outcome[13].

## 5.3 Study Population

### 5.3.1 Number of Participants

We want to follow 22 PwCF with moderate to severe lung function decline who are currently being treated with E/T/I. We expect 10% of subjects to terminate early either due to failure of the BTT or for other reasons. Therefore, we will enroll 25 subjects in our study.

### 5.3.2 Eligibility Criteria/Vulnerable Populations

Inclusion Criteria:

1. Able to provide informed consent
2. Age ≥ 18 at the time of screening
3. Diagnosis of CF
4. Regularly using E/T/I for ≥ 90 days
5. $FEV_1$ between 30% and 70%, inclusive, at time of screening
6. Denies active smoking or vaping
7. Clinically stable with no significant changes in health status within the 28 days prior to and including the screening visit
8. Patients on cycled inhaled antibiotics will need to be either on or off their antibiotic for 7 days prior to Visit 1 and not scheduled to cycle during the 2-week treatment period until after Visit 2
9. Has no other conditions that, in the opinion of the Site Investigator/Designee, would preclude informed consent, make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives

Exclusion Criteria:

1. Use of an investigational drug within 28 days prior to and including the screening visit

2. Unable or unwilling to withhold HS for 4 weeks (2 weeks prior to Visit 1 and 2 weeks between Visit 1 and Visit 2)

3. Unable or unwilling to withhold dornase alfa and bronchodilators on the morning of Visit 1 and Visit 2, until completion of study procedures

4. Initiation of new chronic CF pulmonary therapy (e.g. dornase alfa, azithromycin, inhaled antibiotic) within 28 days prior to and including the screening visit

5. No acute use of antibiotics (oral, inhaled, or IV) or acute use of systemic corticosteroids for respiratory tract symptoms within 28 days prior to and including the screening visit.

6. No chronic use of oral corticosteroids > 10 mg of prednisone or equivalent daily

7. Unable to tolerate albuterol or other bronchodilator

8. History of intolerance to HS or inhaled mannitol

9. Pregnancy or breast feeding

10. Have had more than 2 chest CTs in the past year or a combination of procedures that are believed to have exposed the subject's lungs to >150 mSv

11. History of significant hemoptysis (≥ 60 mL) in the last three months

This study will not enroll children, pregnant women, decisionally-impaired individuals, or other members of vulnerable populations.

# 6 - Methods

## 6.1 Treatment - Drug

### 6.1.1 Identity of Investigational Product/New Drug

The study drug is Bronchitol - a sugar alcohol indicated as an add-on maintenance therapy to improve pulmonary function in adult patients 18 years of age and older with cystic fibrosis.

### 6.1.2 Dosage, Admin, Schedule (if applicable)

Bronchitol should be administered 400 mg (the contents of 10 capsules administered individually) via oral inhalation twice daily. A short-acting bronchodilator should be administered 5-15 minutes before each dose of Bronchitol. The Bronchitol inhaler should be discarded and replaced after 7 days of use.

### 6.1.3 Packaging/Labelling

The inhalation powder is a clear, colorless hard gelatin capsule imprinted with "PXS 40 mg" and contains 40 omg of mannitol per capsule. The accompanying white plastic inhaler is comprised of a mouthpiece, blue piercing buttons, capsule chamber and a removable cap. A blister pack consists of 10 capsules. After a capsule is placed in the capsule chamber and pierced by firmly pressing and releasing the buttons on the side of the device, the powder within the capsule becomes exposed and ready for dispersion into the airstream generated by the patient upon inhalation through the mouthpiece. Under standardized in vitro test conditions, the inhaler delivers 32.2 mg of mannitol per inhalation when tested at a flow rate of 60 L/min for 2 seconds. The actual amount of drug delivered to the lungs will depend on patient factors, such as inspiratory flow profile.

### 6.1.4 Storage Conditions

Bronchitol should be stored between 68°F-77°F (20°C-25°C) with excursions permitted between 59°F-86°F (15°C30°C). The drug should not be refrigerated or frozen.

### 6.1.5 Concomitant therapy

A short-acting bronchodilator should be administered 5-15 minutes prior to each dose of Bronchitol.

Nebulized hypertonic saline should be held 1-2 weeks prior to Visit 1 and for the duration of the study until completion of Visit 2.

Dornase alfa and bronchodilators should be held on the morning of Visit 1 and the morning of Visit 2.

### 6.1.6 Restrictions

There are no other restrictions.

## 6.2 Assessments

### 6.2.1 Efficacy

The primary efficacy outcome will be a significant change in the $MCC_{60}$, the average rate of MCC measured in the whole right lung compartment over 60 minutes by 5.4% (absolute change). A treatment difference of this magnitude is considered to be both clinically meaningful and achievable, as it approximates the sustained effect observed with HS treatment in patients with cystic fibrosis.

### 6.2.2 Safety/Pregnancy-related policy

Women will undergo a urine pregnancy test at each visit to confirm they are not pregnant.

#### 6.2.2.1 Adverse Events Definition and Reporting

*Adverse Events*

An AE is any untoward medical occurrence during study participation that does not necessarily have a causal relationship with the study participation. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with participation in the study whether or not related to that participation. An unexpected AE is one of a type not identified in nature, severity, or frequency than expected.

The Investigator will discuss the subject's health status to identify the occurrence of AEs during each subject visit and record the information in the site's source documents. AEs will be recorded in the subject CRF.

*AE Severity*

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, as modified for CF, should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found on the Study Website. If the experience is not covered in the modified criteria, the guidelines shown in the table below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

| Severity (Toxicity Grade) | Description |
|---|---|
| Mild (1) | Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated |
| Moderate (2) | Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate Instrumental activities of daily living (e.g., preparing meals, using the telephone, managing money) |
| Severe (3) | Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living (e.g., bathing, dressing, feeding self, using toilet, taking medications) |
| Life-threatening (4) | Life-threatening consequences; urgent intervention indicated |
| Death (5) | Death related to AE |

*AE Relationship to Study Procedures*

The relationship of an AE to study procedures should be assessed using the following guidelines.

| Relationship to Treatment Arm Assignment | Comment |
|---|---|
| Definitely | An event that occurs follows a reasonable temporal sequence after a study procedure that is not explained by any other reasonable hypothesis. |
| Probably | An event that follows a reasonable temporal sequence after a study procedure; and that is not explained by any other reasonable hypothesis; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions. |

| | |
|---|---|
| Possibly | An event that follows a reasonable temporal sequence after a study procedure; but that could readily have been produced by a number of other factors. |
| Unrelated | An event that can be determined with certainty to have no relationship to a study procedure. |

**Serious Adverse Experiences (SAE)**

An SAE is defined as any AE that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

*Serious Adverse Experience Reporting*

Study sites will document all SAEs that occur on an SAE Report Form. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit (Visit 2) have been completed.

All SAE Report Forms will be reviewed by the site investigator and sent to the coordinating site (UNC) within one business day of the site learning of the event. Sites will send the SAE report by either:

- Email (scanned copy) to: sasellers@med.unc.edu
- Fax: (984) 974-2968

The site will notify the coordinating site of additional information or follow-up to an initial SAE Report as soon as relevant information is available. The coordinating site PI may request additional information related to the SAE. Follow-up information is reported on an SAE Report Form.

In accordance with the standard operating procedures and policies of the Institutional Review Board (IRB), the site investigator will report SAEs to the IRB.

**6.3 Study Procedures**

*Medical History and Physical Examination:*

During the screening visit, each patient will be questioned concerning medical history. Medical conditions, current medication use, and drug allergies will be recorded. A complete physical examination will be performed by a physician investigator. At Visits 1 and 2, inquiry into adverse events, new symptoms, and medication use will be performed, as will a focused physical examination (vital signs, head, neck, pulmonary and cardiac exams).

*Vital Signs:*

Temperature, respiration rate, heart rate, oxygen saturation, and blood pressure will be recorded at each study visit prior to pulmonary function tests.

*Spirometry:*

Spirometry will be performed at screening to determine eligibility. At visits 1 and 2, spirometry will be performed to assess efficacy. On each occasion, the best of 3 trials, based on ATS/ERS criteria. The best FVC, $FEV_1$ and $FEF_{25-75}$ (from trial with highest FVC+$FEV_1$ sum) will be recorded (absolute value and % of predicted). If subject accidentally uses bronchodilator the morning of visit, spirometry will be collected in the same fashion at the next visit.

*Pregnancy Test:*

Every female of childbearing potential (first menses has occurred) will have a urine pregnancy test conducted before each study visit. If either of these tests is positive, the patient will be withdrawn from the study.

*Measurement of MCC:*

MCC measurement will be performed at baseline (Visit 1) and following 14 +/- 2 days of Bronchitol self-administration (Visit 2). Site staff will confirm that the subject meets other eligibility for the procedure (i.e., is not pregnant, symptomatic and has withheld the required medications before proceeding with the MCC procedure. A transmission scan (solid sheet containing Co57, placed in front of the body) of the subject's lungs will be obtained. Detailed instruction will be included in a Study Specific Procedure document. For each measure of MCC, the subject will inhale an aerosol of sulfur colloid labeled with Tc99m. Inhalation will occur until ~40 µCi of radioactivity is deposited in the lungs of subjects, which typically takes approximately two minutes. An initial deposition scan will then be recorded within 5 minutes of completing the inhalation, after clearing the oropharynx of activity by drinking a small glass of water. The subject will then remain seated in front of the gamma camera while continuous two-minute images are recorded. The subject will be expected to remain aligned on camera for the first 34 minutes of the scan and then for the first 4 minutes out of each 10-minute epoch until 94 minutes has elapsed. During the first 64 minutes of imaging, subjects will be encouraged to suppress spontaneous coughing so that cilia-driven clearance can be assessed. From 64—94 minutes, they will voluntarily cough through a peak flow meter, to achieve a total of 60 coughs during this interval, to assess cough clearance (CC). Voluntary coughs will be dispersed evenly during this interval. The peak airflow rate of every fifth voluntary cough will be recorded and averaged. Spontaneous cough frequency will be

recorded throughout the imaging period. Subjects will return to the lab for a 15-minute continuous scan of residual lung activity at 6 hours (±15 minutes) after the initial deposition scan. The images collected will be uploaded in dicom format to University of North Carolina, Chapel Hill through the secure Accellion MCC fileshare site. Detailed description of this procedure is contained in the SILP MCC Standard Operating Procedure Manual (**Appendix 1**).

*Bronchitol Tolerance Test:*

Following completion of MCC at Visit 1, all subjects will undergo a Bronchitol Tolerance Test (BTT), which will be administered and performed under the supervision of a healthcare provider who is able to manage acute bronchospasm, to identify patients who will tolerate Bronchitol maintenance therapy. Patients will first have their baseline oxygen saturation (SpO2(Oxygen saturation)) measured and then will be instructed to use a short-acting bronchodilator 5-15 minutes prior to the first test dose of Bronchitol. They will then be instructed to inhale the contents of 1 capsule of Bronchitol (40 mg inhaled mannitol). One minute following this, the new oxygen saturation will be obtained; if below 90% of the baseline SpO2, the patient will be considered as having failed the BTT. If above 90% of the baseline SpO2, the patient will be instructed to inhale the contents of 2 capsules, one capsule at a time. After 1 minute, a new SpO2 and FEV1 will be obtained. If the SpO2 is lower than 90% of the baseline or the FEV1 is less than 80% of the baseline FEV1 obtained prior to MCC, the patient will be considered as having failed the BTT. If both measures are above the thresholds, the patient will then be instructed to inhale the contents of 3 capsules, one at a time. After 1 minute, a new SpO2 and FEV1 will be obtained. If the SpO2 is lower than 90% of the baseline or the FEV1 is less than 80% of the baseline FEV1 obtained prior to MCC, the patient will be considered as having failed the BTT. If both measures are above the thresholds, the patient will then be instructed to inhale the contents of 4 capsules, one at a time. After 1 minute, a new SpO2 and FEV1 will be obtained. If the SpO2 is lower than 90% of the baseline or the FEV1 is less than 80% of the baseline FEV1 obtained prior to MCC, the patient will be considered as having failed the BTT. If both measures are above the thresholds, the patient is considered as having passed the BTT and will continue with home use of Bronchitol until Visit 2. If the patient fails the BTT at any time, they will be terminated from the study. Study staff will use the Bronchitol "Healthcare Practitioner Instructions for Use" worksheet to administer the BTT (Appendix 2).

*Quality of Life and Respiratory Symptoms:*

The CFQ-R is a detailed, rigorously designed and validated instrument designed to measure quality of life in patients with CF who are 14 years and older[11]. This instrument was developed as the first CF-specific, health-related QOL measure. The respiratory domain has a 0-100 scale, with higher values signifying less severe symptoms. The CRRSD-CRISS is an 8-item, patient-centered outcome measure evaluating symptom severity over the prior 24 hours in 8 domains central to CF[12]. Both questionnaires will be administered during Visits 1 and 2 to assess efficacy of the treatment on symptom burden as a secondary outcome.

*Treatment Satisfaction:*

The TSQM is a 14-question standardize measure for assessment of treatment satisfaction and has been valued for use of inhaled medications in PwCF[13]. It will be administered prior to MCC measurement at Visit 1 and Visit 2. The TSQM items are answered on 5- or 7-point Likert type scale and cover four domains, corresponding to distinct aspects related to the satisfaction of patients with their treatment (Effectiveness; Side effects; Convenience and Global satisfaction). The questionnaire is scored on a 0-100 scale, with higher values indicated greater satisfaction.

### 6.3.1 Study Schedule

The following procedures at the specified study visit will be performed in the listed order of conduct:

A. Screening Visit

- Informed consent
- Medical history
- Vital signs
- Urine pregnancy test (for females of child-bearing potential)
- Full physical examination

B. Visit 1 (0 or 14+/-3 days after screening)

- Questionnaire administration
- Vital signs (not required if performed same day as screening)
- Abbreviated physical examination (not required if performed same day as screening)
- Urine pregnancy test (for females of child-bearing potential)
- Spirometry
- Mucociliary clearance measurement
- Bronchitol tolerance test

C. Visit 2 (14 +/- 3 days after Visit 1)

- Questionnaire administration
- Vital signs
- Abbreviated physical examination
- Adverse event assessment
- Spirometry
- Mucociliary clearance measurement

### 6.3.2 Informed Consent

The patient agrees to participate in the study by signing and dating the informed consent form after the nature of the study has been fully explained and all questions have been satisfactorily answered.

### 6.3.3 Screening

Screening will be conducted by a member of the study staff. After initial chart review indicating preliminary inclusion/exclusion criteria are met, study staff will discuss the study with the patient in the clinic or via telephone, and if they are agreeable, arrange for a screening visit. At the screening visit, inclusion and exclusion criteria will again be reviewed and if met, informed consent will be obtained. Following this, subjects will undergo medical history review, physical examination, medication review, urine pregnancy testing (if applicable) and spirometry measurement.

### 6.3.4 Recruitment, Enrollment and Retention

Following screening procedures, informed consent and enrollment into the study, a member of the study staff will determine when Visit 1 can be scheduled based on subjects' last reported use of nebulizer hypertonic saline. Visit 1 can be scheduled as early as the same day as screening, but the subject must report no use of nebulized hypertonic saline within 14 days of Visit 1. If the subject has used hypertonic saline on the day of the Screening Visit, Visit 1 must be scheduled at least 14 days from that point and the subject will be required to withhold hypertonic saline during that time. Study staff will send the subject a reminder prior to Visit 1 via telephone or electronic communication.

At the completion of Visit 1, once the subject has been determined to pass the BTT, study staff will schedule Visit 2 in 14 +/- 2 days. Study staff will send the subject a reminder prior to the visit via telephone or electronic communication.

### 6.3.6 End of Study and Follow-up

Subject participation will be complete at the end of Visit 2 or at the time of withdrawal for subjects who elect to withdraw early or do not pass the BTT. No further attempts will be made to contact subjects beyond this point for study purposes.

### 6.3.7 Removal of subjects

Subjects will be withdrawn from the study if they report pregnancy or test positive on pregnancy testing or if they fail the BTT. Subjects may choose to withdraw voluntarily at any time during the study for any reason. If subject has been given Bronchitol to use at home at the time of withdrawal, study staff will ask that the Bronchitol and inhaler be discarded and

that the subject return to use of airway clearance regimen as prescribed by their primary provider. Subjects who prematurely withdraw will be replaced.

**Who6.4 Statistical Method**

### 6.4.1 Statistical Design

To determine the effect of visit, and thus Bronchitol administration on clearance, the $MCC_{60}$ will be analyzed with a paired t-test. As a second step, we will include the effect of skew in a repeated measure model. Inclusion of deposition skew as a covariate in the primary assessment of MCC is an important consideration because increased deposition skew/heterogeneity is associated with faster observed MCC rates. This change in MCC rate reflects increased radio-aerosol deposition in large airways, which have intrinsically faster MCC rates. The described mixed effect model will, therefore, allow us to determine whether there is an effect of treatment (reflected by the visit covariate) on MCC independent of any potential changes in deposition skew. Exploration of other parameters including site location, age, sex, etc. will be performed by visualizing box plots and scatter plots of $MCC_{60}$ organized by each variable. Remaining secondary outcomes will be evaluated using paired t-tests to determine differences before and after treatment with Bronchitol.

### 6.4.2 Sample Size Considerations

The proposed sample size of 22 patients enrolled and completing two visits should provide approximately 80% power at a two-sided significance level of 0.05 to detect a treatment effect of 5.4% (absolute change in MCC60). This calculation is based upon prior data, which revealed a standard deviation of within-patient difference of 8.5%. A treatment difference of this magnitude is considered to be both clinically meaningful and achievable, as it approximates the sustained effect observed with HS treatment in patients with CF.

### 6.4.3 Planned Analyses

#### 6.4.3.1 Primary Analyses

The primary outcome, $MCC_{60}$ will be reported using means and standard deviation, with associated 95% confidence intervals at each visit. The mean change of $MCC_{60}$ between the 2 visits will also be reported with associated 95% confidence intervals. The data will also be plotted in box plots. $MCC_{60}$ between the 2 visits will be analyzed with a 2-sided paired t-test.

We know from previous studies that skew affect $MCC_{60}$. Therefore, $MCC_{60}$ will also be analyzed using a linear repeated measure model including visit number, skew of the deposition histogram as fixed effects. The effect of (visit x skew) as an interaction term will be explored, and if significant, will be included in the model. P-values below 0.05 will be considered significant.

#### 6.4.3.2 Secondary Objectives Analyses

All secondary outcomes, including CC, average clearance over alternative intervals (90 minutes), MCC clearance of central and peripheral lung regions, and pre-bronchodilator FEV1 will all be reported using means and standard deviations, with associated 95% confidence intervals at each visit. Total CFQ-R, CFRSD-CRISS, and TSQ scores will be considered continuous and will also reported using means and 95% confidence intervals.

The effect of treatment will be evaluated using 2-sided paired t-tests on all secondary outcomes. A repeated measures model will also be used to analyze $MCC_{90}$ with visit number and skew as fixed effects. P-values below 0.05 will be considered significant.

Exploration of the effect of site location, age, sex, %FEV1 and CFQ-R and TSQ scores on MCC60 will be explored using repeated measure models.

#### 6.4.3.3 Safety/Pregnancy-related policy

Adverse events will be collected and coded per MedDRA, version 9 or higher, and tabulated by treatment exposure. The proportion of patients experiencing an adverse event for each treatment exposure will be reported, and 95% confidence intervals will be computed for the proportions using exact binomial methods.

#### 6.4.3.4 Analysis of Subject Characteristics

The following clinico-demographic information from subjects will be collected via chart review and at the screening assessment: Age, gender, race/ethnicity, height, weight, BMI, baseline $FEV_1$ % predicted, clinical microbiology, CFTR genotype, CF-related comorbidities, and concomitant CF medications. Data will be tabulated and means with standard deviations reported.

### 6.4.5 Handling of Missing Data

Subjects who do not complete the 2 visits will be listed and reasons for drop-off will be reported. The final dataset will include all subjects who complete both visits per protocol. If any value is missing, it will be considered missing at random and will not be replaced.

# 7 - Trial Administration

### 7.1 Ethical Considerations: Informed Consent/Assent and HIPAA Authorization

This protocol will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

Prior to enrolling a subject in the trial, the Investigator must explain the purpose of the study, describe the procedures that will be performed, how the study medication will be use, and explain the potential risks associated with participation in the trial to the subject. Subjects will be informed that their participation is voluntary, and they may withdraw consent to participate at any time. In order to participate in the study, each subject must sign the informed consent (and other locally required documents) after the nature of the study has been fully explained. The consent form must be signed and dated prior to performance of any study-related activity. Subjects must be given the opportunity to ask questions. The subject will be given sufficient time to read the informed consent and to ask additional questions. After this explanation and before entry into the trial, consent should be appropriately recorded by means of either the subject's dated signature or the signature of an independent witness who certifies the subject's consent in writing. After having obtained the consent, a copy of the informed consent form (ICF) must be given to the subject. If the subject is unable to read, an impartial witness must attest the informed consent.

The collection and processing of personal data from subjects enrolled in this trial will be limited to those data that are necessary to investigate the safety, quality, and utility of the investigational agent used in this trial. These data will be processed with adequate precautions to ensure confidentiality. Confidentiality will be maintained throughout the complete data processing.

### 7.2 Institutional Review Board (IRB) Review

The protocol and consent forms will be reviewed and approved by the IRB on record prior to study initiation. SAEs regardless of relationship to study procedures will be reported to the IRB in accordance with the standard operating procedures and policies of the IRB, and the Investigator will keep the IRB informed as to the progress of the study. The Investigator will obtain assurance of IRB compliance with regulations.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB's written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB's unconditional approval statement will be transmitted by the Investigator to the Sponsor or designee prior to

the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB approval except when necessary to eliminate immediate hazards to the subjects or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

### 7.3 Subject Confidentiality

The collection and processing of data from subjects enrolled in this trial will be limited to those data that are necessary to investigate the safety, quality and utility of the investigational agent(s) used in this trial. These data will be processed with adequate precautions to ensure confidentiality. Only a site number and subject number will identify all study subjects on CRFs and other documentation submitted to the Sponsor. Confidentiality will be maintained throughout the complete data processing.

### 7.4 Deviations/Unanticipated Problems

A protocol violation occurs when the subject, investigator, or the Sponsor fails to adhere to significant protocol requirements affecting the eligibility, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet eligibility criteria
- Defined “major” errors in MCC SOP execution (see Appendix 1)

### 7.5 Data Collection

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject who signs informed consent.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol specific CRF when the information corresponding to that visit is available. Scanned paper CRFs will be uploaded along with study images (DICOM format) to the central reading site via a secure Accellion file transfer system. A REDCap database will be designed to house non-image data. Study images will be stored in a secure file location with daily electronic back-up. Subjects will not be identified by name in the study

database or on any study documents to be collected by the Sponsor (or designee) but will be identified by a site number and subject number. Signed consent forms will be saved in a regulatory binder maintained in a locked office at each site.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator.

### 7.6 Data Quality Assurance

After data have been entered into the study database, data validation checks will be applied on a regular basis. Queries will be generated as needed to clarify and confirm submitted data. The study database will be updated in accordance with the resolved queries.

### 7.7 Study Records

The Investigator must make study data accessible to authorized representatives of the Sponsor (or designee), IRB, and Regulatory Agency (e.g., FDA) inspectors upon request. MCC image data will be maintained electronically. All paper hard copies including ICF, HIPAA authorization, source documents and CRFs will be stored in a regulatory binder at the study site in a locked office. Site PIs must ensure the reliability and availability of source documents from which the information on the CRF was derived.

### 7.8 Data or Specimen Storage/Security

MCC image data will be transmitted to and maintained by the central reading site via a secure Accellion file transfer system. Visit log sheets and paper CRFs will also be uploaded via the Accellion file transfer system. Key data elements will then be recorded into, a secure, web-based REDCap database. Only study staff will have access to the database.

### 7.10 Retention of Records

All study documents must be kept secured for a period of two years after database lock.

### 7.12 Data Safety Monitoring Plan

The Sponsor, Dr. Sellers, is an Assistant Professor of Medicine with experience in clinical research. It will be her responsibility to review any unanticipated problems and adverse events that occur during the conduct of the study.

Adverse events will be collected at study visits by the clinical research coordinator and/or other members of the study team. Each event will be recorded into the study record. The attribution (related, unrelated), severity, and outcome of each event will be reviewed by a site physician investigator. All IRBs of record will be notified of any serious adverse events and any unanticipated, suspected adverse reactions within 48 hours of becoming aware of the event. Other adverse events will be summarized and reported at least annually.

### 7.13 Study Modification

The primary investigator assumes responsibility for reporting protocol amendments and any necessary ICF revisions to the IRB.

### 7.14 Study Completion

The expected completion date of the study is March 1, 2023. The primary investigator will notify the IRB of study completion at that time.

### 7.15 Conflict of Interest Policy

### 7.17 Funding Source

Pending**.**

### 7.18 Publication Plan

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

# Appendix 1

**Slow Inhalation Large Particle (SILP) Procedure**

### A. Introduction and Rationale

The SILP MCC Procedure is designed to deliver large (~9 um MMAD) particle aerosol in a controlled delivery at a slow inhalation flow rate (55 ml/sec). This method increases large and small airway deposition while reducing oral and alveolar deposition, and is accomplished without requiring a dosimeter.

### B. Equipment and supplies

Pari compressor (Trek-S)

Rotameter (range 0-20 L/min)

Steam sterilizer (Phillips Avent)

Large syringe for leak test (e.g. 1L, 3L spirometry syringe)

Nose clips

SILP Aerosol Delivery Assembly: *(provided by MCC NRC Coordinating Center)*

- Mouth piece (disposable)
- Pari SPRINT XLent nebulizer (single patient use)
- Tubing for connecting nebulizer to compressor
- Curved connector (top of nebulizer to exhalation path)
- Short straight connector (curved connector to Pall BB50T filter)
- Pall BB50T exhalation filter (disposable)
- Y-connector (Pall BB50T exhalation filter to exhalation valve and pressure transducer)
- Rudolph one way valve to fit in exhalation tubing
- Tubing to connect Y-valve to Magnehelic pressure gauge
- Magnehelic pressure gauge
- Lead shielded case for delivery components

### C. Camera quality control procedures:

1. Perform sensitivity test for any camera to be used (once; see below) and is known/documented
    a. Take a 10 min image without nearby activity to assess background; record total field of view value (kcps) and save for future reference

   b. Obtain 50 uCi of Tc99m and place in a petri dish in adequate volume to cover bottom of dish
   c. Place dish containing Tc99m on top of horizontally positioned camera head and record for 1 min. Save kcps value for future reference. This should be at least 6x background value
2. Ensure automatic decay correction is turned off (perform test if unknown/uncertain)
   a. Open acquisition protocol to be used in MCC study (serial 2 min images; dynamic)
   b. Place petri dish containing 50 uCi of Tc99m onto camera and record for 60 min
   c. If decay correction is turned off, a ~10% reduction in kcps should be observed at the end of the imaging period relative to t=0
3. Perform flood image to assess camera homogeneity at least monthly, as per local guidelines.

**D. Aerosol delivery setup**

- See Figures 1,2,3.
- Before setting up aerosol delivery assembly, inspect the nebulizer jet and use a fine needle to clean the holes if you observe any residual sulfur colloid or foreign material.

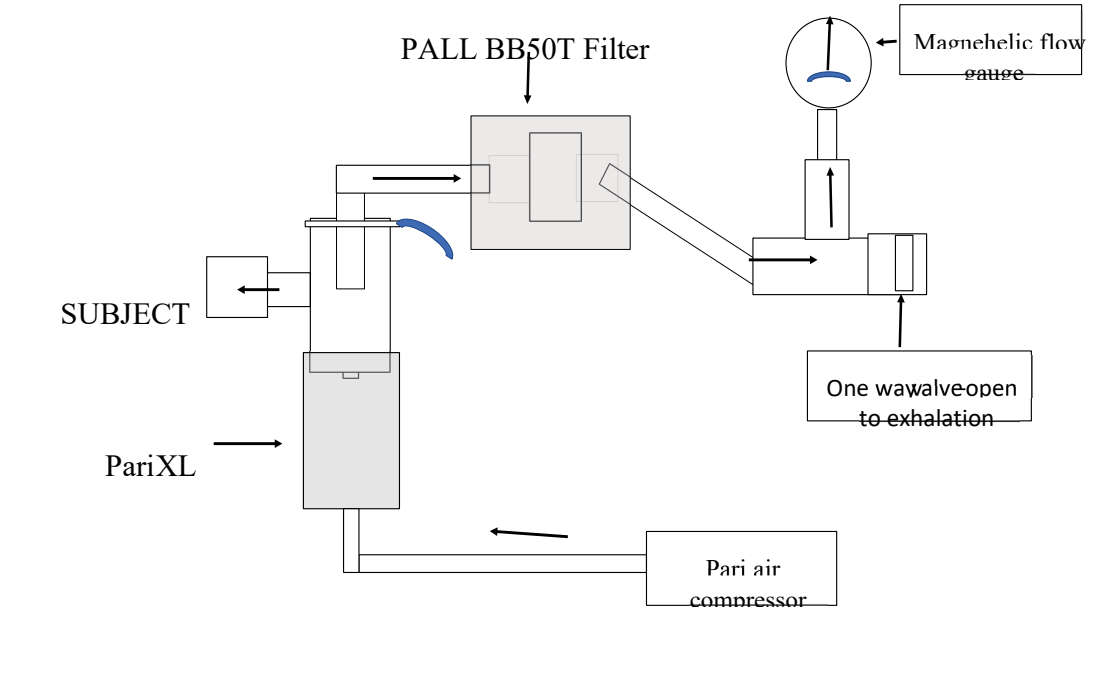



**Figure 1**: SILP Aerosol Delivery Assembly Schematic

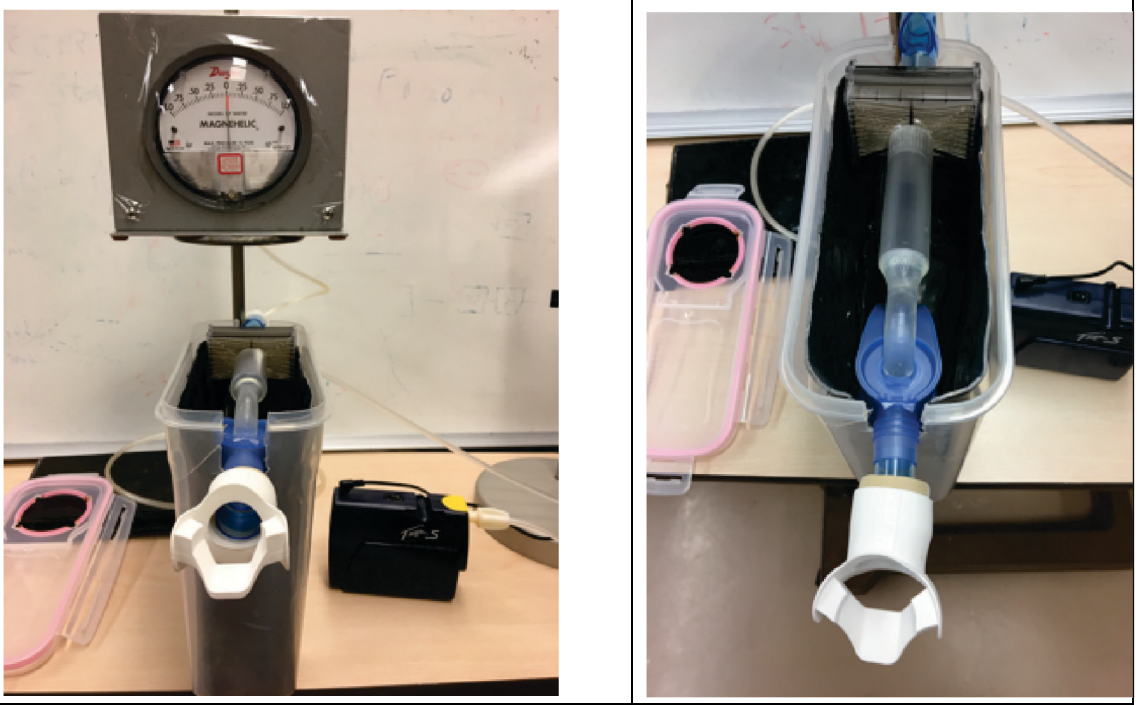


**Figure 2**: SILP Aerosol Delivery Assembly Setup

**Figure 3**: Mouthpiece setup

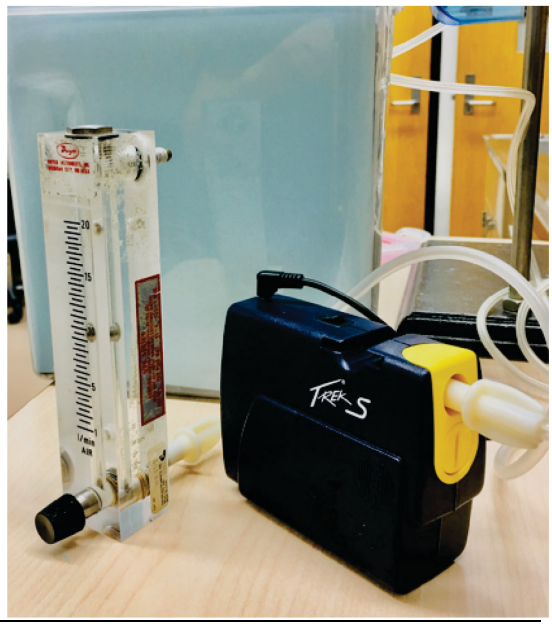



**Figure 4**: Compressor flow test

**E. Testing of equipment**

1. Check compressor flow (Fig. 4): Connect the compressor to the rotameter (flow meter), turn on the compressor, the flow should be between 8-10 L/min.

2. Aerosol delivery system leak test (Fig 5): After assembling the aerosol delivery unit, connect a large syringe (e.g. 1L or 3L spirometry calibration syringe) to the aerosol outlet of the Pari SPRINT XLent nebulizer (in place of mouthpiece).
   a. Gently pull the syringe handle until the needle on Magnehelic pressure gauge deflects toward the left (about ½ way to maximum value);


**Figure 5:** System Leak Test. In left panel, note leftward deflection of needle on Magnehelic pressure gauge while gentle aspirating with large volume syringe from nebulizer aerosol output.

In right panel, note that the needle stabilizes leftward of zero while obstructing the open port on back of Magnehelic with finger, after releasing suction on syringe.

b. With syringe in place, use your fingertip to block the bottom/open outlet of the Magnehelic to determine whether there is a leak – the needle deflection should be stable. Release the fingertip quickly then block again check if the needle moves then again remains stable. If the needle keeps moving towards zero after blocking the outlet it means that there is an air leak somewhere in the nebulizer setup, in which case you need to check all the connections, then repeat the test until a satisfactory result is obtained.

3. Nebulizer airflow test (Fig 6): With assembled aerosol delivery uni, turn on the compressor and use your hand with clean gloves to block the nebulizer mouth piece outlet. The needle on the Magnehelic pressure gauge should increase 0.2 inches of water) which corresponds to about 55 ml/sec in this setup.

**Figure 6**: Nebulizer airflow test

4. Nebulizer aerosol test: Put about 0.2ml normal saline in the nebulizer, turn on the compressor, check if the nebulizer generates the aerosol. Once aerosol generation is confirmed, let the compressor run until there is no more aerosol leaving the nebulizer.

After testing the nebulizer, securely attach the mouthpiece to the front of the inhalation box for subject use.

**F. Placement of Americium-241 sources:** Place two Americium-241 sources (1 uCi; shielded side facing patient) along the centerline of the subject's back. The upper marker should be placed over the C7 vertebra; the lower marker should be placed below the inferior lung margin (~13 inches) but within the camera field of view. Use a surgical marker to circle each source, to allow accurate replacement at the 6 hours and/or 24 hours follow-up scan. Tape the Americium sources in place with paper tape. Record the distance between the two Americium markers (log sheet).

**G. Transmission scan:**

1. Adjust laser pointer to shine on a tape marker placed on subject's neck (near sternal notch) while patient is sitting upright, against camera. Adjust a mirror to allow subject to keep the laser pointer spot on the tape to help maintain alignment later during scanning.
2. Make sure the appropriate camera acquisition protocol is opened (dual isotope state imaging with Co and Am windows). Position the subject in front of camera with the

camera head tilted to 75 degrees. The subject should sit with their back against camera while you place the $Co^{57}$ flat source in front of the subject (~13-15 inches from camera head, resting the $Co^{57}$ source on the chair arms and angling it parallel to camera head (i.e. 75 degrees). Check the image on the computer screen and adjust the camera height to make sure the image is in the right position.

3. Start the scan, noting start time (log sheet) and being aware of approximate $Co^{57}$ exposure time that is expected (see table)
4. Remove the $Co^{57}$ source from camera vicinity once a clear image of the lung outline is seen (record exposure time; log sheet)
5. End the transmission scan acquisition once activity from the $Am^{241}$ sources can be visualized in the appropriate channel. Note time of scan completion (log sheet). Be aware that the total transmission scan time will be longer than the time exposed to $Co^{57}$ in most cases. The $Co^{57}$ exposure time depends on the subject size and activity of the $Co^{57}$ source (see scan time reference table below). Record height and angle of camera head (to be replicated at subsequent studies).

| Current $Co^{57}$ activity | Time for $Co^{57}$ exposure |
|---|---|
| 15-20 mCi | 30 seconds |
| 10-15 mCi | 1 minute |
| 7.5 –10 mCi | 90 seconds |
| 5 - 7.5 mCi | 2 minutes |
| 2.5 – 5 mCi | 2 ½ minutes |
| < 2.5 mCi (time to get a new source) | 3 minutes |

**H. Background scan:**

1. Ensure subject is aligned and laser pointer shines on the tape marker (do not move the tape) while patient is sitting upright against camera. Adjust mirror to allow subject to keep the laser pointer spot on the tape to help maintain alignment during later scanning.
2. With subject appropriately aligned against the camera, begin a 15-minute background scan for use as the current visit background. Ensure that all radioactive sources (except $Am^{241}$ sources on the patient) are removed from the room. Record the average background rate (kcps; read off camera in real time; log sheet).

**I. Inhalation Procedure:**

1. Subject positioning and preparation: Ensure the proper camera protocol (Tc99 and Am241 windows) is selected. Have the subject wear a disposable gown and gloves. Ask the subject to sit in front of the gamma camera, adjust a bedside table to the proper height to make sure the subject's head is level with the aerosol delivery assembly, without needing to bend neck or slouch once the mouthpiece is in their

mouth. During the maneuvers, they should sit with both feet on ground. Place nose clips on subject once ready to proceed with practice manuevers.

Practice maneuvers: Before loading any radioaerosol, the inhalation maneuvers should be practiced. After placing nose clips and putting the mouthpiece in their mouth, have the subject briefly hold their breath as you turn on the compressor. If the subject is indeed holding their breath the magnehelic pressure should increase 0.2 in of water (as with nebulizer airflow test – Fig 6). The subject should then immediately begin to gently inhale from the nebulizer for a period of 6 seconds, using a metronome to indicate the inhalation time. The closed inhalation loop limits their inspiratory flow to that provided by the compressor (55 ml/sec). The magnehelic pressure should decrease from the zero setting (leftward; greater than 0.5 inches) in which case the subject is receiving the 55 ml/sec delivered from the nebulizer. At the end of 6 second inhalation the subject should exhale at a constant rate (rightward 0.5 inches of water on Magnehelic) until the compressor has been turned off. *****Make sure the subject does not come off the mouthpiece until instructed by the operator and the compressor has been turned off; otherwise aerosol will be emitted into the room (causing contamination if done during radioaerosol inhalation).***

2. Radioaerosol Inhalation Maneuver: After the subject has demonstrated the proper breathing technique, load the nebulizer with Tc99m-sulfur colloid (3 mCi in 3ml of sterile saline) and begin the inhalation as described above. The subject will come off the mouthpiece (after compressor is turned off, as described above) after each breath of radioaerosol. Following a momentary rest, repeat the next inhalation breath. Generally, 6-10 breaths are required to deposit 40-50 uCi of Tc-SC in the lungs. Use the gamma camera to monitor the deposited isotope dose in kcps with their back pressed against the camera. The target deposited dose of 40-50 uCi should yield camera counts similar to that observed during camera sensitivity test with 50 uCi of Tc isotope, and should be well above the background rate.

After completing the final inhalation, immediately remove the aerosol delivery assembly from room, have the subject drink a small cup of water to flush mouth activity into the stomach, and remove any contaminated materials (gown, glove, etc.). **J. Dynamic scan:**

1. Ensure the subject is lined up properly, using the laser pointer and tape marker on neck. Alignment should be accomplished as soon as possible (ideally within 2-3 min) of completing the aerosol delivery steps.
2. Once aligned, simultaneously start a digital timer and dynamic image acquisition (consecutive 2 minute images; dual windows for $Tc^{99m}$ and Am241). Record the acquisition start time in study log.
3. Ask the patient to refrain from coughing if possible (for first 64 minutes of scan). Record any spontaneous coughs during the dynamic scan period on the data log sheet.
4. Ensure the patient maintains alignment for the first 34 minutes (17 frames); then allow a <6-minute break. Subsequently, it is extremely important that alignment is achieved for the entirety of the first 4 minutes of each ten min period.
5. Terminate data acquisition at the 94 time point.
6. Remove the $Am^{241}$ sources, ensuring their position is adequately marked for placement at later time points (e.g. 3, 6, and 24 hours).

**K. Static images at later time points (e.g. 3,6, or 24 hours):**

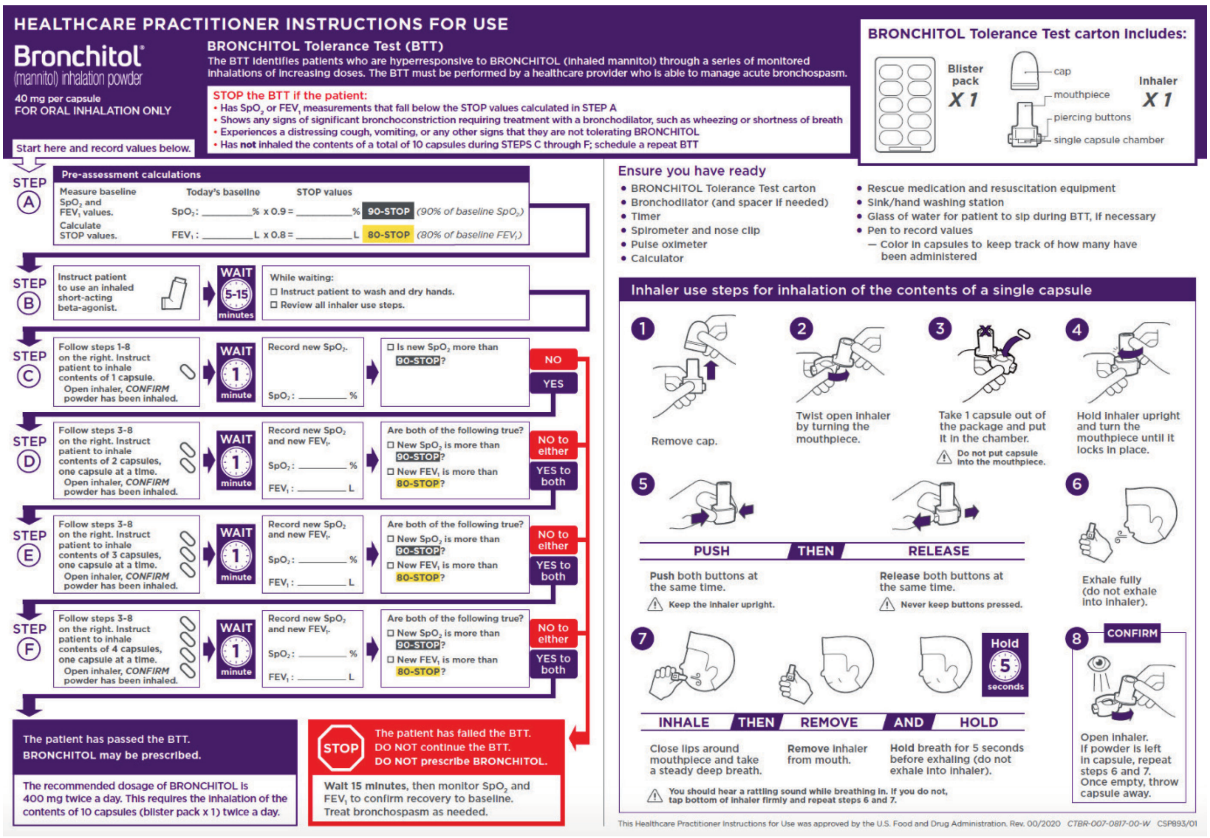
1. Ensure the camera/chair are returned to the prior recorded positions (height and angle).
2. Replace the Am241 sources to their previous locations on the subject.
3. Position the subject in front of the camera using the laser point and mirror.
4. Begin a 15 minute static image acquisition for interval time points $\leq$ 6 hrs after inhalation; use a 30-minute static image acquisition if collecting a later (e.g. 24 hours) time point.
5. Note start/stop time of the image in logsheet. Static images should be performed within 15 min of the nominal timepoint up to 6 hrs; 24-hr images should be within 6 hours of nominal timepoint.

**L. Cleaning procedures**:

1. After completing the radioaerosol inhalation, leave the nebulizer and connectors in a shielded area (e.g. the lead-shielded delivery box) until the activity has decayed to an acceptable level.
2. When ready to clean, disassemble the delivery apparatus. Discard disposable items (Pall BB50T filter; mouthpiece).
3. Separate the nebulizer, connectors, and mouthpiece and rinse with running water, then soak in a warm clear dish soap solution for 5 minutes, followed by a second rinse in running water. Finally, place all re-usable components into the steam sterilizer, following manufacturer directions to disinfect the equipment. After this disinfection step, all components should be allowed to air dry on a clean paper towel, then stored appropriately. You should use the same nebulizer (Pari XLent) for all visits conducted by an individual subject, then dispose.

# Appendix 2

Instructions for Bronchitol Tolerance Test (BTT)



**References**

1. Teper A, Jaques A, Charlton B. Inhaled mannitol in patients with cystic fibrosis: a randomised open-label dose response trial. *Journal of Cystic Fibrosis*. 2011;10(1):1-8.

2. Bronchitol (mannitol inhalation powder) [package insert]. Cary, NC: Chiesi USA, Inc. 2020.

3. Donaldson SH, Bennett WD, Zeman KL, Knowles MR, Tarran R, Boucher RC. Mucus clearance and lung function in cystic fibrosis with hypertonic saline. *N Engl J Med*. Jan 19 2006;354(3):241-50. doi:10.1056/NEJMoa043891

4. Elkins MR, Robinson M, Rose BR, et al. A controlled trial of long-term inhaled hypertonic saline in patients with cystic fibrosis. *N Engl J Med*. Jan 19 2006;354(3):229-40. doi:10.1056/NEJMoa043900

5. Middleton PG, Mall MA, Dřevínek P, et al. Elexacaftor-Tezacaftor-Ivacaftor for Cystic Fibrosis with a Single Phe508del Allele. *N Engl J Med*. Nov 7 2019;381(19):1809-1819. doi:10.1056/NEJMoa1908639

6. Fajac I, Van Brunt K, Daines C, et al. S65 Impact of elexacaftor/tezacaftor/ivacaftor triple combination therapy on health-related quality of life in people with cystic fibrosis heterozygous for F508del and a minimal function mutation (F/MF): results from a Phase 3 clinical study. BMJ Publishing Group Ltd; 2021.

7. Rowbotham NJ, Smith S, Leighton PA, et al. The top 10 research priorities in cystic fibrosis developed by a partnership between people with CF and healthcare providers. *Thorax*. 2018;73(4):388-390.

8. Mayer-Hamblett N, Nichols DP, Odem-Davis K, et al. Evaluating the Impact of Stopping Chronic Therapies after Modulator Drug Therapy in Cystic Fibrosis: The SIMPLIFY Study Design. *Ann Am Thorac Soc*. Jan 19 2021;doi:10.1513/AnnalsATS.202010-1336SD

9. Bilton D, Robinson P, Cooper P, et al. Inhaled dry powder mannitol in cystic fibrosis: an efficacy and safety study. *Eur Respir J*. Nov 2011;38(5):1071-80. doi:10.1183/09031936.00187510

10. Teper A, Jaques A, Charlton B. Inhaled mannitol in patients with cystic fibrosis: A randomised open-label dose response trial. *J Cyst Fibros*. Jan 2011;10(1):1-8. doi:10.1016/j.jcf.2010.08.020

11. Henry B, Aussage P, Grosskopf C, Goehrs J-M. Development of the Cystic Fibrosis Questionnaire (CFQ) for assessing quality of life in pediatric and adult patients. *Quality of Life Research*. 2003;12(1):63-76.

12. Goss C, Edwards T, Ramsey B, Aitken M, Patrick D. Patient-reported respiratory symptoms in cystic fibrosis. *Journal of Cystic Fibrosis*. 2009;8(4):245-252.

13. Regnault A, Balp MM, Kulich K, Viala-Danten M. Validation of the Treatment Satisfaction Questionnaire for Medication in patients with cystic fibrosis. *J Cyst Fibros*. Dec 2012;11(6):494-501. doi:10.1016/j.jcf.2012.04.007