Clinical Research Protocol

A master protocol to test the impact of discontinuing chronic therapies in people with cystic fibrosis on highly effective CFTR modulator therapy (SIMPLIFY)

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Version Date:	February 14, 2020
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Approval: Study PIs noted above have approved the protocol and documentation of approval is on file

NICHOLS

PI Signature (David Nichols, MD)

Feb 14 2020

Date

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing the Sponsor-Investigator with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: SIMPLIFY-IP-19

Protocol Title: A master protocol to test the impact of discontinuing chronic therapies in people with cystic fibrosis on highly effective CFTR modulator therapy (SIMPLIFY)

Protocol Date: February 14, 2020

_____ _____

Site Investigator Signature *Date*

_____

Print Name and Title

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PROTOCOL SYNOPSIS

TITLE	A master protocol to test the impact of discontinuing chronic therapies in people with cystic fibrosis on highly effective CFTR modulator therapy (SIMPLIFY)
NUMBER OF SITES/SUBJECTS	Approximately 80 sites Approximately 920 subjects
STUDY DESIGN	This master protocol is designed to evaluate the independent effects of discontinuing hypertonic saline (Study A) and dornase alfa (Study B) in people with cystic fibrosis (CF) age 12 and older currently taking the highly effective modulator elexacaftor/tezacaftor/ivacaftor (ETI). Study A and Study B are identical open label two-arm randomized trials consisting of a 2-week screening period, randomization to continue or discontinue hypertonic saline (Study A) or dornase alfa (Study B), followed by a 6-week study period. Subjects taking only hypertonic saline (HS) or dornase alfa at trial entry will be randomized 1:1 to either continue or discontinue the applicable therapy (i.e. HS or dornase alfa). Subjects taking both hypertonic saline and dornase alfa at study entry will be randomized to participate in either Study A or Study B and will be randomized (1:1) to continue or discontinue the applicable therapy (i.e. HS or dornase alfa). After completion of the first study, eligible subjects may subsequently enroll in the alternative study. Clinical outcomes (forced expiratory volume in 1 second [FEV_1], antibiotic use, pulmonary exacerbations, and patient reported outcomes), safety (adverse events) and the subject's perception of how stopping HS or dornase alfa (or both) would impact their daily life will be evaluated in all subjects. Additional outcome measurements will be conducted in a subset of subjects at selected study sites: • Multiple Breath Washout (MBW) to evaluate changes in lung clearance index (LCI) • Mucociliary Clearance (MCC) using inhaled radio-labeled particles to evaluate changes in mucociliary clearance
STUDY POPULATION	This master protocol will enroll people with CF who are age 12-17 years old with $FEV_1 \geq 70\%$ predicted, and people with CF who are 18 years and older with $FEV_1 \geq 60\%$ predicted. Subjects must be clinically stable and currently taking ETI in addition to hypertonic saline and/or dornase alfa for at least the 90 days prior to screening. Study A and Study B will enroll approximately 400 subjects each (800 total). After interim safety reviews by the CFF Data Monitoring Committee (DMC), an additional cohort of 120 subjects ≥ 18 years old with FEV_1 between 40 and <60% predicted may be enrolled into Study A (hypertonic saline).
PRIMARY OBJECTIVE	Study A: • To determine whether discontinuing hypertonic saline is non-inferior to continuing hypertonic saline among people with CF on ETI, as measured by the 6-week absolute change in FEV_1 % predicted

	Study B: • To determine whether discontinuing dornase alfa is non-inferior to continuing dornase alfa among subjects on ETI, as measured by the 6-week absolute change in FEV_1 % predicted
PRIMARY ENDPOINT	The absolute change in FEV_1 % predicted from Visit 1 (randomization) to Visit 3 (week 6)
SAMPLE SIZE	It is anticipated from prior CF trials conducted through the TDNCC that the attrition and non-adherence rate will be less than 20%, and thus it is reasonable to expect that a total sample size of 400 per study will enable at least 308 subjects to complete the trial and be included in the per-protocol analysis (PPA) population. The PPA population will be the primary analysis population for test of non-inferiority. Assuming a standard deviation for the change in FEV_1% predicted of 8.4, a total sample size of 308 provides 88% power to detect non-inferiority with a margin of -3% absolute FEV_1 % predicted when there is truly no effect of discontinuation. If the DMC approves enrollment of a lower lung function cohort, approximately 60 per arm in Study A will be recruited for exploratory analyses focused on the safety of discontinuation of hypertonic saline in this cohort.

1 BACKGROUND

Health outcomes in people with CF have steadily improved for decades through high quality, multi-disciplinary clinical care models and expanding therapeutic options. These advances have most often been additive and require people with CF and their families to dedicate significant time and resources toward daily care needs. Since 2012, a small but increasing number of people with CF have benefited from highly effective CFTR modulator treatment (HEMT). This "highly effective" description refers to health benefits which are as good as those typically observed in people with a G551D mutation when treated with ivacaftor: sweat chloride reduction by approximately 50 mmol/L, FEV_1 % increase by ≥ 10%, large reduction in respiratory symptoms, and ≥ 50% reduction in risk of acute pulmonary exacerbation(1, 2). Until recently, < 10% of the US CF population has been eligible for HEMT by age and mutation-based label indication. Through continued progress in CFTR modulator drug development and use in younger populations, this number is dramatically increasing and is expected to reach ≥ 90%.

As transformative modulator drug therapies emerge, many in the CF Community (patients, families and caregivers) are asking if any of the pre-existing therapies can be reduced or eliminated. A survey of several hundred people with CF, their family members, and CF care providers was conducted in the US in 2018. Survey results from both the CF Community and Clinician-Investigator groups indicate very high support for this research and widespread interest in participating (3). Over 80% of people with CF or their parents surveyed reported that trials testing discontinuation of chronic therapies should be conducted in groups benefitting from HEMT. In parallel, 94% of CF clinicians supported such trials and both groups expressed high interest in participating in trials testing withdrawal of either hypertonic saline or dornase alfa. Both of these nebulized, inhaled therapies were also identified by the CF Community as relatively burdensome components of daily care.

2 STUDY RATIONALE

The initial population of people with CF in the US gaining new access to HEMT in the form of elexacaftor/tezacaftor/ivacaftor (ETI) are age 12 years old and older with at least one F508del mutation. Results from Phase 3 clinical trials (4, 5) testing ETI indicate that this drug combination provides clinical improvements as good as, if not better, than those observed with ivacaftor monotherapy in people with the G551D mutation (2). The SIMPLIFY master protocol will study people treated with ETI who also use inhaled hypertonic saline and/or inhaled dornase alfa by clinical prescription. In this adolescent and adult study population, we will be testing the question of treatment discontinuation rather than the question of whether or not to start a therapy in the first place.

The two lead therapies to be studied in the SIMPLIFY master protocol are hypertonic saline and dornase alfa. Both are identified as relatively burdensome, are intended to improve mucociliary clearance and have demonstrated dynamic effects on measures of pulmonary function (5-8). Early studies with either hypertonic saline or dornase alfa demonstrated a return to baseline lung function (i.e., pre-treatment FEV_1 % predicted) within 14 days of stopping the treatment (6). HEMT (i.e., ivacaftor) also improves lung function and mucociliary clearance, with demonstrably greater effects than either hypertonic saline or dornase alfa (1, 2, 7). Data indicate that ETI may be even more effective than ivacaftor monotherapy at improving CFTR function and airway clearance, and ETI will be indicated for a much larger proportion of people with CF. It is not known if hypertonic saline or dornase alfa improve or maintain pulmonary health above what is already gained through ETI use.

FEV_1 % predicted has been the most frequently used outcome measure of lung function in CF clinical trials, including studies of hypertonic saline, dornase alfa, and HEMT (2, 6, 8, 9). However, an alternative

and perhaps more sensitive measure of change in lung function using multiple breath washout (MBW) and the lung clearance index (LCI) has also been used to test pulmonary benefits from muco-active drugs or modulators (7, 10, 11). LCI improvement from ivacaftor in those with G551D was nearly twice the effect measured with either inhaled hypertonic saline or dornase alfa, though study population demographics differed (7, 10, 11). LCI is more useful in those with better preserved lung function and it is unclear how it will perform in the group recruited to SIMPLIFY. LCI is also not available at all study sites. However, the potential to capture even small changes in pulmonary function through LCI, possibly in those with higher baseline lung function, is valuable and LCI will be conducted at SIMPLIFY sites with this capability as a secondary outcome measure.

Including spirometry and LCI may increase the ability of this master protocol to detect and understand the impact of treatment withdrawal on airway function. Additionally, mucociliary clearance (MCC) scans will be done at a small number of SIMPLIFY study sites with this capability and may provide important physiological data related to spirometry and MBW (12). Consistency across all three of these related outcome measures would increase confidence in the interpretation of the trial results. If this master protocol determines that people on ETI who discontinue these therapies have no appreciable loss in lung function or change in MCC, our hypothesis will be supported that certain daily therapies might not be necessary or beneficial in at least some people treated with ETI. If this master protocol demonstrates that people treated with ETI who continue hypertonic saline or dornase alfa have measurably better outcomes, then these data will help to justify the time, effort and cost of continuing such treatments.

Transformative improvement in CF clinical care through HEMT represents an important opportunity to test the impact of withdrawing chronic therapies, specifically hypertonic saline and dornase alfa, in a randomized, controlled setting. Observational studies of treatment withdrawal are prone to confounding by indication and, if insufficiently powered, may be difficult to interpret. SIMPLIFY will limit indication bias through randomization and is powered to test hypotheses of non-inferiority in order to determine whether discontinuation of each therapy is comparable to continuing therapy as measured by change in pulmonary function. As there are currently no data from controlled trials testing the effects of discontinuing these therapies in CF, the results of this protocol will be important when considering individual treatment decisions among the increasingly large percentage of people with CF on highly effective modulator therapies.

2.1 Risk / Benefit Assessment

Drug Intervention: Each study in this master protocol is unblinded and there is no study drug or new exposure to a drug compound. Subjects enrolled in a study will be randomized to either continue or discontinue a medication that has already been clinically prescribed and used prior to enrollment. It is possible that discontinuing a medication (i.e., hypertonic saline or dornase alfa) will result in a decline in health. Determining whether or not this occurs among people on chronic ETI is the purpose of the protocol and subjects will be monitored closely. The length of each study has been minimized to an interval that is thought to be sufficient to determine if a change in lung function occurs. After the 6-week randomized participation period to either Study A (hypertonic saline) or Study B (dornase alfa), subjects and their care providers will decide whether or not to continue use of these medications. Subjects who experience an adverse event (AE) during Study A or Study B should be treated by clinical indication, including the potential to restart any medication that was stopped for this study.

Spirometry Test: Potential shortness of breath or light headedness.

MBW Test*:* The instrument to be used for the MBW test is the Exhalyzer® D by Eco Medics AG (Duernten, Switzerland). There is a very small risk of hyperventilation, which is minimized because each

breath will be visualized on the computer screen in real time and the exhaled gases are monitored. Rarely with hyperventilation, there may be dizziness or fainting.

MCC Test: The following complications could result from inhalation of nebulized aerosols: cough, shortness of breath, hoarseness, throat irritation, temporary decrease in lung function measurements, wheezing and decrease in oxygen saturation. The MCC procedure entails exposure to some radiation. 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 for adults ≥ 18 years old, >15 mSv for children < 18 years 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.

3 STUDY DESIGN

3.1 Study Overview

This is a master protocol with two concurrent randomized trials. It is designed to evaluate the independent effects of discontinuing hypertonic saline (Study A) and dornase alfa (Study B) in people with CF age 12 and older taking ETI for at least 90 days prior to study screening. Individuals with CF ages 12-17 years with FEV_1 70% predicted or greater and those 18 years and older with FEV_1 60% predicted or greater may enroll. There is no upper limit for FEV_1% predicted. Study A and Study B are identical randomized, open label two-arm randomized trials consisting of a 2-week screening period, randomization to continue or discontinue hypertonic saline (Study A) or dornase alfa (Study B), followed by a 6-week study period. Only those that remain clinically stable and maintain adequate reported adherence to inhaled drug therapy between screening and Visit 1 will be eligible for randomization.

Subjects currently being treated with only hypertonic saline or dornase alfa will be enrolled in Study A or Study B (as applicable) and will be randomized 1:1 to either continue or discontinue their current prescribed therapy.

Subjects who are currently being treated with both hypertonic saline and dornase alfa will remain on both therapies during the screening period and then be randomized to Study A (hypertonic saline) or Study B (dornase alfa) as well as randomized (1:1) to continue or discontinue the applicable therapy. The randomization to Study A or Study B among subjects on both therapies is not optional and is essential to reduce indication bias and ensure comparable populations across studies. After completion of the first study, these subjects may subsequently enroll in the alternate study if they meet eligibility criteria.

For subjects randomly assigned to continue their therapy during a given study, this therapy should be taken at least once daily according to each subject's pre-existing, clinically prescribed regimen (e.g., daily, twice daily). The concentration of hypertonic saline will also be according to clinical prescription (e.g., 7% sodium chloride or 3.5% sodium chloride). Hypertonic saline concentration must be at least 3%.

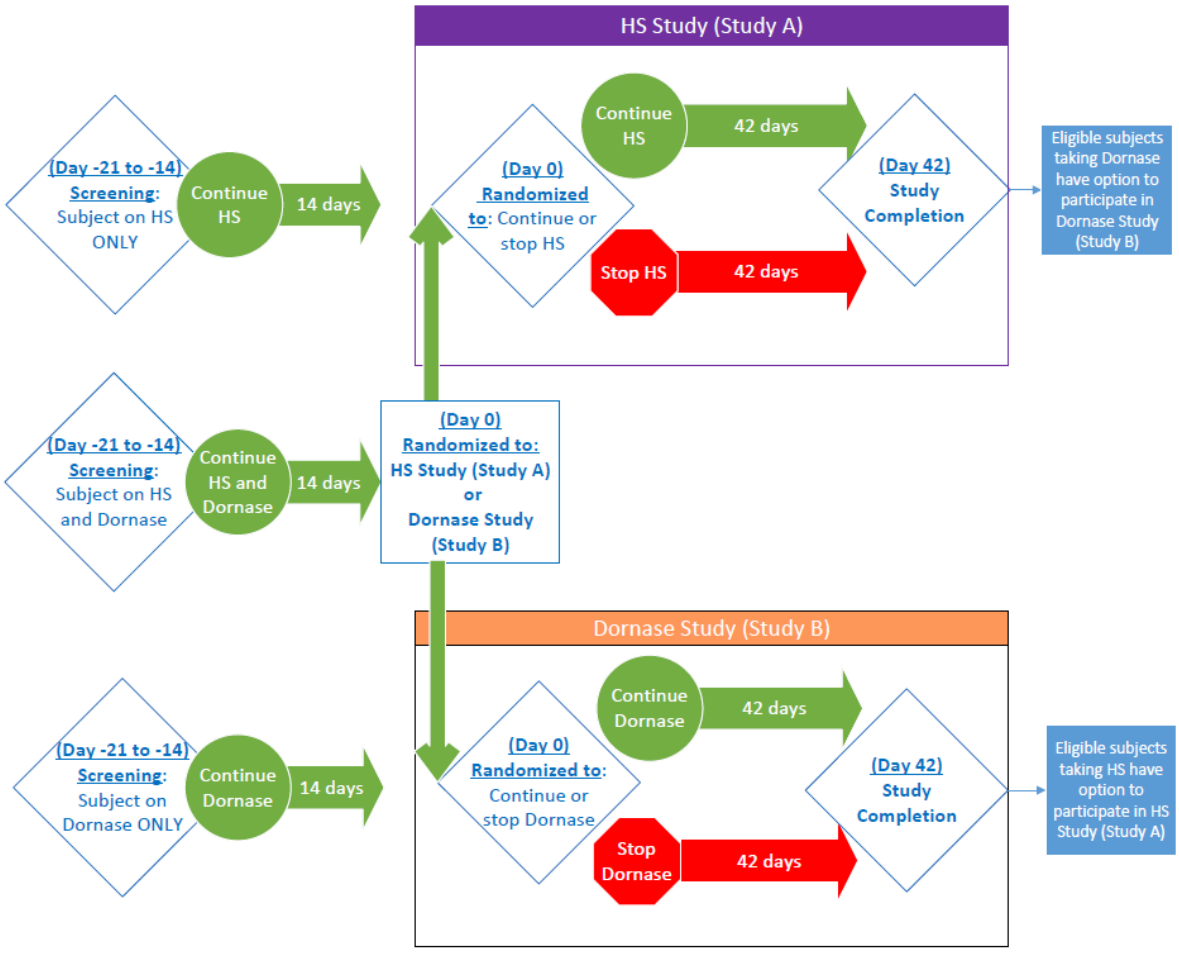
Clinical outcomes (FEV_1, antibiotic use, pulmonary exacerbations, and patient reported outcomes), safety (AEs), and subject perception of how stopping HS or dornase alfa (or both) would impact their daily life will be evaluated at all sites during each study. Additional measurements will be conducted at selected study sites with the capabilities to conduct these procedures:

- MBW to evaluate changes in LCI
- MCC scans using inhaled radio-labeled particles and imaging techniques to evaluate changes in mucociliary clearance

Each study includes four study visits over a period of 8 weeks, including the 2-week screening period.

- Subjects taking only hypertonic saline or only dornase alfa upon study entry at the Screening Visit would participate in study visits for up to 8 weeks.
- For subjects taking both hypertonic saline and dornase alfa upon study entry at the Screening Visit:
    - They have the potential to participate in either one or both studies. Subjects would participate in the first study for up to 8 weeks. After completion of the last visit in the first study, subjects may consider enrollment into the second study. If subjects are eligible and enroll in the second study, they would participate in study visits for up to an additional 8 weeks.
    - Participation in the two studies may be consecutive or separated by any length of time.
- All subjects will additionally be asked to complete electronic questionnaires approximately every 28 days for up to 24 weeks after their final study visit (Visit 3, Week 6). For subjects who participate in a second study within 24 weeks of completing the first, the post study questionnaires from the first study may be stopped.

A data monitoring committee (DMC) will be reviewing safety data on an ongoing basis for both studies. Upon interim review of safety data, an optional cohort with lower baseline lung function may be enrolled into Study A (hypertonic saline). The optional cohort will include people with CF ≥ 18 years old with FEV_1 between 40 and less than 60 % predicted who satisfy the remaining criteria for participation in Study A. Additional endpoints, including LCI and MCC, will not be obtained in this cohort.



4 STUDY OBJECTIVES

4.1 Primary Objective

The primary objectives are:

Study A: To determine whether discontinuing hypertonic saline is non-inferior to continuing hypertonic saline among people with CF on ETI, as measured by the 6-week absolute change in FEV_1 % predicted.

Study B: To determine whether discontinuing dornase alfa is non-inferior to continuing dornase alfa among people with CF on ETI, as measured by the 6-week absolute change in FEV_1 % predicted.

4.2 Secondary Objectives

The secondary objectives of each study are to evaluate:

- The safety of discontinuing vs. continuing hypertonic saline (Study A) or dornase alfa (Study B)
- The effect of discontinuing vs. continuing hypertonic saline (Study A) or dornase alfa (Study B) on lung clearance index (LCI)

- The effect of discontinuing vs. continuing hypertonic saline (Study A) or dornase alfa (Study B) on other clinical outcomes (e.g., antibiotic events, pulmonary exacerbations, and patient reported outcomes)

4.3 Exploratory Objectives

The exploratory objectives of this study are to:

- Evaluate the effects of discontinuing vs. continuing hypertonic saline (Study A) or dornase alfa (Study B) on mucociliary clearance (MCC)
- To estimate and compare the effects of discontinuing vs. continuing both hypertonic saline and dornase alfa among the subgroup of subjects using both chronic therapies
- Among those enrolled in a lower lung function cohort, evaluate the effects of discontinuing vs. continuing hypertonic saline (Study A) on safety and clinical endpoints
- Evaluate hypertonic saline and/or dornase alfa use up to 24 weeks after completion of each study and associate the decision to remain on or off therapy after the study with both randomization assignment and study outcomes
- Evaluate self-reported acute antibiotic use up to 24 weeks after completion of each study, comparing those who choose to use vs. not use inhaled hypertonic saline or dornase alfa
- Evaluate subject perception of how stopping HS or dornase alfa (or both) would impact their daily life

5 STUDY ENDPOINTS

5.1 Primary Endpoint

The primary endpoint in each study is the absolute change in FEV_1 % predicted from Visit 1 (randomization) to Visit 3 (week 6).

5.2 Secondary Endpoints

Secondary endpoints in each study include:

Efficacy

- Change in LCI from Visit 1 (randomization) to Visit 3 (week 6)
- Change in FEV_1 % predicted from Visit 1 (randomization) to Visit 2 (week 2)
- Proportion of subjects initiating acute antibiotics from Visit 1 (randomization) to Visit 3 (week 6)
- Proportion of subjects hospitalized from Visit 1 (randomization) to Visit 3 (week 6)
- Proportion of subjects with a pulmonary exacerbation from Visit 1 (randomization) to Visit 3 (week 6)
- Change in Chronic Respiratory Infection Symptom Score (CRISS) from Visit 1 (randomization) to Visit 3 (week 6)

- Change in the Cystic Fibrosis Questionnaire-Revised (CFQR) Respiratory Domain from Visit 1 (randomization) to Visit 3 (week 6)
- Change in FEV_1 % predicted from Screening to Visit 1 (randomization)

Safety

- Incidence of adverse events occurring between Visit 1 (randomization) to Visit 3 (week 6)
- Proportion of subjects temporarily or permanently changing their assigned therapy regimen between Visit 1 (randomization) to Visit 3 (week 6)

5.3 Exploratory

Exploratory endpoints include:

- Change in MCC from Visit 1 (randomization) to Visit 3 (week 6)
- Proportions of subjects remaining on and off hypertonic saline and dornase alfa for up to 24 weeks after completion of each study
- Proportions of subjects with acute antibiotic use up to 24 weeks after completion of each study
- Average impact score at Visit 3 (Week 6) on subject perception of how stopping hypertonic saline or dornase alfa (or both) would impact their daily life

6 SUBJECT SELECTION

6.1 Study Population

People with a diagnosis of CF who meet all eligibility criteria will be eligible for participation in this protocol.

Study A will enroll approximately 400 subjects (approximately 200 randomized to discontinue hypertonic saline and 200 to continue hypertonic saline).

Study B will enroll approximately 400 subjects (approximately 200 randomized to discontinue dornase alfa and 200 to continue dornase alfa).

Lower Lung Function Cohort: Approximately 120 additional subjects will be enrolled into this cohort in Study A upon approval from the DMC.

It is estimated that approximately 920 unique people with CF will be randomized in this master protocol, and that a subset of people with CF on both hypertonic saline and dornase alfa will enroll in both Study A and Study B.

Additional assessments conducted in only a subset of subjects:

- ***MBW Assessment:*** We anticipate that up to 400 subjects will provide data for the MBW assessment.
- ***MCC Sub-Study****:* Approximately 30 subjects from Study A and 30 subjects from Study B will enroll in the MCC sub-study.

6.2 Eligibility Criteria at Screening

Eligibility criteria will be evaluated at the Screening visit for each study in the protocol. Subjects that enter the SIMPLIFY Master Protocol taking only hypertonic saline or only dornase alfa at the time of entry will only be eligible to participate in one study.

6.2.1 Consent

A. Written informed consent (and assent when applicable) obtained from subject or subject's legal guardian.

B. Enrolled in the CFF Patient Registry.

C. For the 6-week study duration, willingness to either continue or discontinue daily use of hypertonic saline or dornase alfa (as applicable to Study A or Study B) based on randomization and according to the clinically prescribed routine (i.e., at least once daily).

D. Is willing and able to adhere to the study visit schedule and other protocol requirements including willingness and ability to provide information using electronic questionnaires loaded onto a personal device (e.g., smartphone or tablet).

E. For subjects who enter the SIMPLIFY Master Protocol taking both hypertonic saline and dornase alfa at the time of entry into their first study: Willingness to be randomized to either Study A or Study B.

6.2.2 Demographics

A. Age ≥ 12 years at the Screening Visit.

6.2.3 Disease History

A. Diagnosis of CF.

B. Forced expiratory volume in 1 second (FEV_1) ≥ 70 % predicted at the Screening Visit if < 18 years old, and ≥ 60 % predicted at Screening Visit if ≥ 18 years old.

- After interim analysis, if DMC approves, a separate cohort (lower lung function cohort) of approximately 120 subjects ≥ 18 years old with FEV_1 40 to < 60 % predicted will be enrolled into Study A.

C. Clinically stable with no significant changes in health status within the 7 days prior to and including the Screening Visit.

D. No active smoking or vaping.

E. Has no other conditions that, in the opinion of the Site Investigator/designee, would preclude informed consent or assent, make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives.

6.2.4 Concomitant Medications and Treatments

A. Current treatment with elexacaftor/tezacaftor/ivacaftor (ETI) for at least the 90 days prior to and including the Screening Visit and willing to continue daily use for the duration of the study.

B. Currently taking hypertonic saline (at least 3%) and/or dornase alfa for at least the 90 days prior to and including the Screening Visit and willing to continue daily use for the 2-week screening period.

C. Ability to tolerate albuterol or levalbuterol (Xopenex).

D. No use of an investigational drug within 28 days prior to and including the Screening Visit.

E. No changes to chronic therapy (e.g., ibuprofen, azithromycin, inhaled tobramycin, aztreonam lysine) within 28 days prior to and including the Screening Visit. This includes new airway clearance routines.

F. No acute use of antibiotics (oral, inhaled or IV) or acute use of systemic corticosteroids for respiratory tract symptoms within 7 days prior to and including the Screening Visit.

G. No chronic use of systemic corticosteroids at a dose equivalent to ≥ 10mg per day of prednisone within 28 days prior to and including the Screening Visit.

H. No antibiotic treatment for nontuberculous mycobacteria (NTM) within 28 days prior to and including the Screening Visit.

6.3 Eligibility Criteria at Randomization (Visit 1, Day 0)

Eligibility criteria will be evaluated prior to randomization at Visit 1 (Day 0) for each study.

6.3.1 Consent

A. Is willing and able to adhere to the study visit schedule and other protocol requirements.

6.3.2 Disease History

A. No absolute decrease in FEV_1 % predicted of ≥ 10% between the Screening Visit and Visit 1.

B. Clinically stable with no significant changes in health status between the Screening Visit and Visit 1.

6.3.3 Concomitant Medications

A. No acute use of antibiotics (oral, inhaled or IV) or acute use of systemic corticosteroids for respiratory tract symptoms from the Screening Visit to Visit 1.

B. More than 70% compliance with submission of daily ePRO questionnaires in the up to 13 days prior to Visit 1.

C. Among the daily ePRO questionnaires submitted in the up to 13 days prior to Visit 1, at least 70% adherence with taking ETI and as applicable, hypertonic saline and/or dornase alfa, as reported from Screening to Visit 1.

6.4 Additional Eligibility for Mucociliary Clearance (MCC)

A. Able to perform the testing and procedures required for the study, as judged by the investigator.

B. Able and willing to withhold hypertonic saline and dornase alfa for at least 12 hours prior to each MCC scan at Visits 1 and 3.

C. Those able to become pregnant: negative pregnancy test at Visit 1.

D. Those able to become pregnant: able and willing to practice a medically acceptable form of contraception from three days prior to Visit 1 through Visit 3 (acceptable forms of contraception: hormonal birth control, intrauterine device, barrier method plus a spermicidal agent, or abstinence) unless surgically sterilized or postmenopausal.

E. No more than 2 chest CT scans in the 12 months prior to Visit 1 (or a combination of procedures that are believed to have exposed the subject's lungs to >150 mSv for adults ≥ 18 years old, >15 mSv for children < 18 years old).

6.5 Study Specific Tolerance for Eligibility Criteria

Subjects who fail to meet one or more of the eligibility criteria will not be enrolled into a study. Waivers of any of the above study entry criteria will not be granted.

6.6 Screen Fail Criteria

Any consented subject who is excluded from the study before randomization is considered a screen failure. All screen failures must be documented with the reason for the screen failure adequately stated. If a subject screen fails prior to randomization, they can be rescreened up to two times if the site staff feels they meet eligibility criteria. Rescreened subjects will have to complete all screening procedures (i.e., data from previous screenings cannot be used).

7 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications (and airway clearance routines) throughout the entire study period (i.e., Screening Visit through Visit 3), as medically feasible, with no introduction of new chronic therapies or discontinuation of current chronic therapies except those outlined in the protocol. Subjects that take medications that are cycled (e.g., inhaled antibiotics) should continue to do so by clinical prescription. Medications may be changed between Study A and Study B for those participating in both trials (although subjects must meet all eligibility criteria at the screening visit for the second study including recent stability of their chronic medications).

7.1 Required Medications and Treatments

For each study:

- Subjects must continue ETI from Screening to Visit 3 (week 6).
- Subjects must continue hypertonic saline and/or dornase alfa (as applicable to Study A or Study B) from Screening to Visit 1 (randomization).

7.2 Prohibited Medications and Treatments

Subjects should not change or begin new chronic respiratory therapies, except as defined in this protocol, from the Screening Visit to Visit 3. Subjects should not begin investigational treatments from the Screening Visit to Visit 3.

MCC Sub-study: Subject must withhold hypertonic saline and/or dornase alfa within 12 hours prior to each MCC scan as noted in the Schedule of Events. Subjects randomized to continue with these therapies in either Study A or Study B should resume treatment upon completion of the MCC scan and are encouraged to do so the day of Visit 1 or 3, following the study visit.

8 STUDY TREATMENTS

8.1 Method of Assigning Subjects to Studies and Treatment Groups

At the randomization visit (Visit 1), those subjects who are eligible and taking only either hypertonic saline or dornase alfa will be enrolled in the appropriate study (A or B). If both studies remain open to enrollment, those taking both and enrolling for the first time will be randomized to study (A or B). If only one study is open for enrollment, eligible subjects taking both therapies may be enrolled into only the open study. Those taking both and who are eligible and enrolling for the second time may enroll in the study that they were not previously in if that study remains open for enrollment.

At Visit 1, subjects will be randomized 1:1 to continue or discontinue the applicable therapy. Randomization will occur by a predetermined scheme using block randomization with the goal of ensuring balance between treatment arms as detailed in the Statistical Analysis Plan (SAP). Within each study, randomization will be stratified by Visit 1 FEV_1 % predicted (≥90%, <90%), treatment combination at screening (single or concurrent use of hypertonic saline and/or dornase alfa), prior study participation (yes/no), and age (≥18 vs < 18). Subjects enrolled in the lower lung function cohort will be similarly randomized to continue or discontinue hypertonic saline and stratified by current dornase alfa use. Any deviations from the randomization schema will be documented in the SAP.

Randomization will occur centrally using a web-based randomization system linked to the electronic data capture (EDC) system. Only authorized site personnel are given access to the randomization module in EDC. Authorized site personnel will enter subject eligibility information and whether or not the subject signed informed consent. If the information entered into the EDC system is consistent with eligibility criteria for the study, the system will provide the appropriate authorized site personnel with a randomization assignment for that subject that matches a specific treatment arm.

8.2 Blinding

Each study in the protocol is open label; no blinding to treatment assignment for individual subjects or their care-providers will be performed. However, aggregate study results will be blinded and tightly controlled until the end of each study.

9 STUDY PROCEDURES AND GUIDELINES

The procedures described below will be performed at the visits as noted in the Schedule of Events (Appendix 2).

9.1 Clinical Assessments within each Study

9.1.1 Informed Consent

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject or subject's legal representative. If appropriate, assent must also be obtained prior to conducting any study-related activities.

As part of the consent process, subjects will be asked if they wish to provide contact information and other data collected during SIMPLIFY to researchers from the Success with Therapies Research Consortium (STRC) to assess treatment burden and thoughts about withdrawal studies. This is optional.

9.1.2 Concomitant Medications

All concomitant medications and concurrent therapies will be documented as noted in the Schedule of Events. Dose, route, unit, frequency of administration, indication for administration and dates of medication will be captured. History of modulator use (ETI and other), hypertonic saline, and dornase alfa use will also be captured.

9.1.3 Demographics and CFF Registry Number

Demographic information (date of birth, sex at birth, race, ethnicity) will be recorded. Additionally, the CFF Registry number will be recorded.

9.1.4 Medical History

Relevant medical history, including history of current disease, post ETI sweat test results (if available), other pertinent respiratory history, and information regarding underlying diseases will be recorded.

9.1.5 CF Diagnosis and Genotype

CF diagnostic sweat test date(s) and results will be recorded. CF genotype will be recorded, if known.

9.1.6 Microbiology History

Results of respiratory microbiology cultures during the 12 months prior to Screening will be reviewed and positive cultures for key CF pathogens will be recorded.

9.1.7 Physical Examination

An abbreviated physical examination will be performed by a licensed professional (MD, NP, RN, PA) as noted in the Schedule of Events. The abbreviated exam includes respiratory, cardiovascular, and abdominal assessments.

After Visit 1, new clinically significant abnormal physical exam findings must be documented as AEs.

9.1.8 Weight and Height

Weight will be measured on the same scale and recorded as noted in the Schedule of Events. Adults and children may remain in clothes (without shoes). A standing height will be measured and recorded as noted in the Schedule of Events.

9.1.9 Spirometry

Spirometry will be performed as noted in the Schedule of Events and in accordance with the current American Thoracic Society recommendations for the performance and interpretation of tests.

Two puffs of albuterol or levalbuterol (Xopenex) will be administered 15 minutes to 2 hours prior to spirometry during all study visits. Subjects that have already self-administered albuterol or levalbuterol (Xopenex) within 2 hours prior to spirometry do not need to re-administer; however those that have taken a long-acting bronchodilator (e.g., salmeterol, formoterol as contained in Advair®, Symbicort®, or

similar medications) would be required to administer albuterol or levalbuterol (Xopenex) 15 minutes to 2 hours prior to spirometry at the visit.

Detailed instructions on the order of procedures will be provided in supplemental study materials.

9.1.10 Subject Questionnaires (paper):

- **CRISS and CFQR:** Subjects will complete paper questionnaires (CRISS and CFQR) to assess CF-specific symptoms at the beginning of each visit as noted in the Schedule of Events. These questionnaires include less than 20 questions in total and take less than five minutes to complete.
- **Barriers to Adherence Survey:** Subjects will complete a paper CF-specific survey to ascertain barriers to adherence to their prescribed clinical therapies as noted in the Schedule of Events.
- **Impact of Changing Daily Therapies Questionnaire:** All subjects will be asked to complete a paper questionnaire about their perception of how stopping either hypertonic saline or dornase alfa (or both) would impact their daily lives at Visit 3 (week 6).

9.1.11 Subject Questionnaires (electronic):

- **Daily Study Adherence Questionnaire**: Subjects will be requested to complete a daily electronic questionnaire to document their use of ETI, hypertonic saline, dornase alfa, and mechanical airway clearance therapy. The electronic application will be downloaded to a subject's phone or tablet at the Screening Visit and completed as noted in the Schedule of Events. Information about whether or not a subject has met the eligibility criteria related to percent of completed questionnaires and percent adherence to current treatments will be provided to the study team prior to randomization at Visit 1 to confirm the subject meets the eligibility criteria. After Visit 1, the study team will be able to see if the subject has completed the daily questionnaires, but will not be able to see responses.
- **Monthly Post Study Participation Questionnaire:** All subjects will be asked to complete electronic questionnaires regarding use of ETI, hypertonic saline, dornase alfa, mechanical airway clearance therapy, and antibiotics approximately every 28 days for up to 24 weeks after their final visit (Visit 3, week 6) in each study. These questionnaires may not be administered during the time period of participation in a second study (Screening to Week 6) among those subjects participating in both Study A and Study B. The study team will be able to see if the subject has completed the monthly questionnaires, but will not be able to see responses.

9.1.12 Research Coordinator Review of Electronic Questionnaires

The Site Research Coordinator will review the completion status of the Daily Study Adherence Questionnaire as noted in the Schedule of Events to confirm that the subject has completed the questionnaire(s). If a subject has not completed the questionnaires, Research Coordinators will remind the subject to complete future questionnaires.

9.1.13 Signs and Symptoms Evaluation

Between the Screening Visit and Visit 3 (week 6), whenever a subject initiates a new IV, oral or inhaled antibiotic, the presence of specific signs and symptoms will be extracted from the medical record and documented in addition to physician's assessment of whether the event is a pulmonary exacerbation.

9.1.14 MBW/Multiple Breath Washout Measurement

The Exhalyzer D (Eco Medics, AG) will be used to assess the efficiency of ventilation distribution and gas mixing by measuring the rate of clearance of an inert gas from the lungs in compliance with the Multiple Breath Washout SOP. During the test, subjects will be in an upright seated position and will use a mouthpiece and nose clip. Subjects will be asked to breathe normally and a tight seal must be maintained on the mouthpiece throughout the test. Each test takes about five to 10 minutes. Subjects will do between three and five tests with a 5-minute rest between them. Subjects will be instructed not to drink carbonated beverages for 30 minutes before and between tests as the CO2 may cause inaccurate results. MBW consists of following a tracer gas over two phases, a wash-in and a washout phase. In the wash-in phase, subjects will breathe room air through the mouthpiece connected to the Exhalyzer D. Subjects will be asked to take breaths until O2 concentration on expiration drops to below 17%. After three consecutive breaths in the acceptable CO2 target range are reached, the subjects will start breathing 100% oxygen that comes from a hospital oxygen tank. In the washout phase, subjects will be asked to breathe room air until the N2 concentration is 1/40th of the start concentration for three consecutive breaths.

9.1.15 MCC/Cough Clearance (CC) Measurement

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 [See Protocol Section 6.4]) 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. For each measure of MCC/CC, the subject will inhale an aerosol of sulfur colloid labeled with Tc99m [40 microcuries (µCi)]. Inhalation will occur until ~40 µCi of radioactivity is deposited in the lungs of subjects and takes approximately two minutes. An initial deposition scan will then be recorded and, as the subject remains seated in front of the gamma camera, continuous two-minute images will be recorded for 34 minutes, and then every 10 minutes for an additional 60 minutes to monitor particle clearance from the lung. 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 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 downloaded in dicom format and sent to University of North Carolina, Chapel Hill.

9.1.16 Adverse Events

Information regarding occurrence of AEs will be captured from randomization through Visit 3. Duration (start and end dates), grade, seriousness, outcome, treatment, and relation to study arm treatment will be recorded on the case report form (CRF).

In addition, any overnight admissions to the hospital that occur between randomization and Visit 3 will be captured on the CRF.

9.2 Clinical Laboratory Measurements

9.2.1 Pregnancy Test (MCC Sub-Study Only)

For those participating in the MCC Sub-study, urine or blood (approximately 2 cc) will be collected from those able to become pregnant for a pregnancy test as noted in the Schedule of Events and tested in clinic according to site standard procedures.

10 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

10.1 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 Table 1 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.

Table 1. AE Severity Grading

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 Treatment Arm Assignment

The relationship of an AE to the subject's treatment arm assignment should be assessed using the following guidelines in Table 2.

Table 2. AE Relationship to Treatment Arm Assignment

Relationship to Treatment Arm Assignment	Comment
Definitely	An event that occurs follows a reasonable temporal sequence after randomization to a treatment arm that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence after randomization to a treatment arm; 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 randomization to a treatment arm; 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 the treatment arm assignment.

10.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring in either treatment arm assignment 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.

10.2.1 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 3) have been completed.

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

- Email (scanned copy) to: cfsaesfacsys@seattlechildrens.org
- TDNCC SAE Fax: (206) 985-3278

The site will notify the TDNCC of additional information or follow-up to an initial SAE Report as soon as relevant information is available. The TDNCC Medical Monitor 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.

10.3 Medical Monitoring

The TDNCC Medical Monitoring Group should be contacted directly at this number to report medical concerns or questions regarding safety:

- (800) 341-0961

11 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

11.1 Early Withdrawal of Subjects from a Study

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for early subject withdrawals. The reason for the subject's early withdrawal from the study will be specified in the subject's source documents. Subjects who withdraw early from the study (prior to Visit 3) should be encouraged to come in for a final early study withdrawal visit (and the procedures to be followed would include those at Visit 3).

Subjects who for any reason modify their hypertonic saline or dornase alfa treatment such that it deviates from their assigned treatment regimen within a study will not be withdrawn from the study and are encouraged to complete all scheduled study visits.

11.2 Replacement of Subjects

Subjects who withdraw from the study will not be replaced.

12 PROTOCOL VIOLATIONS

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
- Subject did not follow their randomization assignment

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Sponsor will determine if a protocol violation should result in early permanent discontinuation of study treatment for a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor representative and the Site Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files. The site will report the violation to their IRB in accordance with their IRB reporting requirements.

13 DATA SAFETY MONITORING

The CFF Data Safety Monitoring Board (DSMB) will establish a DMC to review data relating to safety, to conduct and review interim analyses, and to ensure the continued scientific validity and merit of the study, according to the CFF DSMB Operations Manual and a DMC Charter created for this protocol. Further details regarding the interim reviews will be fully specified in the DMC charter and approved by the study appointed DMC.

14 STATISTICAL METHODS AND CONSIDERATIONS

14.1 General Considerations

Prior to the analysis of the final study data, a detailed SAP will be written, describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

For the purposes of analyses, baseline will be defined as Visit 1 (Day 0). All estimates of differences between treatment arms will be reported with corresponding 95% confidence intervals, and unless otherwise noted, statistical tests when performed will be two-sided and determined at the 0.05 level. Missing data methods for the primary and key secondary endpoints will be described in the SAP. No adjustment for multiple comparisons will be made beyond group sequential methods for interim monitoring.

Each study will be analyzed independently as specified below, with select exploratory analyses performed with data pooled across studies.

14.1.1 Data Sets Analyzed

The intent to treat (ITT) population, defined as all subjects randomized at Visit 1 (Day 0), will be used for all subject disposition, secondary, exploratory, and safety summaries.

The per-protocol analysis (PPA) population, defined as all subjects who meet criteria as specified in the SAP, will be used for the primary analyses in both Study A and Study B, with sensitivity analyses repeated on the ITT population. Select secondary analyses will also be run on the PPA population.

Demographic and Baseline Characteristics

Treatment arms within Study A and Study B will be descriptively summarized with respect to baseline demographic and clinical characteristics including sex at birth, CFTR genotype, race, height, weight, and all randomization strata.

14.2 Analysis of Primary Endpoint

The primary hypothesis for both Study A and Study B is that discontinuing therapy is non-inferior to continuing therapy, as measured by the 6-week change in FEV_1 % predicted. The primary endpoint for each study is the difference between arms in the change in FEV_1 % predicted from randomization to

week 6. The primary analysis for non-inferiority will be conducted on the PPA population. A linear model will be used to adjust for randomization strata as specified in Section 8.1. The estimated effects of discontinuation and corresponding 95% confidence intervals will be reported for both studies A and B, and p-values will be evaluated for a one-sided alpha-level 0.025 test of non-inferiority with a margin of -3% absolute change in FEV_1 % predicted for each study.

The lower lung function cohort, if enrolled, will not be included in the primary analysis, but will be analyzed as a separate cohort for which the primary and secondary analysis within this cohort will focus on the evaluation of safety. Further details will be provided in the SAP.

14.3 Analysis of Secondary Endpoints

Differences between treatment arms will be estimated within each study for i) subjects initiating acute antibiotics, ii) subjects hospitalized, iii) subjects with a pulmonary exacerbation, and iv) subjects who meet adherence criteria. These differences will be estimated using logistic regression methods and reported with confidence intervals accounting for randomization strata.

Differences in the between treatment arms from randomization to week 6 will be estimated in each trial for i) CRISS, ii) CFQR, and iii) LCI using general linear regression adjusting for randomization strata. Change in FEV_1 % predicted from Screening to randomization will also be compared between treatment arms within each study using linear regression adjusting for randomization strata. Descriptive summaries of the change from Screening to randomization, and randomization to week 2 will also be provided for all continuous endpoints.

14.4 Analysis of Safety Endpoints

All reported SAEs and AEs will be coded using MedDRA and grouped by body system. SAEs and AEs will be tabulated by treatment arm using standard coding terms sorted by body system. The incidence of AEs in each treatment arm will be tabulated by seriousness, severity, and relationship to treatment arm assignment. If an AE is reported more than once during the study period for a given subject, the greatest severity and the worst-case relationship will be presented in tables.

The number of SAEs and AEs will be summarized for each treatment arm as follows: (i) The proportion of subjects with at least one (S)AE, (ii) The average number of (S)AEs per subject, and (iii) The rate of (S)AEs per subject week of follow-up. Histograms showing the frequency of the number of (S)AEs in each treatment group will be included. Rates of (S)AEs by System Organ Class (SOC) will be presented by treatment group. Poisson regression modeling will be used to derive rate ratios and 95% CIs for each SOC. The rate ratios will be compared using a two-sided 0.05 level test for Poisson count data.

In addition to AEs, the proportion of subjects temporarily or permanently changing their assigned therapy will be summarized.

14.5 Analysis of Exploratory Objectives

Differences between treatment arms in the change in MCC from randomization to week 6 will be estimated using general linear regression adjusting for randomization strata and reported with associated confidence intervals. The average impact score on subject perception of how stopping HS or dornase alfa (or both) would impact daily life will be summarized at week 6 by treatment arm.

Among those enrolled in the lower lung function cohort, descriptive safety summaries will be provided for the differences between treatment arms in the change in FEV_1 % predicted, AE rates, and proportion

of subjects re-initiating therapy in the discontinuation arm. Treatment arms will be formally compared within this cohort as further specified in the SAP to determine if discontinuation of therapy results in clinically meaningfully inferior outcomes as compared to remaining on therapy.

Among those who are currently taking both hypertonic saline and dornase alfa at study entry, exploratory analysis will be performed pooling data across studies. Data from the arms randomized to remain on therapy in both Study A and Study B will be pooled to form a single comparator arm of subjects on both hypertonic saline and dornase alfa across trials. This comparator arm will be compared separately to the subgroups of subjects in each study randomized to discontinue therapy using the same methodology as for the primary objective.

The proportions of subjects remaining on and off hypertonic saline and dornase alfa for up to 24 weeks after completion of each study will be descriptively summarized. The odds of staying with the same regimen as assigned during the previously completed study will be modeled using logistic regression for each treatment arm with covariates derived from both baseline and clinical outcome data during the study. Data across studies may be pooled into the same model and repeated measures methodology utilized. Similar methodology will be used to summarize and model the proportion of subjects with acute antibiotic use after completion of each study.

14.6 Interim Analysis

A pre-defined monitoring plan for safety and study stopping rules will be formalized in conjunction with the DMC and will be in place prior to initiating the study. On each study, a formal interim evaluation of harm associated with discontinuing therapy will be conducted as detailed in the DMC charter.

Each study (A and B) will have separate monitoring evaluations for safety and a decision to stop; one will not be conditional on the other.

14.7 Sample Size

The primary endpoint for each study is the difference between arms in the change in FEV_1 % predicted from randomization to week 6. Data from previous studies were used to estimate the standard deviation of change in FEV_1 % predicted, resulting in an estimate of 8.4% per group (4,5).

It is anticipated from prior CF trials conducted through the TDNCC that the attrition and non-adherence rate will be less than 20% and thus it is reasonable to expect that a total sample size of 400 per study will enable at least 308 subjects to complete the trial and be included in the PPA population. The PPA population will be the primary analysis population for test of non-inferiority.

A total sample size of 308 provides 88% power to detect non-inferiority with a margin of -3% absolute FEV_1 % predicted when there is truly no effect of discontinuation. It provides 74% power when, in truth, discontinuation on average decreases FEV_1 % predicted by 0.5% absolute. These results were based on a simplified conservative analysis that did not include covariate adjustment.

If the DMC approves enrollment of a lower lung function cohort, approximately 60 per arm in Study A will be recruited for exploratory analyses focused on the safety of discontinuation of hypertonic saline.

15 DATA COLLECTION, RETENTION AND CLINICAL MONITORING

15.1 Data Collection Instruments

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 electronic CRF when the information corresponding to that visit is available. 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.

If a correction is required for a CRF, the time and date stamp tracks the person entering or updating CRF data and create an electronic audit trail.

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. At the completion of the study, a copy of the CRF data will be provided to the site to be retained at the Investigator's site.

MBW Assessment: Subjects who are participating in the MBW portion of the SIMPLIFY study will be assigned the same unique identification number as the main study. Sites will provide MBW measurements to the MBW Core Center at the University of Toronto via a secure file transfer application after each study visit for over-reading and quality control. Data transfer details from the sites to the University of Toronto will be provided in the MBW SOP. The MBW Core Center will send over-read MBW data via a secure file transfer application to the TDNCC for analysis.

MCC Sub-Study: Subjects who are participating in the MCC sub-study will keep the same unique identification number as the main study. The MCC images will not be entered into the CRF but will be provided directly by participating sites to the MCC sub-study principal investigator for evaluation and analysis via secure mail or electronic transfer. The data files will be kept on a password protected computer and routinely backed up. At the end of the study, the sub-study principal investigator will provide relevant sub-study data to the TDNCC to be incorporated into the overall study database. The data will be transferred via a secure file transfer application.

Data transfer from TDNCC to MCC sub-study Lead Investigator and STRC Investigators: At varying intervals during the study, the TDNCC will provide both the MCC sub-study lead investigator and the STRC ancillary study investigators (See section 9.1.1) with relevant SIMPLIFY CRF data for subjects who provide consent for the additional research to enable the sub-study/ancillary study specific analyses. The data will be transferred via a secure file transfer application. Details of the data transfer (including, but not limited to, frequency of transfer, format of data and query process) will be documented in a Data Transfer Agreement between the sub-study/ancillary study investigator institutions and the TDNCC. Before data are released to these investigators, documentation of IRB approval of their investigation must be provided to the TDNCC.

15.2 Data Management Procedures

TDNCC utilizes Medidata Solutions, Inc. (Medidata) Rave® for their EDC studies. The Medidata Rave EDC system is designed to be US Code of Federal Regulations (CFR) 21 Part 11 compliant, with a robust audit trail system and electronic signature capabilities. Study personnel at each site will enter data from a

subject's visit onto electronic CRF screens via a web browser. Study subjects will not be identified by name in the study database or on any data capture screens but will be identified by a unique subject identification number. Only study personnel at the individual sites will be able to link the study ID to the subject's name. TDNCC also utilizes the Medidata Rave eCOA/ePRO system, a regulatory compliant system which allows subjects in a study using Medidata Rave EDC to complete and submit forms and data for patient-reported outcomes electronically on a mobile device to the Medidata Rave EDC System. Study personnel at each site will register subjects using their unique subject identification number which generates an activation code unique to that subject. Study site personnel provide the subjects with their activation code. The subject downloads the Medidata Rave eCOA/ePRO app to their mobile device and uses their unique activation code to create their ePRO login and password.

The Biostatistics and Clinical Data Management group of the TDNCC will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed. All procedures for the handling and analysis of data will be conducted using good computing practices for the handling and analysis of data for clinical trials.

Data Quality Control and Reporting

After data have been entered into the study database, data validation checks will be applied on a regular basis. Queries are entered, tracked, and resolved through the EDC system directly. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented in an audit trail.

15.3 Security and Archival of Data

The EDC and Rave eCOA/ePRO systems are hosted by Medidata; the data are stored at Medidata's primary data center in Houston, Texas, with fail-safe data centers in New Jersey. Data are regularly backed up by Medidata.

Medidata maintains 21 CFR Part 11-compliant electronic systems, with procedures in place to safeguard against unauthorized acquisition of data. Any authorized communication with the Medidata servers at the Houston Data Center is conducted via SSL (128-bit) encryption. Robust password procedures, consistent with 21 Part 11, are in place. Robust physical security procedures are in place at the Houston Data Center to prevent unauthorized personnel physical access to the server rooms. EDC account access is maintained and monitored by the Biostatistics and Clinical Data Management group of the TDNCC.

Other databases will be stored on Seattle Children's servers and are safeguarded against unauthorized access by established security procedures. Network accounts are password protected and maintained and monitored by Seattle Children's. Data is backed up regularly according to the Information Services group's procedures.

15.4 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed informed consent, HIPAA Authorization and assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (e.g., patient files, signed informed consent forms, copies of CRFs, Essential Document and Study Reference Binders) must be kept secured for a period of two years after database lock. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

15.5 Monitoring

By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation. Monitoring visits will be conducted by representatives of the Sponsor according to the U.S. CFR 21 Part 312 and International Conference on Harmonisation (ICH) Guidelines for GCP (E6) and to ensure investigator compliance to 21 CFR Parts 50, 56 and 312 and to GCP.

15.6 Subject Confidentiality

In order to maintain subject confidentiality, only a site number and subject number will identify all study subjects on CRFs and other documentation submitted to the Sponsor. The subject's CFF Registry number will also be collected. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

16 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

This master 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).

To maintain confidentiality, all evaluation forms, reports and other records will be identified only by a coded number. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

16.1 Protocol Amendments

Any amendment to the protocol will be written by the Sponsor. Protocol amendments cannot be implemented without prior written IRB approval except as necessary to eliminate immediate safety hazards to subjects. A protocol amendment intended to eliminate an apparent immediate hazard to subjects may be implemented immediately, provided the IRBs are notified within five working days.

16.2 Institutional Review Boards

The protocol and consent forms will be reviewed and approved by the IRB on record prior to study initiation. SAEs regardless of relationship to treatment arm assignment 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.

16.3 Informed Consent Form

Informed consent will be obtained for each study in accordance with the Declaration of Helsinki, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), HIPAA (if applicable), and local regulations.

The Investigator will prepare the informed consent form and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB. The written consent document will embody the elements of informed consent as described in the ICH GCP and will also comply with local regulations. The Investigator will send an IRB-approved copy of the informed consent form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into a study. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the IRB, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

During the course of the study, if modifications are made to the consent form that impact the subject, the subject will be re-consented as described above.

16.4 Consent for Collection and Use of CFF Registry Number

To facilitate possible future evaluation of retrospective and prospective information from all patients who screen for this study, the subject's CFF Registry number will be collected. The CFF Registry collects data on all CF patients who consented to participate in the CFF Registry and who are followed at CFF-accredited care centers. The Registry data includes information from clinical encounters, hospitalizations courses of antibiotics, and year-end surveys. Data also include microbiology results,

spirometry results, CF genotype and other information. The patient's CFF Registry number will be recorded in the CRF.

16.5 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written 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.

16.6 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the studies in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the studies.
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs that occur in the course of the studies, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the studies are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the master protocol.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

17 REFERENCES

1. Rowe SM, Heltshe SL, Gonska T, Donaldson SH, Borowitz D, Gelfond D, Sagel SD, Khan U, Mayer-Hamblett N, Van Dalfsen JM, Joseloff E, Ramsey BW, Network GIotCFFTD. Clinical mechanism of the cystic fibrosis transmembrane conductance regulator potentiator ivacaftor in G551D-mediated cystic fibrosis. *Am J Respir Crit Care Med* 2014; 190: 175-184.
2. Ramsey BW, Davies J, McElvaney NG, Tullis E, Bell SC, Drevinek P, Griese M, McKone EF, Wainwright CE, Konstan MW, Moss R, Ratjen F, Sermet-Gaudelus I, Rowe SM, Dong Q, Rodriguez S, Yen K,

Ordonez C, Elborn JS, Group VXS. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. *N Engl J Med* 2011; 365: 1663-1672.
3. Gifford AH, Mayer-Hamblett N, Pearson K, Nichols DP. Answering the call to address cystic fibrosis treatment burden in the era of highly effective CFTR modulator therapy. *J Cyst Fibros* 2019.
4. Heijerman HGM, McKone EF, Downey DG, Van Braeckel E, Rowe SM, Tullis E, Mall MA, Welter JJ, Ramsey BW, McKee CM, Marigowda G, Moskowitz SM, Waltz D, Sosnay PR, Simard C, Ahluwalia N, Xuan F, Zhang Y, Taylor-Cousar JL, McCoy KS, Group VXT. Efficacy and safety of the elexacaftor plus tezacaftor plus ivacaftor combination regimen in people with cystic fibrosis homozygous for the F508del mutation: a double-blind, randomised, phase 3 trial. *Lancet* 2019.
5. Middleton PG, Mall MA, Drevinek P, Lands LC, McKone EF, Polineni D, Ramsey BW, Taylor-Cousar JL, Tullis E, Vermeulen F, Marigowda G, McKee CM, Moskowitz SM, Nair N, Savage J, Simard C, Tian S, Waltz D, Xuan F, Rowe SM, Jain R, Group VXS. Elexacaftor-Tezacaftor-Ivacaftor for Cystic Fibrosis with a Single Phe508del Allele. *N Engl J Med* 2019; 381: 1809-1819.
6. Eisenberg JD, Aitken ML, Dorkin HL, Harwood IR, Ramsey BW, Schidlow DV, Wilmott RW, Wohl ME, Fuchs HJ, Christiansen DH, Smith AL. Safety of repeated intermittent courses of aerosolized recombinant human deoxyribonuclease in patients with cystic fibrosis. *J Pediatr* 1997; 131: 118-124.
7. Davies J, Sheridan H, Bell N, Cunningham S, Davis SD, Elborn JS, Milla CE, Starner TD, Weiner DJ, Lee PS, Ratjen F. Assessment of clinical response to ivacaftor with lung clearance index in cystic fibrosis patients with a G551D-CFTR mutation and preserved spirometry: a randomised controlled trial. *Lancet Respir Med* 2013; 1: 630-638.
8. Donaldson SH, Bennett WD, Zeman KL, Knowles MR, Tarran R, RC B. Mucus clearance and lung function in cystic fibrosis with hypertonic saline. *N Engl J Med* 2006: 241-250.
9. Elkins MR, Robinson M, Rose BR, Harbour C, Moriarty CP, Marks GB, Belousova EG, Xuan W, Bye PT, National Hypertonic Saline in Cystic Fibrosis Study G. A controlled trial of long-term inhaled hypertonic saline in patients with cystic fibrosis. *N Engl J Med* 2006; 354: 229-240.
10. Amin R, Subbarao P, Lou W, Jabar A, Balkovec S, Jensen R, Kerrigan S, Gustafsson P, Ratjen F. The effect of dornase alfa on ventilation inhomogeneity in patients with cystic fibrosis. *Eur Respir J* 2011; 37: 806-812.
11. Amin R, Subbarao P, Jabar A, Balkovec S, Jensen R, Kerrigan S, Gustafsson P, Ratjen F. Hypertonic saline improves the LCI in paediatric patients with CF with normal lung function. *Thorax* 2010; 65: 379-383.
12. Donaldson SH, Laube BL, Corcoran TE, Bhambhvani P, Zeman K, Ceppe A, Zeitlin PL, Mogayzel PJ, Jr., Boyle M, Locke LW, Myerburg MM, Pilewski JM, Flanagan B, Rowe SM, Bennett WD. Effect of ivacaftor on mucociliary clearance and clinical outcomes in cystic fibrosis patients with G551D-CFTR. *JCI Insight* 2018; 3.

APPENDIX 1. LIST OF ABBREVIATIONS AND ACRONYMS

AE	adverse event
CC	cough clearance
CF	cystic fibrosis
CFF	Cystic Fibrosis Foundation
CFQR	Cystic Fibrosis Questionnaire-Revised
CFR	Code of Federal Regulations
CFTR	cystic fibrosis transmembrane conductance regulator
CRF	case report form
CRISS	Change in Chronic Respiratory Infection Symptom Score
CT	computed tomography
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
eCOA/ePRO	electronic Clinical Outcome Assessment/ electronic Patient Reported Outcome
EDC	electronic data capture
ETI	elexacaftor/tezacaftor/ivacaftor
FDA	Food and Drug Administration
FEV_1	forced expiratory volume over one second
GCP	Good Clinical Practice
HEMT	highly effective modulator therapy
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
ITT	intent to treat
LCI	lung clearance index
MBW	Multiple Breath Washout
MCC	Mucociliary Clearance
PPA	per-protocol analysis
PI	Principal Investigator
RC	Research Coordinator
SAE	serious adverse experience
SOC	System Organ Class
SAP	statistical analysis plan
STRC	Success with Therapies Research Consortium
TDNCC	Therapeutics Development Network Coordinating Center

APPENDIX 2. SCHEDULE OF EVENTS FOR EACH STUDY

The following table shows the required activities at each study visit.

	Screening		Treatment			Follow-Up Questionnaires
	Screening Visit (Day -21 to -14)	Visit 1 Randomization (Day 0)	Visit 2 Week 2 (Day 14 ± 3 Days)	Call Week 4 (Day 28 ± 3 Days)	Visit 3/EW Week 6 (Day 42 ± 3 Days)	6 total Questionnaires (1 every 28 days for 24 weeks) *
History & Interviews & PROs						
Informed Consent - Optional Consent for STRC future contact	X					
Review Eligibility	X	X				
CF Diagnosis	X					
12 month Microbiology History [a]	X					
Collect Conmed History: ETI, hypertonic saline, dornase alfa, previous modulator use	X					
Post-ETI Sweat Test Results (if available) [b]	X					
Collect Medical History, Demographics, CFF ID	X					
Concomitant Medication Review	X	X	X	X	X	
Adverse Events Review			X	X	X	
Hospitalization Review	X	X	X	X	X	
RC to help subject download mobile app for: - Daily Study Adherence Questionnaire - Monthly Post Study Participation Questionnaire	X					
Subject completes the Daily Study Adherence Questionnaire (electronic)	START	Completed by subjects daily			STOP	
RC to review subject's Daily Study Adherence Questionnaire		X	X	X	X	
CRISS + CFQR (paper)	X	X	X		X	
Barriers to Adherence Survey (paper)	X					
Impact of Changing Daily Therapies Questionnaire (paper)					X	
Monthly Post Study Participation Questionnaire (electronic)						X [c]

	Screening		Treatment			Follow-Up Questionnaires
	Screening Visit (Day -21 to -14)	Visit 1 Randomization (Day 0)	Visit 2 Week 2 (Day 14 ± 3 days)	Call Week 4 (Day 28 ± 3 days)	Visit 3/EW Week 6 (Day 42 ± 3 days)	6 total Questionnaires (1 every 28 days for 24 weeks) *
Local Procedures and Tests						
Height	X					
Weight	X	X	X		X	
Abbreviated Physical Exam	X	X				
Spirometry [d]	X	X	X		X	
Randomization to Study A or Study B [e]		X				
Randomization to continue or discontinue Hypertonic Saline or Dornase Alfa (as applicable to Study A or Study B)		X				
Sign and Symptoms Assessment	START	Completed by RC/PI any time antibiotics are started during the study			STOP	
Multiple Breath Washout Procedure [f]	X	X			X	
MCC SUB-STUDY						
Remind Subject of Study Requirements Pre-MCC Test [g]	X			X		
Pregnancy Test [h]		X			X	
Confirm Eligibility [i]		X			X	
MCC Transmission Scan [j]		X			X	
Perform MCC Procedure		X			X	

[a] Presence of key CF pathogens from respiratory samples during the 12 months prior to screening will be recorded.

[b] Data entry of post ETI sweat result, if available.

[c] Performed every 28 days for 24 weeks following Visit 3 (Day 42). Note: Questionnaires may be stopped if subject enrolls into second study within 6 months.

[d] All spirometry testing should be performed between 15 min to 2 hours post-bronchodilator administration.

[e] Only subjects taking both hypertonic saline and dornase alfa.

[f] Will be done at a sub-set of centers with the capability of performing the MBW procedure.

[g] Subjects participating in MCC sub-study will be contacted prior to the visit in order to be reminded about MCC sub-study requirements (i.e., discontinue use of excluded medications, maintained use of contraception for those able to become pregnant, if applicable).

[h] Only those able to become pregnant.

[i] Before performing MCC measurement, confirm subject meets the criteria to perform the test, subject has held the required medications, those able to become pregnant are not pregnant and have used an acceptable form of contraception for 3 days prior to Visit 1 through Visit 3.

[j] A transmission scan is performed prior to the MCC procedure.