Study Title: Functional Studies of Novel Genes Mutated in Primary Ciliary Dyskinesia II: Genotype to Phenotype

NCT number NCT04901715
Document Date 02/08/2023

Study Title: Functional Studies of Novel Genes Mutated in Primary Ciliary Dyskinesia II: Genotype to Phenotype

UNC Principal Investigator (PI): Lawrence E. Ostrowski

UNC PI Department/Division: Pediatrics/Pulmonary

Number of Sites: 1

Lead Site: Meadowmont/Eastowne

Lead Site Principal Investigator: William Bennett, PhD

Additional Site: USEPA Human Studies Facility

UNC Co-Investigator(s) and Department/Division

William Bennett, PhD; Medicine/Pulmonary

Margaret Leigh, MD; Pediatrics/Pulmonary

Michael Knowles, MD; Medicine/Pulmonary

Sara Abu-Nasser, DO; Pediatrics/Pulmonary

Peadar Noone, MD; Medicine/Pulmonary

Study Statistician

Agathe Ceppe
Marsico Hall Room 2204,
125 Mason Farm Road
Chapel Hill, NC 27599

Sponsor(s) N/A

Source(s) of Funding : NIH; NHLBI

I HAVE READ AND APPROVE THIS VERSION OF THE PROTOCOL.

[electronic signature accepted]

Principal Investigator: Laurence E. Ostrowski **Date:** 08/Feb/2023

Statistical Co-investigator: Agathe Ceppe Date: 1/26/2021

Table of Contents

Statement of Compliance

This study will be conducted in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6) and the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46). The statistical analysis plans will be consistent with guidances such as the CONSORT Statement [1] or STROBE Statement [2], ICMJE recommendations [3], the 2016 and 2019 statements of the American Statistical Association [4,5], and recommendations in Nature [6,7].[1] All personnel involved in the conduct of this study have completed human subjects protection training.

[1] [1] www.consort-statement.org [2] www.strobe-statement.org [3] www.icmje.org [4] Wasserstein RL, et al. (2016), The ASA's Statement on p-Values, ***The American Statistician***, 70:2, 129-133 [5] Wasserstein RL, et al. (2019), Moving to a World Beyond p < 0.05, ***The American Statistician***, 73:sup1, 1-19 [6] Amrhein, et al. (2019) Scientists rise up against statistical significance, ***Nature*** 567, 305-307 [7] Editorial (2019) It's time to talk about ditching statistical significance: Looking beyond a much used and abused measure would make science harder, but better. ***Nature*** 567, 283-283.

Abbreviations and Definitions of Terms

Abbreviation/Acronym	Definition
AE	Adverse Event/Adverse Experience
AED	Automated External Defibrillator
ATS	American Thoracic Society
Ave60Clr	Average Clearance at 60 minutes
Ave120Clr	Average Clearance at 120 minutes
BP	Blood Pressure
CC	Cough Clearance
CEMALB	Center for Environmental Medicine, Asthma and Lung Biology
CF	Cystic Fibrosis
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
Co57	Cobalt 57
CONSORT	Consolidated Standards of Reporting Trials
C/P	Central/Peripheral
CRF	Case Report Form
CRO	Contract Research Organization
CTCAE	Common Terminology Criteria for Adverse Events
DSMB	Data and Safety Monitoring Board
EMS	Emergency Medical Services
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
FEF 25-75	Maximal Mid-Expiratory Flow Rate
FEV1	Forced Expiratory Volume in one second
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICMJE	International Committee of Medical Journal Editors
IRB	Institutional Review Board
IUD	Intra Uterine Device

HR	Heart Rate
KeV	Kilo electron volts
LAIV	Live Attenuated Influenza Vaccine
N	Number (typically refers to subjects)
MCC	Mucociliary Clearance
mCi	Millicurie
MDI	Metered Dose Inhaler
MMAD	Mass Median Aerodynamic Diameter
NHLBI	National Heart, Lung and Blood Institute
NIH	National Institute of Health
PE	Physical Exam
PEF	Peak Expiratory Flow
PFT	Pulmonary Function Test
PHI	Protected Health Information
PI	Principal Investigator
RR	Respiratory Rate
QA	Quality Assurance
QC	Quality Control
SABA	Short Acting Beta Agonist
SAE	Serious Adverse Event/Serious Adverse Experience
SD	Standard Deviation
SE	Standard Error
SOP	Standard Operating Procedures
SpO2	Peripheral capillary oxygen saturation
STROBE	STrengthening Reporting of OBservational studies in Epidemiology
Tc99m-SC	Technetium99m - Sulfur colloid
uCi	Microcurie
UP	Unanticipated Problem

1. Protocol Synopsis

Study Title	Functional Studies of Novel Genes Mutated in Primary Ciliary Dyskinesia II: Genotype to Phenotype
Objectives	**Primary** To measure mucociliary clearance (MCC) in subjects with primary ciliary dyskinesia (PCD) caused by mutations in genes associated with a milder clinical phenotype and subjects with PCD caused by mutations in genes associated with a more severe clinical phenotype. To compare the 2 groups with each other and with healthy individuals. **Secondary** To measure the effect of a short acting beta adrenergic (SABA) on MCC in in subjects with PCD caused by mutations in genes associated with a milder clinical phenotype and subjects with PCD caused by mutations in genes associated with a more severe clinical phenotype. **Tertiary/Exploratory** To measure cough clearance in subjects with PCD caused by mutations in genes associated with a milder clinical phenotype and subjects with PCD caused by mutations in genes associated with a more severe clinical phenotype.
Target Population	The target population is patients with PCD having confirmed mutations in genes associated with a milder clinical phenotype and subjects with PCD caused by mutations in genes associated with a more severe clinical phenotype. Subjects for this study will be recruited based on genotype. **Key Inclusion Criteria** **PCD Patients** 1. PCD subjects with mutations in genes associated with a milder clinical phenotype. This includes subjects with 2 confirmed mutations in *RSPH1*, *RSPH9*, *RSPH4a*, and *DNAH11*.This group may also include subjects with mutations in newly identified genes that are associated with a milder clinical phenotype. OR PCD subjects with mutations associated with a more severe clinical phenotype. This includes subjects with 2 confirmed mutations in *DNAH5*, *DNAI1*, *CCDC39*, and *CCDC40*.This group may also include subjects with mutations in newly identified genes that are associated with a more severe clinical phenotype. 2. Age 12 - 90 years old 3. Subjects of childbearing potential must be non-pregnant and non-lactating. **Healthy Controls** 1. ≥ 18 years old 2. No pre-existing lung disease (asthma, cystic fibrosis, etc.) 4. Subjects of childbearing potential must be non-pregnant and non-lactating.

	Key Exclusion Criteria 1. Clinically severe disease (requiring oxygen; FEV1<30%) 2. Currently smoking or vaping 3. Radiation exposure in the past year that would cause them to exceed federal radiation safety guidelines 4. Free of sino-nasal pulmonary exacerbation for 4 weeks.
Numbers of Participants	**Number to be recruited for screening** : 40 **Number of eligible participants enrolled**: 32
Clinical Phase	I
Intervention	Short acting beta agonist inhaler (albuterol by metered dose inhaler)
Study Description	Participants will inhale aerosolized radiolabeled sulfur colloid (Tc99m-SC) and will undergo gamma scintigraphy to measure mucociliary and cough clearance (MCC/CC) of the radiolabeled particles from the airways. During the first hour of MCC imaging subjects will attempt to avoid coughing. Subjects will then inhale 4 puffs of albuterol (90 mcg/puff via metered dose inhaler (MDI) with spacer) to assess the effect of beta-adrenergic stimulation on MCC over the second hour of MCC imaging. After the two hours of MCC imaging. Subjects will perform spirometry and directed coughing over a 30-minute period during which CC will be measured, again by gamma scintigraphy. Subjects will then rest (approximately 3.5 hours) and be scanned for 15 minutes at 6 hour post initial MCC/deposition scan.
Outcome Measures	Baseline MCC: Ave60Clr Change in MCC following albuterol: Ave120Clr-Ave60Clr Change in MCC following cough clearance: Ave150Clr-Ave120Clr
Study Duration	The entire study is expected to last up to 4 years.
Subject Participation Duration	Each subject's participation will last 1 day.
Estimated Time to Complete Enrollment	4 years
Statistical Analysis Plans	Aim 1 will provide estimates of the mean and standard deviation of baseline MCC (Ave60Clr) for each group of PCD subjects. The 2 disease groups will be compared to data from healthy subjects using ANOVA followed by multiple comparisons, using the Tukey Kramer correction. Age, gender and the initial deposition C/P will be added as covariates to PCD groups in a linear regression model on MCC. In Aim 2, the change pre- to post-albuterol (Ave120Clr-Ave60Clr) will be calculated for each subject. Estimates of the mean and standard deviations will be reported for each group and compared with paired t-tests. The difference of change between the 2 groups will first be analyzed with an independent t-test. Age, gender and C/P will then be added as covariates in linear regression model.

	In Aim 3, the change pre- to post-cough clearance (Ave150Clr-Ave120Clr) will be calculated for each subject. Estimates of the mean and standard deviations will be reported for each group and compared with paired t-tests. The difference of change between the 2 groups will be analyzed with an independent t-test. Age, gender and C/P will then be added as covariates in linear regression model. All tests will be 2-sided, with α=0.05. Tests with p values above 0.05 will be considered inconclusive.

2. Introduction: Background and Scientific Rationale

2.1. Background Information

Primary Ciliary Dyskinesia (PCD) is a heterogeneous genetic disease that results in impaired function of the cilia [1]. Pulmonary MCC is dependent on airway secretory cells and submucosal glands and ciliated cells to propel mucus upwards through the airways. Rates of MCC are dependent on ciliary beat frequency, hydration, and the rheologic properties of mucus [2]. Abnormalities in ciliary function causes reduction in mucociliary clearance (MCC), which contributes to mucus plugging and worsening of lung function. Due to cilia dysfunction, mucociliary clearance (MCC) and cough clearance (CC) are affected in PCD. PCD varies in phenotypic presentation and ranges from mild to severe loss of lung function. PCD patients develop airway obstruction and bronchiectasis as disease advances. While some patients present with bronchiectasis in childhood, virtually all adult patients have bronchiectasis [3]. PCD is a progressive disease causing a range of respiratory disease from poor lung function to chronic respiratory failure requiring lung transplant [3].

As more genetic mutations are discovered, milder phenotypes have been associated mutations in certain genes. For example, it has been reported that individuals with mutations in *RSPH1* (radial spoke head homolog 1) have a milder presentation than other genes [4]. Individuals with *RSPH1* have a higher nasal nitric oxide value, decreased incidence of neonatal respiratory distress and higher FEV1 when compared to subjects with mutations in genes encoding outer dynein arm structures. In addition, cilia from subjects with mutations in *RSPH1* can have normal appearing structure and maintain an almost normal ciliary beat frequency, although they often beat in a circular pattern [5]. In contrast, mutation in other genes have been associated with a more severe clinical phenotype. For example, subjects with mutations in *CCDC39* or *CCD40* were significantly younger at diagnosis and had significantly lower percent predicted FEV1 than subjects with mutations in other genes [6].

Currently, there are no treatments that have been clinically approved specifically for PCD. In other disease processes, albuterol has been reported to improve mucociliary clearance. Laube et al reported decreased MCC in patients after lung transplant which significantly improved with administration of albuterol [7]. Additionally, beta agonists have been reported to improve ciliary beat frequency in human bronchial epithelial cells in vitro and in certain animal models [8-9]. The effect of beta agonists on MCC or CC has not been studied in those with mild or severe phenotypes of primary ciliary dyskinesia. A recent retrospective study did investigate bronchodilator response (BDR) with a SABA in patients with

PCD (adults and children), and reported a higher prevalence of BDR in adults and those with defects of the microtubule compared to younger patients and those with other defects [12].

2.2.Supporting Pilot / Unpublished Data

N/A

2.3.Scientific Rationale

We have previously reported that PCD patients with *RSPH1* mutations have a milder pulmonary phenotype and better lung function than patients with PCD caused by other mutations [3]. Further, we demonstrated that although cilia lacking RSPH1 beat in an abnormal, circular pattern, *Rsph1*$^{-/-}$ mice exhibited a low level of MCC [13]. This is in contrast to *Dnaic1*$^{-/-}$ mice, who exhibit essentially immotile cilia and a complete lack of MCC [14]. In this aim, we will test the hypothesis that PCD subjects with mutations in *RSPH1* and certain other mutations that have a milder phenotype [4, 11] maintain a low level of MCC that is likely responsible for their milder disease phenotype. In addition, we will test the hypothesis that stimulating ciliary beat frequency (CBF) with a beta-agonist will increase MCC in this patient group. These studies may result in the development of new personalized treatments for this rare disease.

3. Objectives

3.1.Specific Aim 1

To estimate how MCC measurements vary in subjects with PCD caused by mutations in genes associated with mild and severe clinical phenotypes relative to healthy subjects, and compare the 3 populations in terms of Average Clearance.

3.2.Specific Aim 2

To characterize the effect of a short acting beta agonist (SABA) on MCC subjects with PCD caused by mutations in genes associated with mild and severe clinical phenotypes and compare the 2 populations in terms of change in Average Clearance.

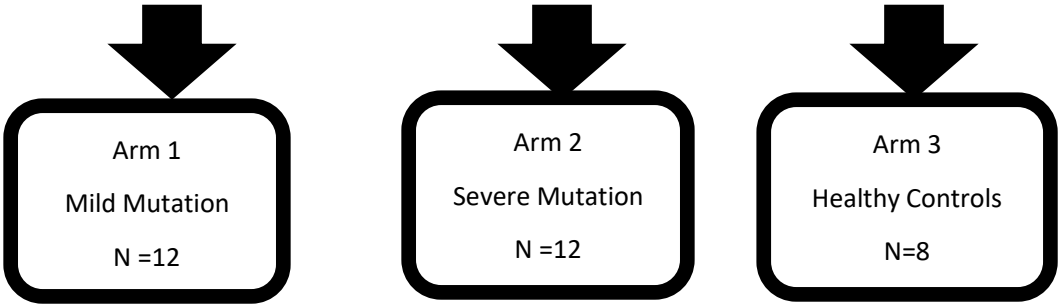
3.3.Specific Aim 3

To characterize effect of cough clearance in subjects with PCD caused by mutations in genes associated with mild and severe clinical phenotypes and compare the 2 populations in term of change in Average Cough Clearance.

4. Study Design

Prior to Enrollment

Total N: Obtain informed consent over the phone or by virtual meeting. Screen potential participants by inclusion and exclusion criteria; obtain medical history, document over the phone or by virtual meeting.



Perform screening and spirometry. Perform mucociliary (MCC) baseline measurement. Continue MCC measurement after SABA administration. Repeat spirometry followed by controlled cough maneuvers to assess cough clearance (CC). Subject will rest for approximately 3.5 hours followed by a final MCC/CC static measurement, 6 hours (±30 minutes) post initial scan.

4.1 Treatment Design

Type of Design: Open label; two group study with healthy controls

Screening/Baseline: Participants will undergo a general medical screening visit to include vital signs, physical exam and pregnancy test (if applicable), then proceed with spirometry.

Intervention/Treatment

Participants will inhale aerosolized radiolabeled sulfur colloid (Tc99m-SC) and will undergo gamma scintigraphy to measure mucociliary and cough clearance (MCC/CC) of the radiolabeled particles from the airways in both groups. During the first hour of MCC imaging patients will attempt to avoid coughing. Patients will then inhale 4 puffs of albuterol (90 mcg/puff via metered dose inhaler (MDI) with spacer) to assess the effect of beta-adrenergic stimulation on MCC over the second hour of MCC

imaging. After the two hours of MCC imaging patients will perform spirometry and directed coughing over a 30 minute period during which CC will be measured, again by gamma scintigraphy. Subjects will then rest (app 3.5 hours) and be scanned for 15 minutes at 6 hour ±30 minutes post initial MCC/deposition scan.

Table of procedures

	PCD MCC/CC study flow sheet	minutes needed	cumulative minu
1	Vital signs, PE,pregnancy test, PFT	30	30
2	Put Americium fiducial markers on	5	35
2	Co57 Transmission scan	5	40
3	Background scan	15	55
4	Radioaerosol inhalation	5	60
5	MCC scan (no cough)	64	124
6	Albuterol MDI (4 puffs with spacer)	6	130
7	MCC scan post albuterol	54	184
8	PFT and directed coughing (30 coughs including any spontaneous induced byPFTs)	20	204
9	CC scan post PFT/cough	4	208
10	Rest until final scan 6 hours after beginning of #5	210	418
11	6 hour post initial radioaerosol MCC scan	15	433

Unscheduled Visits: There is no expectation for unplanned visits. Subjects will simply be rescheduled if they have a change in health.

4.1. Outcome Measures

Primary Outcome(s): Baseline MCC (Ave60Clr) in subjects with PCD caused by mutations associated with mild and severe clinical phenotypes.

Secondary Outcome(s): Change in MCC (Ave120Clr-Ave60Clr) after the administration of albuterol in subjects with PCD caused by mutations associated with mild and severe clinical phenotypes PCD patients with mild disease compared to PCD patients with more severe disease.

Tertiary/Exploratory Outcome(s): Change in cough clearance (Ave150Clr-Ave120Clr) in subjects with PCD caused by mutations associated with mild and severe clinical phenotypes PCD patients with mild disease compared to PCD patients with more severe disease.

5. Study Participants

5.1. Number of Participants

Number to be recruited for screening: 40

Number of eligible participants enrolled 32

5.2. Eligibility

Inclusion Criteria for PCD Patients

1. Age 12-90 of both genders

2. Confirmed PCD diagnosis with identified genetic mutations.
3. Negative pregnancy test for females who are not s/p hysterectomy with oophorectomy.
4. FEV1 of at least 30% of predicted.

Inclusion Criteria for Healthy Controls:

1. Age ≥ 18 years old.
2. Subjects must have an FVC, FEV1 and FVC/FEV1 of at least 80% of predicted. Subjects who fall out of the normal range will be offered a copy of the test to share with their personal physician.
3. No pre-existing lung disease (asthma, cystic fibrosis, etc.).
4. Negative pregnancy test for females who are not s/p hysterectomy with oophorectomy.

Exclusion Criteria for all participants:

Clinical Contra-indications:

1. Any chronic medical condition considered by the PI as a contraindication to the exposure study including significant cardiovascular disease, diabetes, chronic renal disease, chronic thyroid disease, immunodeficiency, history of tuberculosis
2. Any acute infection requiring antibiotics within 4 weeks of study.
3. Mental illness or history of drug or alcohol abuse that, in the opinion of the investigator, would interfere with the participant's ability to comply with study requirements.
4. Medications which may impact the results of the study treatment, or may interfere with any other medications potentially used in the study (to include steroids, beta antagonists, non-steroidal anti-inflammatory agents)
5. Active smoking to include e-cigarettes within 1 year of the study, or lifetime of > 10 pack years of smoking
6. History of vaping or current vaping.
7. Allergy/sensitivity to study drugs, or their formulations.
8. Viral upper respiratory tract infection within 4 weeks of challenge.
9. Radiation exposure history in the past year which would be outside the safe levels
10. **Pregnant or lactating women** will also be excluded since the risks associated with radiation are unknown and cannot be justified
11. Use of the following medications:
    a. Use of beta blocking medications
    b. Receipt of LAIV (Live Attenuated Influenza Vaccine), also known as FluMist , within the prior 30 days, or any vaccine within the prior 5 days
    c. Multivitamins, Vitamin C or E or herbal medications in the 4 days prior to the treatment visit
    d. Non-steroidal anti-inflammatory drugs in the 4 days prior to the treatment visit.
12. Known IgE mediated hypersensitivity to albuterol

13. Physical/laboratory indications:
    a. Temperature > 37.8
    b. Subjects >15 y.o - Systolic BP >150 mm hg or < 90 mm Hg or diastolic BP> 90 mm Hg or < 50 and Subjects 12-15 y.o – Systolic BP > 130 mmHg or < 80 mmHg or diastolic BP > 80 or <40
    c. Oxygen saturation of < 93%
14. Inability or unwillingness of a participant to give written informed consent.

5.3.Strategies for Recruitment and Retention

Recruitment Strategy:

We will recruit subjects who have a known diagnosis of PCD caused by mutations in genes associated with a milder clinical phenotype (RSPH1, RSPH9, RSPH4a, and DNAH11) and subjects with PCD caused by mutations in genes associated with a more severe clinical phenotype (DNAH5, DNAI1, CCDC39, and CCDC40). In addition, we will recruit healthy controls from the general public to include fliers around UNC campus, e-mails, and/or by word of mouth. PCD subjects may be recruited from pediatric or adult PCD clinics at UNC by physicians listed on the protocol including Dr. Peader Noone, Dr. Margaret Leigh, and Dr. Sara Abu-Nasser. In addition, subjects may have had diagnostic information obtained from other research studies completed at our institution and will be recruited from previous PCD research studies. The research studies below will provide diagnostic PCD information for patients: 98-1015 (1395), 05-2997 (5901), 05-2979 (5902), 08-0764 (5903), 10-1523 (5904), 14-1225 (5905), and 20-0508 (5906). Once patients with the above genetic mutations are identified, patients will be called by a study coordinator or a study physician to recruit subjects for the study.

Retention Strategy:

We will perform the initial screening over the phone or virtually, in order to decrease the number of in person visits required. The study is to be performed over the span of one day, and will not require follow-up, therefore it is less likely for subject to drop out.

Screen Failures:

For subjects who do not meet enrollment criteria, all study related activity will stop once it is determined that the subject does not meet criteria.

5.4.Consent Process

Once subjects have been identified, a study coordinator or physician will call the patient to present and describe the study. Due to COVID-19 pandemic, precautions to limit in person contact include using phone calls or virtual calls to explain and discuss the study. The study coordinator/physician will also review the consent form with the subjects in the same method if they agree to the study. If subject agrees, a signature for the consent form will be required on the day of the study.

For children under the age of 18, their guardian or parent will be approached in the same manner as above. After explanation of the study, the consent form will be explained to the parent or guardian. Parent or guardian will sign the consent form the day of the study. The study procedures will also be

explained to the subject in age appropriate language during the call. Written and signed assent will be obtained the day of the procedure. When reasonably available, two parent or guardians will be required to provide consent for minors to participate. For situations when a parent is available able to consent but obtaining the written signature is not feasible, DocuSign may be used to document parental permission electronically.

6. Study Intervention

6.1.Intervention – Test Article

Description: Albuterol; HFA 90 mcg/puff; 4 puffs; one time. It is available commercially as ProAir HFA or Ventolin HFA.

Formulation/Packaging/Labeling: [As supplied by manufacturer/Sponsor]

Open label study

Storage and Stability:

Albuterol MDI will be delivered to and stored at a secure location within the research office. Albuterol MDI is stable at room temperature.

Preparation and Administration:

Albuterol MDI inhaler will require a spacer to be administered. Dose is 4 puffs one time for patients of all ages.

Modification:

No anticipated modification necessary.

Accountability:

Albuterol MDI inhaler will be disposed of after the completion of the study.

6.2.Assignment Procedures

Matching and Stratification: N/A

Randomization, Concealment, and Blinding: (N/A)

Masking (Blinding): N/A

6.3.Intervention – Procedural (if applicable)

Description: [N/A

Training on Procedural Intervention: N/A

6.4. Concomitant Therapy (if applicable)

N/A

6.5. Rescue Medications and Procedures (if applicable)

N/A

6.6. Compliance Checks

N/A

6.7. Withdrawal / Discontinuation of Enrolled Participants

Patient's participation will be discontinued if:

1. Subject develops new or worsening respiratory symptoms including shortness of breath or decrease in FEV1 of 20% or greater.
2. Subject develops a condition that is an exclusion criterion.
3. Subject is noncompliant with the recommended withholding of SABA or ICS/LABA for 12 hours prior to study.

If any of the above are met, then patient will not continue.

6.8. Voluntary Withdrawal (Drop-Out) of Enrolled Participants

Participants may voluntarily withdraw participation at any time, for any reason, with no penalty or loss of rights. Patients will not require follow up if they withdraw and can continue routine medical care.

7. Study Procedures and Schedule

7.1.Table of Events

Procedures		MCC Visit (Day 1)
Informed Consent		X
Medical History, med review		X
Concomitant Medications		X
COVID-19 Screen		X
Physical Exam	Complete	X
	Symptom-Directed	X
	Vital Signs	X
Urine Pregnancy Test		X
Spirometry		X
MCC		X

7.2.Pre-Screening / Screening

Subjects will be screened by phone for potential eligibility and interest once they are identified using the recruitment strategies discussed in Section 5.3. Screening will review inclusion and exclusion criteria. Approximately 48 hours before the study visit, subject will be screened for COVID-19 using the screening questions stated in section 7.4 and reminded to withhold use of SABA or LABA 12 hours prior to visit, and airway clearance medications (e.g. Hypetonic Saline or Pulmozyme) for 24 hours before the visit.

7.3.Enrollment / Baseline (Day 0)

On the day of the study, medical history and exclusion criteria will be reviewed again. Consent will also be signed on day of the study.

7.4.Study Visits

Intervention/Treatment procedures for MCC (by visits)

Adverse events are assessed at each visit.

Screening Call: Approximately 48 hours before the study, patient will undergo a COVID-19 screen with the questions below:

Do you feel feverish or have a fever?

Do you have a new or worsening cough?

Do you have a new or worsening sore throat?

Are you newly short of breath?

Have you had a new onset of loss of taste or smell in the last 5 days?

Do you have new onset vomiting or diarrhea?

Do you have new onset of repeated shaking with chills not related to another medical condition?

If patient answer yes to any of the questions above, study will be cancelled.

MCC/Treatment visit: On day of the study, medical history will be updated, including any new medications or new medical history. Vital signs and baseline spirometry will be collected, and a negative urine pregnancy test will be required for all women.

Participants will inhale aerosolized radiolabeled sulfur colloid (Tc99m-SC) and will undergo gamma scintigraphy to measure mucociliary and cough clearance (MCC/CC) of the radiolabeled particles from the airways. During the first hour of MCC imaging patients will attempt to avoid coughing. Subjects will then inhale 4 puffs of albuterol (90 mcg/puff via metered dose inhaler (MDI) with spacer) to assess the effect of beta-adrenergic stimulation on MCC over the second hour of MCC imaging. After the two hours of MCC imaging, patients will perform spirometry and directed coughing over a 30-minute period during which cough clearance will be measured, again by gamma scintigraphy. Subjects will then rest (app 3.5 hours) and be scanned for 15 minutes at 6 hour (±30 minutes) post initial MCC/deposition scan.

All personnel performing aerosolizing procedures (MCC scans and spirometry) will wear eye protection and N95 masks. A HEPA filter will also be placed in the room where the mucociliary scans will be performed.

7.5.Phone Contact (Day Day X ± Y)

N/A

7.6.Final Visit (Day Day X ± Y)

N/A

7.7. Follow-Up Contact (Day Day 1 ± 3)

The study team will contact participants between 1 and 4 days after the study visit to confirm when study payment card has been loaded and to follow up on any questions or concerns.

7.8. Premature Discontinuation

Study is considered completed after the last scan of the day. Subjects may withdraw at any time. Subjects may be withdrawn at any time for safety concerns. Subjects who have any unanticipated problems (UPs) with the study procedures will be monitored until resolution of the symptoms.

7.9. Collection and Management of Tissue Specimens

Sample Preparation: N/A

Record Keeping and Monitoring: N/A

Sample Storage and Security: N/A

8. Study Measurements and Evaluations

List variables that will be abstracted from medical charts: Medical records will be reviewed to obtain previous FEV1 measurement and to confirm concomitant medications.

Describe screening evaluation: Vital signs, including heart rate, respiratory rate, temperature and blood pressure will be collected. Oxygen saturation levels will be noted, as well as breath sounds. A brief physical examination, including but not limited to the cervical lymph nodes, eyes, ears, nose, throat, cardiovascular and respiratory systems will be performed.

Describe how measurements will be taken:
Spirometry:
This test measures the volume of air that can be exhaled and the rate of airflow during exhalation after a maximal inhalation and is performed to American Thoracic Society (ATS) guidelines. Subjects will inhale as deeply as possible, then exhale as rapidly and completely as possible into the spirometer. Measurements obtained from each maneuver include the forced vital capacity (FVC), the forced expiratory volume in the first second (FEV1), the maximal mid-expiratory flow rate (FEF 25-75%) and the peak expiratory flow (PEF). The largest FVC and FEV1, from at least 3 acceptable trials, are selected for analysis; the flow rates are selected from the trial with the largest sum of FVC and FEV1.

Albuterol Inhalation:

The subject will be instructed on the proper use of MDI Inhaler, an approved device for inhalation of albuterol. The subject will use the inhaler to administer 4 puffs of albuterol.

MCC procedures:

Prior to each MCC study, a transmission Co57 scan will be performed to define the lung boundaries, to assign regions of interest, and to normalize these regions for lung volume differences. A rectangular phantom containing the radioisotope Co57 (< 25 mCi) will be placed in front (5cm) of the subject sitting with his/her back to the gamma camera for 30 seconds. The transmission scan has been used by us (e.g., 05-2358, 08-0795, 06-1016, 13-1605, 15-938) and others to provide a delineation of lung boundaries for generating the central (C) and peripheral (P) regions of interest (ROIs) used to assess initial particle deposition (C/P ratio) and subsequent clearance of the inhaled radio-aerosol [15-16]. Prior to the transmission scan on each study day, we will place 2 spot markers of Americium241 (0.9 microcurie (uCi) each, gamma 66 KeV) on the upper and lower back of each subject during scanning (both Tc99m-SC deposition/retention and Co57 transmission). With dual isotope imaging, these spot markers will allow alignment of images for more accurate determination of regional deposition/retention. These very low radiation sources have been obtained from commercially available home smoke alarms. The placement of these markers will be determined to be outside the lung field during the transmission scan. Their location will be marked in semi-permanent ink for later placement during Tc99m deposition/retention scans. The shielded side of this source will be placed/taped onto the subject's skin. Radiolabeled Tc99m-sulfur colloid will be delivered using a modified Pari-LC Star nebulizer (MMAD 9.5 um). This is a closed delivery system that produces 80 ml/sec air flow, and therefore limits the inspiratory flow rate to this value. While seated in front of a gamma camera subjects will perform single inhalations lasting ~10 seconds each from the delivery system and will exhale at 500 ml/sec (using feedback from a flow meter in the breathing circuit). Approximately 5 of these inhalation maneuvers will be required to deposit an adequate isotope dose to the lung. Subjects will be allowed to breathe normally (off the nebulizer) in between each inspiratory maneuver. Each volunteer will practice these maneuvers prior to the actual radio-aerosol inhalation to guarantee his/her proficiency. The activity of Tc99m-SC loaded in the nebulizer will be adjusted to provide an estimated 40 uCi deposited in the lung for the MCC/CC scan. Patients between the age 12-18 years old will receive ¾ of the adult dose to account for the smaller lung volume. A single crystal detector will be placed at the subject's back during inhalation to monitor dose to the lung. Total inhalation time should be less than 5 minutes in all cases. Immediately following isotope inhalation, the subject will gargle and drink water to clear activity that deposited in the mouth into the stomach. The subject will then (within a minute of final inhalation maneuver) be seated in front of a large-field-of-view gamma camera to begin acquiring consecutive 2 minute images. The first two-2-minute images will provide initial, time zero activity (i.e. 100% retention) followed by the same imaging at the start of every 10-minute period until 1 hour has passed to assess baseline MCC. Subjects will then inhale 4 puffs of albuterol from the MDI inhaler and consecutive 2 min imaging continues for the next hour to assess the effect of albuterol on MCC. PFTs will then be performed and subjects will cough a total of 30 times over a 30-minute period to assess CC by gamma imaging over that period. Finally, a static 15-minute image will be measured at 6 hours (±30 minutes) after the initial radio- aerosol deposition scan. (See table of procedures in section 4.1).

8.1.Outcome Measures for Evaluation of Feasibility / Tolerability

N/A

8.2. Outcome Measures for Evaluation of Efficacy

First estimates of the efficacy of SABA for accelerating MCC immediately in PCD subjects, will be obtained by comparing the baseline rate of MCC and the rate of MCC immediately after albuterol inhalation.

8.3. Outcome Measures for Evaluation of Safety

Vital signs are monitored throughout the study as needed, including heart rate, respiratory rate, temperature, and blood pressure.

We will assess vital signs at baseline, and then as needed when indicated by study physician.

9. Statistical Analysis Plans

9.1. Strategies that Apply to all the Specific Aims

To describe each measure of MCC by retention vs. time data set, the average % clearance (or 100* (1-Retention)) over the 0-60 and 60-120 min period of observation are computed (i.e. average of the 10 minute clearance values from 0 to 60 and 60-120 minutes). These computed values are signified as Ave60Clr and Ave120Clr respectively. Each calculated AveClr value utilizes all 10-minute data points, rather than simply reporting a clearance value at a single time, and represents the average clearance at the midpoint of each period of retention vs. time observation. 6 hour clearance is determined by (100* (1- 6 hour retention)) and provides an index of initial airway (6hr Clr) vs. alveolar (6hr retention) deposition that along with the central/peripheral (C/P) ratio provides regional deposition data that can be included as covariates in analysis of MCC comparisons between groups and within individuals.

Distributions of the outcomes is expected to be normal. We will plot the data in histograms and qqplot to evaluate any extreme datapoints or outliers. Their impact if any will be analyzed by performing the analysis with and without these datapoints.

9.2. Description of the Study Cohort

The study cohort will consist of 12 PCD subjects with mutations in genes associated with a mild pulmonary phenotype (RSPH1, RSPH9, RSPH4a, or DNAH11) and 12 PCD subjects with mutations in genes associated with a more severe pulmonary phenotype (DNAH5, DNAI1, CCDC39, or CCDC40). A subject with mild pulmonary phenotype will typically have later onset of respiratory symptoms, better lung function, and milder bronchiectasis [4]. The age of patients will range between 12-90 years old. Eight healthy patients will be enrolled and undergo the same MCC protocol to obtain data for comparison. All subjects following the protocol will be included in the database. Subjects not

completing the protocol will be replaced. Baseline measurements of population (age, gender, race, FEV1) will be tabulated, for the whole population and per group (mild genotype, severe genotype, healthy) using mean and standard deviation and frequencies with percentages as appropriate. Data will be plotted in graphical figures including as histograms, bar plots, and scatter plots.

9.3.Aim-Specific Plans

Aim 1: To estimate how MCC measurements vary in PCD subjects with mutations in genes that are associated with mild or severe disease, relative to healthy subjects, and compare the 3 populations in term of Average Clearance.

Aim 1 is measuring MCC in PCD subjects having confirmed mutations in genes associated with a milder clinical phenotype and subjects with PCD caused by mutations in genes associated with a more severe clinical phenotype. These measurements will be reported as estimates of the means and standard deviations of Ave60Clr for each group, with associated confidence intervals. The baseline MCC in PCD subjects with "mild genotypes" and "severe genotypes" will be compared to healthy subjects. Our expected result is that subjects with mutations in genes associated with mild disease will display lower MCC rates than healthy subjects, and subjects with mutations in genes associated with severe disease will be even lower. For this comparison, we will use an ANOVA followed by multiple comparisons, using the Tukey Kramer correction. Then, to control for other possible sources of variations, age, gender and the initial deposition C/P will be added as covariates to PCD groups in a linear regression model on MCC.

Aim 2: To characterize the effect of a short acting beta agonist (SABA) on MCC in PCD subjects with mutations in genes that are associated with mild or severe disease, and compare the 2 populations in terms of change in Average Clearance.

In aim 2, we will be measuring the effect of albuterol on MCC in PCD subjects. We will look at the change between Ave60Clear (before albuterol) and Ave120Clr (after albuterol) for each subject. Estimates of the means and standard deviations with associated confidence intervals will be reported for each group. Using paired t-tests, we will verify if the change is significantly different than 0. The difference between the 2 PCD groups will then be analyzed with an independent t-test. Age, gender and the initial deposition C/P will be added as covariates to PCD groups in a linear regression model on MCC.

As this is not a randomized crossover design nor a parallel-arm design, our proposed comparison pre- to post-albuterol is uncontrolled and cannot assess the efficacy of SABA. The goal of our study is to obtain first estimates of the immediate effect albuterol on MCC in a small, controlled population.

Aim 3: To characterize cough clearance in PCD subjects with mutations in genes that are associated with mild or severe disease, and compare the 2 populations in terms of change in Average Clearance.

We will compare cough clearance in PCD subjects with mutations associated with mild disease with PCD subjects with mutations in genes associated with more severe disease. Cough clearance will be measured as the change in retention between 120 and 150 min post deposition. This change between Ave120Clr and Ave150Clr will be tabulated for each PCD group in term of means and standard deviations with associated confidence intervals and compared to 0 using paired t-tests. The difference between the 2 PCD groups will then be analyzed with an independent t-test. Age, gender and the initial deposition C/P will be added as covariates to PCD groups in a linear regression model on MCC.

For all aims, all tests will be 2-sided, with α=0.05. Tests with p values above 0.05 will be considered inconclusive.

Expected results, potential problems, and alternatives. We expect to observe a significant rate of MCC (different from 0) in the group of PCD patients with mutations in genes associated with a milder phenotype. Based on previous studies of ciliary activity in PCD subjects with mutations associated with a more severe phenotype, we expect to see little or no MCC in this group of PCD patients. Further, we expect the rate of MCC to be significantly greater in subjects in the "mild genotype" group compared to the PCD patients in the "severe genotype" group. We expect that subjects with a mild genotype will respond to albuterol with an increase in MCC while the PCD patients with the severe genotype will not. We expect that subjects with mutations in genes associated with a mild phenotype will have better cough clearance compared to subjects with mutations in genes associated with a severe phenotype. As we have performed many similar studies in the past, including studies of MCC in PCD subjects, we anticipate no technical difficulties in carrying out these studies. If there is no difference detected between the groups of PCD patients, this would suggest that the less severe clinical phenotype observed in some patients is not due to the maintenance of a low level of MCC. Nevertheless, this would be an important experimental finding, as it would indicate that some other mechanism is responsible for the difference in phenotype.

9.4. Planned Interim Analyses (If Applicable)

N/A

9.5. Plans for Coping with Screen Failures, Withdrawals and Loss-To-Follow-Up

Participants will be screened for key eligibility criteria over the phone prior to the study visit. Only subjects that meet the key eligibility will be scheduled for a visit. If any eligibility criteria is not met on day of the visit (eg. Vital signs, sprirometry results), subject will be considered a screen-failure and no additional procedures will be completed. Additional participants will be recruited to replace screen failures in order to meet target enrollment numbers.

Due to the short duration of the study, we do not except any withdrawal or loss to follow up patient. In the case a subject would withdraw consent, we will recruit another subject to reach our desired sample size for analysis.

9.6. Sample Size Rationale

Sample size:

Based on the results of mucociliary transport (MCT) rates (healthy: 6.58 ± 1.39, *Rsph1*$^{-/-}$: 1.63 ± 0.78, other PCD: 0) in our mouse model [13], we expect subjects with a mild genotype to average 25% of the MCC of healthy subjects (healthy: Ave60Clr =14.3 ± 7.2% ; mild expected: 3.6 ± 2%), while subjects with a severe genotype will not show any MCC results, i.e. Ave60Clr not different from 0 (expected 0.5 +/- 0.25). With n=10, we will be able to observe significant differences between all groups, with α=0.05 and power=0.8.

Moreover, we expect the mild genotype group to react to albuterol treatment by doubling their Ave60 Clr, while the group with severe mutations will not. With 10 subjects, we will be able to find a significant response in the mild genotype group with expected Ave60Clr = 7.2 ± 4%. Accounting for 20% possible dropout, we plan to enroll 12 subjects.

10. Safety Monitoring and Management

10.1. Risk / Benefit Assessment

Potential Risks:

Potential Risks of Spirometry:
Potential risks include possible lightheadedness or wheezing.

Potential Risks of Mucociliary clearance scan:
The radiation risks for the MCC scan, including the Cobalt 57 transmission scan and the Americium241 disks, which are used as fiducial markers is approximately 44 mRems. Adults in the New York City area receive about 300mRems per year in natural radiation exposure.

Potential Benefits:

Potential Benefits includes looking at possible treatment to improve mucociliary clearance and/or cough clearance in patients with PCD.

10.2. Assessment of Safety

The study procedures are low risk and are not expected to cause any adverse events, however, to ensure that subjects are tolerating the study well we have listed the screening and assessments below. General screen including physical exam at the start of the study and symptom-based exams during the study.

- Spirometry will be completed at baseline and after administration of SABA.
- Pulse oximetry will be checked at the start of the study and as needed.
- Vital Signs will be obtained at the start of the study and as needed.

Protections to Minimize Risk of Spirometry:
- Subjects will be seated in a non-rolling chair when spirometry is performed and standard methodology conforming to the American Thoracic Society guidelines for measurement of spirometry will be used. Subjects will be instructed to notify the study staff if they feel lightheaded, and albuterol will be available in the event the subject experiences any unanticipated

bronchoconstriction.

Protections to Minimize Risk of Mucociliary clearance scan:
- Radiation history is collected. Any subject who will exceed Federal guidelines for safe annual exposure limits will not be enrolled.

10.3. Unanticipated Problems, Adverse Events, Serious Adverse Events

Definition of Adverse Event (AE) and Serious Adverse Event (SAE)
An adverse event for a given volunteer will be defined as failure of any of the safety criteria outlined above. Decrease in lung function (FEV1) greater than 20% will be considered an adverse event. Any symptoms that induce a volunteer to seek medical attention from any provider within 96 hours of a study visit will be considered an adverse event. A serious adverse event will be defined as any event that requires hospitalization or results in life threatening illness or injury, permanent (or likely to be permanent) illness or injury, or death if these events occur within 96 hours of a study visit (or if the clinical scenario leading up to hospitalization, illness, injury or death begins within 96 hours of a study treatment visit).

Grading criteria

In addition to determining whether an adverse event fulfills criteria for a serious adverse event or not, the severity of adverse events experienced by study participants will be graded according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events Version 4.0. This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events.

All adverse events whether or not listed in the NCI-CTCAE will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual (A semi-colon indicates 'or' within the description of the grade.):

Grade 1 = Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 = Moderate; minimal, local or noninvasive intervention indicated; limiting age- appropriate instrumental ADL (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc).

Grade 3 = Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL (bathing, dressing and undressing, feeding self, using he toilet, taking medications, and not bedridden).

Grade 4 = Life-threatening consequences; or urgent intervention indicated.

Grade 5 = Death related to AE.

Adverse Event/Serious Adverse Event reporting procedures: Any SAE's and UP's will be reported to the IRB and the Medical Monitor. The funding agency will be notified of any SAEs.

Medical Emergency procedures: There is a study physician available for all inhalation procedures. A code cart and Automated External Defibrillator (AED) are available in the event of a cardiopulmonary

event. Albuterol, both inhalers and nebulizers, are available in the event of bronchospasm. If a subject has an event that is does not immediately respond to care in the research lab, he/she will be transported via EMS to UNC Healthcare for treatment.

10.4. Safety Monitoring

[Discuss how, in what time frame, and by whom subject safety will be monitored and the real-time plan for responding to adverse reactions, injury, and new safety concerns. Include criteria to be used for early study stopping. In addition to real-time evaluation, include consideration for a DSMB, Safety Monitoring Committee, or **Independent Medical Monitor**, as applicable, and describe in detail what their obligations will be. For a multi-site study, describe centralized safety oversight of the sites and communication of safety and quality issues]

Medical Emergency procedures: There is a study physician available for all inhalation procedures. A code cart and Automated External Defibrillator (AED) are available in the event of a cardiopulmonary event. Albuterol, both inhalers and nebulizers, are available in the event of bronchospasm. If a subject has an event that is does not immediately respond to care in the research lab, he/she will be transported via EMS to UNC Healthcare for treatment.

Data Safety Monitoring Plan: Data is initially recorded on paper documents, and the documents are maintained in a binder which is kept in a locked research office of study staff. This data is then entered into REDCap by an initial data entry person, and confirmed by a second user.

10.5. Study Suspension / Early Termination of the Study

[Describe circumstances that may warrant suspension/termination, e.g., unexpected, significant or unacceptable risk to participants; incomplete or unevaluable data; determination of futility. Provide actionable study stopping rules.]

Criteria for safety of a given individual during the study which would suspend the individual from further participation in the study will include:

1. A 20% or greater decline in FEV1 from baseline
2. Symptoms of respiratory distress.
3. Heart rate, blood pressure, or respiratory rate increase of more than 30% from baseline values (established on the same day).
4. SpO2 < 90% for > 1 hour.

11. Supporting Documentation and Operational Considerations

11.1. Regulatory, Ethical, and Study Oversight Considerations

11.1.1. Informed Consent Process

11.1.1.1. Consent/Assent and Other Informational Documents Provided to Participants

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials are submitted with this protocol:

1. Consent and Assent form for research study

11.1.1.2. Consent Procedures and Documentation

Describe the procedure that will be used to obtain informed consent/HIPAA authorization and assent (if applicable): Initial contact with potential subjects will be phone or virtual meeting. The study will be described in detail to the subject, including why the study is being conducted, the medication being studied, the risks and benefits, and what is expected of the subject. Briefly, the subject will be informed that they will undergo a screening exam and lung function testing. Subject will inhale a radiolabel substance and undergo medical imaging to measure the clearance of mucus from the airways. Albuterol will be administered and lung function testing will be performed. The imaging will be repeated two more times. The study will be completed in one day and will last 6 hours. The major risk of the study is exposure from the imaging and inhaling the radiolabeled substance. Radiation history will be collected and if it is more than the allowable safety limit, the subject will be excluded. The major benefit is to understand how subjects with PCD clear mucus.

The subject will be given adequate time to read the consent, and consent will be obtained prior to any study procedures. Consent may take place on a separate day from the baseline procedures. Due to COVID-19, consent forms will be reviewed by phone or virtual visit prior to the day of procedure. Subject will sign on the day of the procedure.

For subjects between the age of 12-17 years old, an assent form will be provided with age appropriate language. A parent will need to sign a consent form for the subjects.

Who will obtain consent: Consent will be obtained by a study coordinator or a study physician.

Where will consent process take place: The consent process and review will take place over the phone or virtual visit due to COVID-19 precautions and limiting in person interactions. Patient will sign the consent form on the day of the study. **T**his study will take place at the UNC Meadowmont/Eastowne location. If necessary, the UNC CEMALB, located in the Human Studies Facility of the US EPA on Mason Farm Road in Chapel Hill will serve as an alternate location.

How will investigator assure that subjects comprehend the nature of the study, procedures, the risks and benefits: The subject will be encouraged to ask questions regarding the study and procedures. Open ended questions will be asked of the subject to solicit correct responses, to help ensure that the subject understands the study commitment, procedures and risks and benefits

11.2. Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to <study participants, investigator, funding agency. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).]

No adverse events are expected from the study procedures. Criteria for safety of a given individual which would suspend the individual from further participation in the study will include:
1. A 20% or greater decline in FEV1 from baseline
2. Symptoms of respiratory distress.
3. Heart rate, blood pressure, or respiratory rate increase of more than 30% from baseline values (established on the same day).
4. SpO2 < 90% for > 1 hour.

Criteria for safety within the entire protocol (failure of which would result in suspension of further study until consultation with the IRB) will include the following:

1. If 2 of the first 10 participants fail the individual safety criteria outlined above, the entire protocol will be suspended pending review by the IRB.
2. No occurrence of any Serious Adverse Event

Subjects will be under direct supervision throughout MCC measurements and will either have contact information for after-hours access to a study physician or the UNC Health 24 hour line.
Screen failure procedures. For subjects who do not meet enrollment criteria, all study related activity will stop once it is determined that the subject does not meet criteria.

11.3. Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible. The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Certificate of Confidentiality (if applicable)

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

11.4. Future Use of Stored Specimens and Data

No specimens will be collected as part of this study.
We may use de-identified data from this study in future research without additional consent.

11.5. Key Roles and Study Governance

Principal Investigator	**Medical Monitor**
Lawrence E. Ostrowski, PhD	Scott H. Donaldson, MD
University of North Carolina	University of North Carolina
Address 6123 Thurston Bowles Bldg, Chapel Hill, NC 27599	2204 Marsico Hall, Chapel Hill, NC, 27599
919-843-7177	919-966-8980
ostro@medu.unc.edu	Scott_donaldson@med.unc.edu

11.6. Safety Oversight

Data Safety Monitoring Plan: Data is initially recorded on paper documents, and the documents are maintained in a binder which is kept in a locked office within the Meadowmont/Eastowne or EPA Human Studies facility. Data will be stored in a secure facility with a guarded entrance and requires identification for entry. This data is then entered into REDCap by an initial data entry person and confirmed by a second user.

Medical Monitor

Scott H. Donaldson, MD, has agreed to serve as an independent medical monitor (MM) for the human subjects studies associated with Dr. Ostrowski's RO1, titled "Functional Studies of Novel Genes Mutated in Primary Ciliary Dyskinesia II: Genotype to Phenotype". Dr. Donaldson is a Professor of Medicine, Division of Pulmonary Diseases and Critical Care Medicine, and the Associate Director of the Marsico Clinical and Translational Research Center, has experience with multiple clinical trials investigating mucociliary clearance in cystic fibrosis, and so is ideally suited to provide safety oversight for this project.

In regards to this proposal, the independent MM has the responsibility to 1) review and evaluate information relevant to the safety of the study. Specifically, this involves reviewing the protocols to be followed, including protocol halting rules, and addressing any safety concerns. 2) The MM will advise the protocol team on safety oversight. 3) The MM will be also be responsible for monitoring, collecting and reporting data related to unanticipated problems and adverse events.

In practice, the MM will review the protocols with the appropriate investigators before the studies begin. The MM will be notified of any unanticipated problems and adverse events immediately, and will report SAEs to the UNC IRB and to the NHLBI program officer within 48 hours. In the absence of any unanticipated problems and adverse events, the MM will review the protocol and results of the studies on a regular basis. Because of the irregular timing of subject recruitment, this may occur more frequently, but at least once yearly and after every 6 subjects complete the study.

11.7. Clinical Monitoring

N/A

11.8. Quality Assurance and Quality Control

N/A

11.9. Data Handling and Record Keeping

11.9.1. Data Collection and Management Responsibilities

Monitoring Plan: We will use REDCap for data management. The data will be entered by one person (typically the coordinator or, for lab analysis, a lab staff member) and then checked by a second person. The data is not marked "complete" until the 2nd person verifies the entry. REDCap creates a data dictionary when the database is established, this is used as the codebook.

Database documentation: All databases using equipment generated have a date and user attached to the electronic file. Data will be linked with a codebook (i.e. sample ID) using Excel. A second entry is done manually in a lab notebook, which includes date, samples assayed, assay used, any issues (like missing samples/data), reference to where data is stored, and a printout of the data (all as hardcopies in a lab notebook). Adherence to the codebook is ensured by having a second individual to check the entries. The PI takes responsibility for data management computations.

Case report forms: Case report forms will be developed by the study team, using templates from previous studies. These forms are maintained by study coordinators and reviewed by investigators as needed.

How will confidentiality be maintained: Subjects will be issued a subject number when they enroll into the study, and this number will only be used to label samples. All PHI will be maintained by the study coordinator or the investigators and will be kept in locked areas when not in use. The identifiers that go into REDCap will only be accessible to those who need them for their job, specifically study physicians, investigators and coordinators. Subjects sometimes undergo procedures – such as sitting in front of the gamma camera – at the same time, however other than basic introductions, nothing about one subject is disclosed to the other subject.

11.9.2. Study Records Retention

Study documents will be retained for a period of 3 years from the dates of Federal Financial Report (FFR) Submission.

11.10. Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP). The noncompliance may be either on the part of the participant, the investigator, or the study site staff. Protocol deviations are not permitted. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

11.11. Publication and Data Sharing Policy

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

11.12. Conflict of Interest Policy

N/A

12. Additional Considerations

N/A

13. References

1. Adam J. Shapiro, Stephanie D. Davis, Deepika Polineni, Michael R. Knowles, Margaret W. Leigh, and Valery Lavergne, et al. Diagnosis of Primary Ciliary Dyskinesia: An Official ATS Clinical Practice Guideline: Executive Summary. Am J Respir Crit Care Med. 2018; 197: 1524–1533.
2. Wanner A, Salathe M, O'Riordan TG. Mucociliary clearance in the airways. Am J Respir Crit Care Med. 1996;154:1868–1902.
3. Lobo J, Zariwala MA, Noone PG. Primary ciliary dyskinesia. Semin Respir Crit Care Med. 2015;36(2):169-179. doi:10.1055/s-0035-1546748

4. Knowles MR, Ostrowski LE, Leigh MW, Sears PR, Davis SD, Wolf WE, *et al*. Mutations in RSPH1 cause primary ciliary dyskinesia with a unique clinical and ciliary phenotype. *Am J Respir Crit Care Med.* 2014;189:707–717.

5. Frommer A, Hjeij R, Loges NT, Edelbusch C, Jahnke C, Raidt J, *et al*. Immunofluorescence analysis and diagnosis of primary ciliary dyskinesia with radial spoke defects. *Am J Respir Cell Mol Biol.* 2015;53:563–573.

6. David SD, Rosenfeld M, Lee HS, Ferkol TW, Sagel SD, Dell SD, Milla C, Pittman JE, Shapiro AJ, Sullivan KM, Nykamp KR, Krischer JP, Zariwala MA, Knowles MR, Leigh MW. Primary Ciliary Dyskinesia: Longitudinal Study of Lung Disease by Ultrastructure Defect and Genotype *Am J Respir Crit Care Med*. 2019.

7. Laube BL, Karmazyn YJ, Orens JB, Mogayzel PJ Jr. Albuterol improves impaired mucociliary clearance after lung transplantation. J Heart Lung Transplant. 2007 Feb;26(2):138-44. doi: 10.1016/j.healun.2006.11.005. PMID: 17258147.

8. Tamoki J, Chiyotani A, Sakai N, Konno K. Simulation of ciliary motility mediated by atypical B-adrenoceptor in canine bronchial epithelium. Life Sci. 1993; 53; 1509-1515.

9. Devalia JL, Sapsford RJ, Rusznak C, Toumbis MJ, Davies RJ. The effects of salmeterol and salbutamol on ciliary beat frequency of cultured human bronchial epithelial cells, in vitro. Pulm Pharmacol. 1992; 5: 257-263.

10. Bennett WD, Wu J, Fuller F, Balcazar JR, Zeman KL, Duckworth H, Donn KH, O'Riordan TG, Boucher RC, Donaldson SH. Duration of action of hypertonic saline on mucociliary clearance in the normal lung. *Journal of applied physiology* 2015; 118: 1483-1490. – methods reference

11. Noone PG, Leigh MW, Sannuti A, Minnix SL, Carson JL, Hazucha M, Zariwala MA, Knowles MR. Primary ciliary dyskinesia: diagnostic and phenotypic features. Am J Respir Crit Care Med. 2004 Feb 15;169(4):459-67. doi: 10.1164/rccm.200303-365OC. Epub 2003 Dec 4. PMID: 14656747.

12. Mosquera RA, Nielsen KG, Gardner L, Holgersen, MG, Hogg C, Carr S, Richardson, Olm M, Athanazio RA, Rached S, Castillo DJ, et al. Prevalence of Reversible Airway Obstruction for Specific Ultrastructural Defects in Primary Ciliary Dyskinesia. Am J Respir Crit Care Med 2020;201:A5344.

13. Yin W, Livraghi-Butrico A, Sears PR, Rogers TD, Burns KA, Grubb BR, Ostrowski LE. Mice with a Deletion of Rsph1 Exhibit a Low Level of Mucociliary Clearance and Develop a Primary Ciliary Dyskinesia Phenotype. Am J Respir Cell Mol Biol. 2019 Sep;61(3):312-321. doi: 10.1165/rcmb.2017-0387OC. PMID: 30896965; PMCID: PMC6839924.

14. Ostrowski LE, Yin W, Rogers TD, Busalacchi KB, Chua M, O'Neal WK, Grubb BR. Conditional deletion of dnaic1 in a murine model of primary ciliary dyskinesia causes chronic rhinosinusitis. Am J Respir Cell Mol Biol. 2010 Jul;43(1):55-63. doi: 10.1165/rcmb.2009-0118OC. Epub 2009 Aug 12. PMID: 19675306; PMCID: PMC2911571.

15. Bennett WD, Laube BL, Corcoran T, Zeman K, Sharpless G, Thomas K, Wu J, Mogayzel PJ Jr, Pilewski J, Donaldson S. Multisite comparison of mucociliary and cough clearance measures using standardized methods. J Aerosol Med Pulm Drug Deliv. 2013 Jun;26(3):157-64. doi: 10.1089/jamp.2011.0909. Epub 2013 Mar 21. PMID: 23517172.

16. Zeman KL, Wu J, Donaldson SH, Bennett WD. Comparison of 133 xenon ventilation equilibrium scan (XV) and 99m technetium transmission (TT) scan for use in regional lung analysis by 2D gamma scintigraphy in healthy and cystic fibrosis lungs. J Aerosol Med Pulm Drug Deliv. 2013;26(2):94-100. doi:10.1089/jamp.2012.0982

17. Zeman KL, Wu J, Bennett WD. Targeting aerosolized drugs to the conducting airways using very large particles and extremely slow inhalations. J Aerosol Med Pulm Drug Deliv. 2010 Dec;23(6):363-9. doi: 10.1089/jamp.2008.0711. Epub 2010 Sep 23. PMID: 20863250.

14. List of Appendices

This section should include a listing of any tables, questionnaires, investigator brochure, device manual, subject 'handouts', etc that should accompany the study protocol.

Appendix 1: Adult Consent Form

Appendix 2: COVID-19 Screening Hand-out: Questions which will be used for COVID-19 screening.

Appendix 3: Safety Outcome Screening: Questions to screen any changes in health status before and during the study

Appendix 1: Adult Consent Form

University of North Carolina at Chapel Hill
Consent to Participate in a Research Study
Adult Participants

Consent Form Version Date: 02/08/2023
IRB Study # 20-3465
Title of Study: Functional Studies of Novel Genes Mutated in Primary Ciliary Dyskinesia II: Genotype to Phenotype
Principal Investigator: Lawrence Ostrowski, PhD
Principal Investigator Department: Pediatric Pulmonary Division
Principal Investigator Phone number: 919-843-7177
Principal Investigator Email Address: ostro@med.unc.edu

Funding Source and/or Sponsor: NIH

Study Contact Telephone Number: 919-962-9841
Study Contact Email: corinne.lawler@unc.edu

This is a consent form. It provides a summary of the information the research team will discuss with you. If you decide that you would like to take part in this research study, you would sign and date this form to confirm your decision. If you sign and date this form, you will receive a signed and dated copy of this form for your records.

CONCISE SUMMARY

The purpose of this study is to determine how patients with primary ciliary dyskinesia (PCD) clear mucus differently based on their genetic mutation, and to determine if albuterol can help them clear mucus from their airways better.

Participants will undergo screening with basic physical exam and lung function testing. Participants will then inhale a radiolabeled substance and undergo medical imaging to measure the clearance of mucus in the airways. Albuterol will be administered after lung function testing will be repeated. Finally, imaging will be repeated two more times. The study will be completed in one day and will last about 6 hours.

The major risk of the study is radiation exposure from the inhaled radiolabel substance. The radiation exposure is very small, but radiation history will be collected and if it is more than the safety limits, then you will not be able to participate in the study. The major benefit of the study is to try to understand how patients with PCD clear mucus and if albuterol treatment will help.

You should not join this research study until all of your questions are answered.

If you are interested in learning more about the study, please continue reading below.

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary.

You may choose not to participate, or you may withdraw your consent to be in the study, for any reason, without penalty.

Things to know before deciding to take part in a research study:

- The main goal of a research study is to learn things to help patients in the future.
- The main goal of regular medical care is to help each patient.
- The decision to join or not join the research study will not cause you to lose any medical benefits. If you decide not to take part in this study, your doctor will continue to treat you.
- Your medical records may become part of the research record. If that happens, your medical records may be looked at and/or copied by the sponsor of this study and government agencies or other groups associated with the study.

Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study.

You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
The purpose of this research study is to compare how patients with primary ciliary dyskinesia with different genetic mutations clear mucus from your lungs differently. It will also test whether albuterol, an inhaled medication, can help clear mucus better.

Are there any reasons you should not be in this study?
You should not be in this study if you:

1. Are pregnant or nursing a child.
2. Take medications in the beta blocker family (labetalol, propranolol, etc.)
3. Smoke or vape tobacco products

How many people will take part in this study?
Approximately 32 people who are 12 years of age or older at this institution will take part in this study.

How long will your part in this study last?

The study will approximately 8 hours. However, you will also receive screening and reminder phone calls in the days leading up to the study.

How will you be contacted during the study?

In addition to phone calls, the study team would like to communicate with you by text message and e-mail, however, you may say "no" to receiving these messages and still participate in this study. If you say "yes", messages may contain personal information about you and may be sent or received by the study team's personal electronic devices or in a method that is not be able to be encrypted (protected) and there is the risk your information could be shared beyond you and the study team. This information may include study visit reminders and notifications to contact the study team. Communication via text message or e-mail will only be used with adult participants or parent/legal guardian(s) of minors.

If you wish to stop receiving unprotected communication from the study team or have lost access to your device, please notify the study team using the study contact information on the first page of this consent form. After the study is complete and all research activities finished, or you withdraw from the study or request to stop receiving unprotected communication, you will no longer receive un-encrypted (un-protected) messages specific to this study.

_____ **Yes**, I consent to the study team utilizing the following email to send communication.

List Email: _____

_____ **Yes**, I consent to the study team utilizing the following cell phone to send text message communication. List Cell Phone: _____

_____ **No**, I do not consent to receive un-protected communication from the study team.

What will happen if you take part in the study?

Informed Consent: We will explain the study to you. If you decide to participate you will be asked to review, sign, and date the consent and HIPAA authorization forms.

Medical History: Your medical chart will be reviewed and you will be asked questions about your health and Primary Ciliary Dyskinesia including current and past illnesses, and use of medications.

Demographic Information: We will collect information about you including, sex, birthdate and race.

Physical Examination: Your study doctor will listen to your lungs and heart. They will briefly examine nose, mouth and neck for any abnormal signs.

Pregnancy Test: Pregnancy testing is required for participation in this study; all girls and women age 12 and older will be tested for pregnancy. Only those testing negative will be allowed to participate.

Spirometry: This test measures how much air your lungs can hold and how fast you can breathe out. You will take a deep breath and then blow into a mouthpiece as hard as you can and for as long as you can. You might have to wear soft nose clips during the test to stop air from escaping through your nose. You will be asked to repeat this test at least 3 times.

Mucociliary Clearance Scan: This procedure is a scan or image of your lungs to see how mucus is being cleared. We will first place a source of Cobalt 57 in front of your chest for up to 5 minutes to obtain a transmission scan (picture) of your lungs. This scan will identify various regions of your lungs to help the researchers analyze data from all the gamma camera scans. You will then have a background scan of your lungs that lasts 15 minutes. Immediately after this scan you will inhale aerosolized radiolabeled solution that will help us see where mucus goes and how it moves in the lungs and airways. This procedure lasts about 5 minutes. You will then have scans of your lungs during the next 6 hours. You will inhale 4 puffs of albuterol using an inhaler and take another scan (picture) of your lungs to see how it affect mucus moving in lungs and airways. After the 2 hour scan you will perform spirometry (lung function test) and 30 voluntary coughs over the next 30 minutes of scanning.

Pre-Visit Reminders: We will provide reminders before your study visits. We will also remind you about stopping certain medications 12 hours before the study if applicable.

Current Medications: You will be asked about all medications and non-drug therapies you are currently taking.

Medication Restrictions: Please inform your study doctor of any medications and non-drug therapies that you are currently taking. Your study doctor will tell you whether you can continue using a particular medication or non-drug therapy while you are participating in this study. You will be asked not to take the following medications while you are participating in the study:

- Albuterol Inhaler/MDI to be stopped 12 hours before study
- Inhalers which have salmeterol, formoterol or other medicines in the same family to be stopped 12 hours before the study
- Airway Clearance Medications such as Hypertonic Saline or Pulmozyme to be stopped 24 hours before the study.

Explanation of Study Visit			
Procedures		General Screen (phone)	Study Visit
Informed Consent		X	X
Medical History, med review		X	X
Medications		X	X
COVID-19 Screen		X	X
Physical Exam	Complete		X

	Symptom-Directed		X
	Vital Signs		X
Urine Pregnancy Test			x
Spirometry			x
Mucociliary Clearance Measurement			x
Approximate Length of Time		~1 hour	~6 hours

What are the possible benefits from being in this study?
Research is designed to benefit society by gaining new knowledge. You will not benefit personally from being in this research study.

What are the possible risks or discomforts involved from being in this study?
There are some minimal risks related to the procedures used in the study. Some of these procedures you may have had at your regular clinic visits. There may be uncommon or previously unknown risks. You should report any problems to the researcher.

Procedure	Possible Risk Associated with Procedure
Spirometry	There is a small risk of wheezing, shortness of breath and lightheadedness.
Mucociliary Clearance Measurement (Gamma scintigraphy and radiolabeled compound)	The mucociliary clearance scan has radiation exposure risks and is estimated to be 44 mRems. For context, adults in Chapel Hill area receive about 300 mRems per year in natural radiation exposure. More information on radiation risks are below.
Albuterol	Common risks are headache, dizziness, fast heartbeat, sore throat, runny nose Less common risks are change in blood pressure and other cardiovascular changes. There is a rare risk of paradoxical bronchospasm (airway spasms) and anaphylaxis (allergic reaction) that may be life threatening.

Confidentiality:
Being in any research study has the risk of loss of privacy or confidentiality. Below tells you how your information will be protected.

Risks associated with radiation exposure:
This research study involves exposure to radiation from inhaled radiolabeled sulfur colloid for gamma scintigraphy scans. While radiolabeled sulfur colloid is FDA approved for oral and IV administration, its use by inhalation is experimental. There is, however, no significant increased radiation risk for our experimental vs. FDA-approved use. Please note that this radiation exposure is not necessary for your medical care and is for research purposes only. Since radiation

can be especially harmful to a developing fetus, it is important that pregnancy be avoided during this study by using effective birth control measures (either hormonal contraceptives, like birth control pills; or a barrier method, like condoms.

The radiation dose you receive will be 44mRems. The risk from the radiation dose received from this procedure is too small to be detected. The radiation exposure described here is what you will get from this research study only. It does not include any exposure you may have received or will receive from other tests outside this study that are a part of your medical care.

The average person in the United States receives a radiation exposure of 0.3 rem (or 300 mRem) per year from natural background sources, such as the sun, and from radioactive materials that are found naturally in the earth's air and soil. The dose that you will receive from participation in this research study (44 mRem) is less than amount you receive from these natural sources in one year (equivalent to 15% of exposure from background radiation in one year).

The amount of radiation you will receive in this study has a minimal risk and is below the dose guideline established by The University of North Carolina Radiation Safety Committee for research subjects. You must inform one of the investigators if you have had any x-rays or other radiation exposure within the past year so that we do not exceed the yearly dose limits. If you wish, Dr. Bennett will provide you with additional information and answer any questions you may have. If desired, additional information can be obtained from Marija Ivanovic, Ph.D, Chairman of the Radiation Safety Subcommittee of UNC Hospitals at 984-974-7779.

What are the risks to a pregnancy or to a nursing child.
The risks to pregnancy or a nursing child are unknown. If you are a woman and are pregnant or nursing a child, you should not be in the study.

If you choose not to be in the study, what other treatment options do you have?
There is no treatment in this study. Your alternative is not to be in this study and continue your normal care.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

The imaging we are using in this research study is not the same quality as imaging that you may have as part of your health care. The images will not be reviewed by a doctor who normally reads such images (such as a radiologist). As a result, you may not be informed of any unexpected findings. The results will not be placed in your medical record. Occasionally the technologist or principal investigator may notice something abnormal on the imaging. If this does occur, the images will be reviewed by a qualified doctor to determine if there is anything of clinical importance. If something is found to be important then you, and/or your primary care provider will be notified. Any further follow up and costs associated with the incidental finding will be your responsibility. There may be benefits to learning such results (such as early detection and treatment of a medical condition), but there are risks as well (such as problems

with getting insurance or a job, or feeling worried about a finding for which no treatment is required or appropriate).

Do you wish to be informed in case of clinical/relevant unexpected findings? Please initial in the box below if you do not wish to be notified of clinical/relevant unexpected findings. If you do not initial in the box, you will be notified of any findings.

_____ I do not wish to be notified.

Will I receive any other clinical results?

Other clinically relevant results of this research will be communicated with you:

- Lung Function testing

How will information about you be protected?

If you take part in this study, we will make every effort to keep your information confidential.

We will store all of your research records in locked cabinets and secure computer files. We will not put your name on any research data. Instead, we will label your information or samples with a study number. The master list that links a person's name to their study number is stored in a locked cabinet or on a secure computer file.
Absolute confidentiality cannot be guaranteed because of the need to give information to these parties. If results of this research are published, we would not use information that identifies you. We would only use your information for research.

These are some reasons that we may need to share the information you give us with others:

- If it's required by law.
- If we think you or someone else could be harmed.
- Sponsors, government agencies or research staffs sometimes look at forms like this and other study records. They do this to make sure the research is done safely and legally. Anyone who reviews study records would keep your information confidential. Agencies or sponsors that may look at study records include:
  - National Institute of Health (NIH)
  - Institutional Review Board (IRB) and others responsible for watching over the safety, effectiveness, and conduct of the research.

Participants will not be identified in any report or publication about this study. We may use de-identified data and/or specimens from this study in future research without additional consent.

What is a Certificate of Confidentiality?

This research is covered by a Certificate of Confidentiality. With this Certificate, the researchers may not disclose or use information, documents or biospecimens that may identify you in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings in the United States, for example, if there is a court subpoena, unless you have consented for this use.

The Certificate cannot be used to refuse a request for information from personnel of a federal or state agency that is sponsoring the study for auditing or evaluation purposes or for information

that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

The Certificate of Confidentiality will not be used to prevent disclosure as required by federal, state, or local law, such as mandatory reporting requirements for child abuse or neglect, disabled adult abuse or neglect, communicable diseases, injuries caused by suspected criminal violence, cancer diagnosis or benign brain or central nervous system tumors or other mandatory reporting requirement under applicable law. The Certificate of Confidentiality will not be used if disclosure is for other scientific research, as allowed by federal regulations protecting research subjects or for any purpose you have consented to in this informed consent document.

You should understand that a Certificate of Confidentiality does not prevent you from voluntarily releasing information about yourself or your involvement in this research. If an insurer, employer, or other person obtains your written consent to receive research information, then the researchers may not use the Certificate to withhold that information.
You will be asked to sign a separate form ("HIPAA Authorization") to allow researchers to review your medical records.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. If you are hurt, become sick, or develop a reaction from something that was done as part of this study, the researcher will help you get medical care, but the University of North Carolina at Chapel Hill has not set aside funds to pay you for any such injuries, illnesses or reactions, or for the related medical care. Any costs for medical expenses will be billed to you or your insurance company. You may be responsible for any co-payments and your insurance may not cover the costs of study related injuries.

If you think you have been injured from taking part in this study, call the study physician at the phone number provided on this consent form. They will let you know what you should do.

By signing this form, you do not give up your right to seek payment or other rights if you are harmed as a result of being in this study.

What if you want to stop before your part in the study is complete?
You can stop being part of the study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

If you withdraw or are withdrawn from this study all data collected up until the point of withdrawal will be retained, however no additional information will be collected unless you provide additional written permission for further data collection at the time of your withdrawal.

Will you receive anything for being in this study?
You will be receiving $150.00 for taking part in this study. Any payment provided for participation in this study may be subject to applicable tax withholding obligations.

All study payments will be paid through the University of North Carolina Disbursement Services office. Receipt of payment can take up to 4 weeks or longer. Your name, address, and U.S. tax payer identification number (SSN or ITIN) are required to process payments and/or to report taxable income to the IRS. You must complete a W-9 (for U.S. persons) or W-8BEN and the Foreign Vendor Withholding Assessment with supporting documents (for non-resident aliens) in order to receive payment for participation.

U.S. person participants must complete Form W-9 in order to receive payment for participation. If payment by UNC equals or exceeds $600 per calendar year for U.S. persons, UNC will report the amount to the Internal Revenue Service on Form 1099. Nonresident alien participants must complete Form W-8BEN and the Foreign Vendor Withholding Assessment with supporting documents in order to receive payment for participation. Payments to nonresident alien participants may be subject to tax withholding and are generally reported to the Internal Revenue Service on Form 1042-S. This information will not be linked to any of the study data and will only be used for payment purposes.

If you do not provide your SSN or ITIN, or complete the appropriate documentation noted above, we cannot issue you a payment for participation. However, you may still choose to participate in this study.

We will also reimburse you for any travel costs needed for the study such as parking, transportation, and hotel if applicable.

Will it cost you anything to be in this study?
It will not cost you anything to be in this study.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by National Institutes of Health (NIH). This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions about the study (including payments), complaints, concerns, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

A description of this clinical trial will be available on www.clinicaltrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

What if you have questions about your rights as a research participant?

All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject, or if you would like to obtain information or offer input, you may contact the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.

Participant's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

_____
Signature of Research Participant

____/____/____
Date

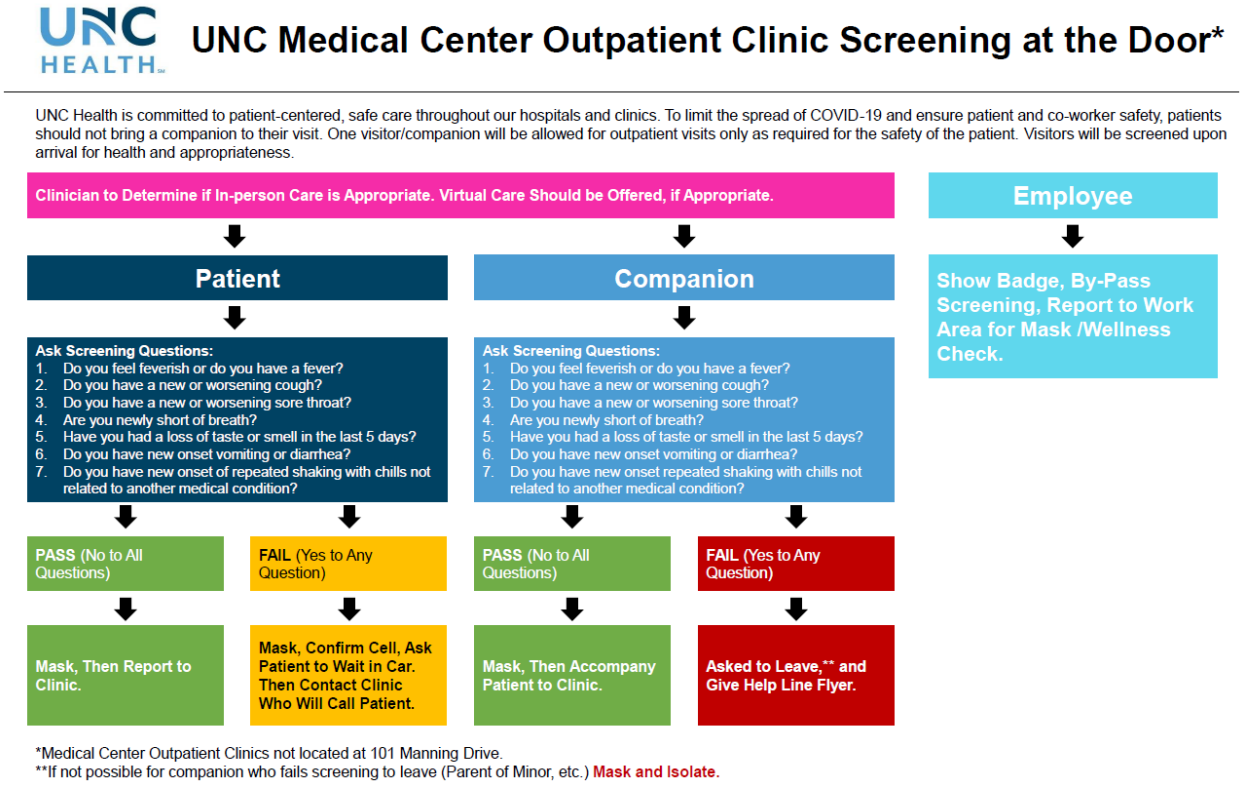
_____
Printed Name of Research Participant

Researcher's Signature
I have fully explained the research study described by this form. I have answered the participant and/or parents'/guardians' questions and will answer any future questions to the best of my ability. I will tell the family and/or the person taking part in this research of any changes in the procedures or in the possible harms/possible benefits of the study that may affect their health or their willingness to stay in the study.

_____ _____
Printed Name of Person Conducting the Informed Consent Discussion — Position

_____ ____/____/____
Signature of Person Conducting the Informed Consent Discussion — Date

Appendix 2: COVID-19 Screening Hand-out:



Appendix 3: Safety Outcome Screening

Symptom Questionnaire:

We will use the following symptom questionnaire:

1. Do you have any health concerns since your last visit?
2. Have you needed to see a doctor for any reason since your last visit?
3. Have you needed to use any over the counter medications?
4. Have you had any specific problems with cough, wheezing, or needed any extra allergy or asthma medications?
5. If the answers to questions 1, 2, 3, or 4 are “yes”, the subject will be assessed by a study physician.