University of North Carolina, Chapel Hill
Committee on the Protection of the Rights of Human Subjects (Medical IRB)

APPLICATION FOR APPROVAL OF RESEARCH INVOLVING HUMAN SUBJECTS

DATE: November 16, 2001 **IRB STUDY NUMBER (leave blank if new submission)**: _____

TITLE OF STUDY: Characterization of mucus and mucins in bronchoalveolar lavage fluids from infants with cystic fibrosis

NAME AND DEGREE(S) OF
PRINCIPAL INVESTIGATOR: Terry L. Noah, M.D. **DEPT**: Pediatrics
SOCIAL SECURITY NUMBER OF PRINCIPAL INVESTIGATOR: xxx-xx-xxxx
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NAMES AND DEGREE(S) OF CO-INVESTIGATORS: Margaret W. Leigh, M.D., George Z. Retsch-Bogart, M.D., C. William Davis, Ph.D.

NAME AND PHONE NUMBER OF
RESEARCH COORDINATOR, IF APPLICABLE: N/A

NAME OF FUNDING SOURCE: NIH; Cystic Fibrosis Foundation (pending grant application)

I. Agreements

Principal Investigator:
I certify that each of the above-named co-investigators has accepted his/her role in this study. I agree to a continuing exchange of information with the Committee on the Protection of the Rights of Human Subjects (IRB). I agree to obtain IRB approval before making any changes or additions to the project. I will provide progress reports at least annually, or as requested. I agree to report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. A copy of the consent form will be given to each subject and the signed original will be retained in my files. If the study involves treatment of UNC Hospitals patients, a copy of the consent form will be placed in each subject's medical record.

_____ _____
Signature of Principal Investigator Date

_____ _____
Signature of Faculty Advisor if P.I. trainee or Non-Faculty Date

Department Chair of P.I. (or Vice-Chair if Chair is investigator or otherwise unable to review):
I have reviewed this research study. I believe the research is sound, that the study design and methods are adequate to achieve the study goals, and that there are appropriate resources (financial and otherwise) available to the investigator. I support it, and hereby submit it for further review.

_____ _____ _____
Signature of Department Chair Department Date

II. Summary Checklist

ARE THE FOLLOWING INVOLVED?	YES	NO
Surveys, questionnaires or interviews *If research is limited to use of surveys, questionnaires or interviews, Submit **Exemption Application Form** instead of this application.*	☒	☐
Existing Patient Records and/or Specimens *If research is limited to study of existing medical records and /or samples, Submit **Short Form** instead of this application.*	☒	☐
Investigational Drug(s) **IND#** *If "yes", do you intend to use the UNC Hospitals Investigational Drug Service?*	☐ ☐	☒ ☐
Approved drugs for "non-FDA-approved" conditions	☐	☒
Placebo(s)	☐	☒
Experimental devices, instruments, machines **IDE#**	☐	☒
Genetic studies on subjects' specimens	☐	☒
Storage of subjects' specimens for future, as-yet-undesignated research *If "yes", see **Instructions for Submitting IRB Applications for Research that Includes the Storage of Human Biologic Specimens**.*	☒	☐
Fetal tissue	☐	☒
Videotaping, audiotaping, filming of subjects	☐	☒
Non-patient volunteers	☐	☒
Patients as subjects	☒	☐
Minors (less that 18 years old) *If "yes", indicate:* **Age range 0 to 2 years**	☒	☐
Do you intend to target your enrollment at: -Students or staff as subjects?	☐	☒
-Non-English-speaking subjects?	☐	☒
-Decisionally impaired or mentally incompetent subjects?	☐	☒
-Prisoners, parolees and other convicted offenders as subjects?	☐	☒
-Pregnant subjects?	☐	☒
Will HIV tests be performed?	☐	☒
Will subjects be studied at off-campus sites?	☐	☒
Is this a multicenter study? *If "yes", is UNC-CH the sponsor or coordinating center?*	☐ ☐	☒ ☐
Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise *If "yes", approval by the Radiation Safety Committee is required.*	☐	☒
Recombinant DNA or gene transfer to human subjects *If "yes", approval by the Biologic Safety Committee is required.*	☐	☒
Is this an oncology study? *If "yes", submit this application directly to the Oncology Protocol Review Committee.*	☐	☒
Will subjects be studied in the General Clinical Research Center? *If "yes", obtain GCRC Addendum from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.*	☐	☒

III. Required Education in Human Subjects Protection

UNC policy requires that all persons engaged in research involving human subjects must complete training in ethical conduct of research and protection of subjects. This applies to all research, regardless of funding source. For further information, including what options are acceptable in fulfillment of these requirements, see http://www.med.unc.edu/irb/Education2.htm

Individuals who have completed training should have been entered into the Human Subjects Training Database maintained by the Office of Research Services (ORS). To print documentation, visit http://zeppo.admin.unc.edu/isapi/certweb.dll and enter the names of each individual involved with this research project. Names not returned by the database are not recognized as having satisfied the education requirement. For questions regarding the database, please contact ORS at 962-7757.

WITH THIS APPLICATION, please submit the printout from the ORS database, verifying that each individual involved in the research (including faculty, staff, students and outside collaborators, if responsible to this IRB) has satisfied the education requirements.

IV. Potential Conflict of Interest

The following questions apply to any investigators or study staff involved with industry-sponsored research, and/or their immediate family members (spouse, dependent children, others). Within the past 12 months or the next 12 months, have you or will you:

Receive any form of personal compensation from the Sponsor, including salary, consulting fees, honoraria, royalties, equipment, etc.?	☐ YES	☒ NO
If so, does or will that compensation exceed $10,000?	☐ YES	☐ NO
Have an ownership interest of any nature in the Sponsor or product under study, including equity, stock options, etc.?	☐ YES	☒ NO
If so, does or will that interest exceed $10,000 in value?	☐ YES	☐ NO
If so, does that interest represent more than 5% ownership in the Sponsor?	☐ YES	☐ NO
Hold any position with the Sponsor, including officer, director, trustee, consultant, member of advisory board, etc.?	☐ YES	☒ NO
Have an intellectual property interest on any technology or invention used in this study, including patent rights, copyright, etc.?	☐ YES	☒ NO
Have a conflict of interest disclosed through the University's annual evaluation policy that relates to this research study?	☐ YES	☒ NO

If the answer is "YES" to any of the questions above, please include an explanation with this application. As with any changes to the research itself, relationships or interests that develop later should be brought to the IRB's attention for further consideration.

V. Description of Proposed Research Activity

Entire application should not usually exceed 5 single-spaced pages using a 12-point font.

1. **Purpose and Rationale:** Provide a brief summary of the background information, state the research question(s), and tell why the study is needed. Avoid an extensive literature review.

Cystic fibrosis (CF) results from mutation in the gene for CFTR, a protein which normally transports chloride ions out of epithelial cells. Children with CF are born with histopathogically normal lungs, but over the first weeks or months of life develop chronic bacterial infections of the conducting airways, along with intensive, neutrophil-dominated inflammation, and obstruction of the airways. It is still unclear exactly how the CFTR defect results in these changes. The best evidence at present favors a "drying" of airway surface liquid (ASL) in the absence of normal CFTR function, due to hyperabsorption of sodium and water secondary to the lack of chloride efflux. This drying severely impairs mucociliary clearance of inhaled bacteria and leads to chronic infection. Data from sputum studies and cell culture models also suggest several abnormalities of mucus biochemistry and expression of mucin peptides in CF. However, it is unclear whether these abnormalities are inherently related to CFTR dysfunction, or are secondary to chronic infection and inflammation.

Due to lack of a completely analogous animal model for CF, direct confirmation of hypotheses on the early events in the lung relies heavily on sampling of ASL from the infant lung using bronchoalveolar lavage fluid (BALF). A precise knowledge of the order of pathogenetic events will greatly focus efforts toward early therapy interrupting the primary processes leading to established infection and inflammation. At present, there is almost no information characterizing mucus or mucins in CF infants prior to the onset of infection and inflammation.

Published BALF studies from our laboratory and others suggest that a short "window" of infection- and inflammation-free lungs occurs in the weeks after birth in children with CF. Preliminary work in our laboratories suggests that mucins can be quantified in BALF using an immunoassay method, and mucus "plugs" can be isolated, fixed, and biochemically characterized by careful processing of BALF samples.

The goals of the proposed study are:

- Quantify mucin in BALF and compare quantities before infection vs. after infection onset in CF; and compare CF vs. nonCF.

- Correlate mucin quantity with measures of infection (quantitative bacteriology) and inflammation (cell numbers, neutrophil products, inflammatory cytokines).

- Isolate mucus plugs and characterize their histology before and after infection, in order to more accurately describe early relationships among mucus obstruction, infection, and inflammation.

2. **Subjects:** Specify number, age, gender, ethnicity, and whether healthy volunteers or patients. If patients, specify the disease or condition and indicate how potential subjects will be identified. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided. NIH applications require that women, minorities, and children be included or that their exclusion be justified. If children are involved, refer to "Children as Research Subjects".

Infants with a clinical diagnosis of CF in the neonatal period, who are typically immediately referred to the UNC CF Center for further diagnosis and clinical care. These infants will mainly consist of those born with meconium ileus, a perinatal bowel obstruction syndrome which is nearly unique to CF and occurs in roughly

15% of newborns wth CF. Additional subjects could include siblings of known CF patients who undergo diagnostic testing prenatally, or in the first weeks of life. Subjects will be recruited at age 2 months or less. As per the expected epidemiology of CF, gender distribution will be equal, and Caucasians will outnumber African-Americans and Hispanics approximately 20:1. It is anticipated that 2-8 patients per year will be recruited from the UNC CF Center and its satellite centers in Asheville and Charlotte. We will aim to study 8-10 infants total in order to be sure we can include 4-5 with no infection prior to the first bronchoscopy. It is anticipated based on previous rates of meconium ileus that we can complete this study in 3 years or less. Control (non-CF) BALF will be obtained through an existing approved protocol (94-PED-275). Control BALF will be selected from among children < age 12 months undergoing bronchoscopy and BALF for clinical indications.

3. **Inclusion/Exclusion criteria:** List required characteristics of potential subjects, and those that preclude enrollment.

Inclusion (CF subjects): Diagnosis of CF by pilocarpine iontophoresis, or genotype diagnostic of CF. (controls): Clinically indicated bronchoscopy, less than or equal to 12 months of age.

Exclusion (CF, controls): Use of oral or inhaled corticosteroids, NSAIDs, or leukotriene inhibitors within 1 week prior to time of bronchoscopy. (CF): History of adverse reaction to sedatives or to bronchoscopy. History of bleeding disorder.

4. **Full description of the study design, methods and procedures:** Include the type of experimental design; study procedures; sequential description of what will be asked of/done to subjects; assignment of subjects to various arms of the study if applicable; doses, frequency and route of administration of medication and other treatment if applicable; kinds of data to be collected; primary outcome measurements; and follow-up procedures. If the study involves treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls. This section (4) should generally not exceed 2 single-spaced pages using 12-point type.

The proposed study is a longitudinal study of BALF changes over the first year of life in infants with CF. Subjects will be tested at 3 tme points: 1) after diagnosis, within the first 6 weeks after birth; 2) at 6 months of age; 3) at 12 months of age.

At each study visit, subjects will be asked to come to UNC Children's Hospital where they will undergo flexible fiberoptic bronchoscopy according to standard clinical protocol. Subjects will be held NPO for 4 hours prior to time of procedure, and will be processed through pre-care in identical fashion to children undergoing clinically-indicated bronchoscopy. An intravenous line will be placed prior to procedure, and presedation with chloral hydrate will be given p.o. Once the patient is transported to the bronchoscopy laboratory, baseline monitoring parameters will be established then the subject will receive i.v. fentanyl and versed at standard doses. When sedation is adequate, 2% lidocaine drops and Otrivin drops will be placed in the nose, and the scope passed to the larynx where additional 2% lidocaine will be instilled via the scope onto the vocal cords. The scope will be passed to the trachea and the lower airways briefly examined. BALF will be obtained by instilling 10 ml into the right middle lobe bronchus then suctioning intermittently. Identical procedure will be done in lingula and the 2 samples pooled. Bronchoscope will then be removed and patient recovered from sedation in the bronchoscopy laboratory and PACU as per standard clinical protocol.

BALF will be processed as follows: Aliquot sent to the UNCH Clinical Microbiology Labs for bacterial, viral, fungal cultures. Cell count will be performed using hemocytometer, and slides for differential cell counts prepared using modified Wright stain. Visible mucus plugs will be removed from BALF using

sterile glass pipettes, then fixed immediately for future histochemical and immunohistochemical staining. The remainder of the BALF will be divided into aliquots of unprocessed and centrifuged, cell-free BALF supernatant, and frozen at –80^{o}C for future assays of mucins and inflammatory markers.

Any remaining BALF after use in the studies described above will be kept at –80 C for possible use in future studies. This is done because of the valuable and rare nature of these specimens for CF research. Parents of subjects will receive the appropriate addendum to the consent form for storage of specimens for as-yet-undesignated research, as per IRB rules.

Parents of subjects will also be asked to complete a questionnaire regarding respiratory symptoms and medication use in the period preceding the procedure.

5. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable**: Include the number of required visits and approximate duration of each visit.

Entire study: 3 years. Individual participation: 3 procedures over 12 months. Each visit will require approximately 4 hours from arrival at Pre-Care to discharge from PACU.

6. **Where will the subjects be studied?** If off UNC-CH campus, list locations.

UNC Children's Hospital Bronchoscopy Laboratory; recovery from sedation in UNC Hospitals PACU.

7. **Full description of risks and measures to minimize risks:** Include risk of psychosocial harm (e.g. emotional distress, embarrassment, breach of confidentiality, etc.) economic harm (e.g. loss of insurability) and legal jeopardy (e.g. disclosure of illegal activity) as well as known side effects of study medication, if applicable, and risk of pain and physical injury.

- Bronchoscopy can cause some irritation of the walls of the nose, throat or bronchial passage. If this occurs, it can cause some bleeding, cough, or wheezing (1-2% of patients). Pneumothorax and atelectasis can also occur but it is much rarer (less than 1%). These complications are minimized by using a very small bronchoscope (1/8 inch wide) and having the procedure done only by experienced bronchoscopists. The investigators are very experienced at doing this procedure.

- The bronchoscope will partially block the breathing passage while the procedure is being done. In some cases this casues significant respiratory distress, but this is rare in the absence of structural airway anomalies, due to the small caliber of the scopes used. Risk is minimized by continuous monitoring of pulse oximetry, respiratory and heart rates, as well as direct visual monitoring by a Respiratory Therapist, as per strandard bronchoscopic procedure. If desaturation or bradycardia occurs, the procedure is halted and support given as necessary.

- Insertion of the bronchoscope may cause a risk of infection in the lung. The risk of this is very small (less than 1%) because the bronchoscope is sterile, although the bronchoscope may transfer infection from the nose to the lung.

- Bronchoscopy can cause a transient slowing of the heart rate in 1-2% of cases. This can occur even if the child's oxygen level is kept high. Risk of complications from this are minimized by continuous heart rate monitoring.

- Bronchoalveolar lavage (BAL) can cause transient fever during the 24 hour period following the procedure. This is seen more commonly in young children only when there is a significant amount of inflammation already present in the airways. Fever after bronchoscopy (BAL) is treated with acetaminophen (Tylenol®).

- Medications used to sedate for bronchoscopy (chloral hydrate, fentanyl, and versed) can be associated with slowing of breathing or apnea, at high doses. This risk is minimized by dosing within standard weight-based guidelines. Complications from this are minimized by the monitoring described above. If desaturation or bradycardia occurs, the procedure is halted and support given as necessary.

- Risk for breach of confidentiality is minimized by encoding samples with access to subject's identity limited to the investigators.

8. **Benefits to subjects and/or society:** The possibility of benefit to society should be clearly distinguished from the possibility of benefit to the individual subject, if any. If there is no direct benefit to the individual subject, say so. Do not list monetary payment as a benefit.

It is widely believed that early treatment of bacterial infections in the CF airway is likely to slow progression of lung disease. Bronchoscopy(BAL) is the only procedure available to obtain reliable microbiologic cultures from the lower airway of young patients with CF. Therefore the culture results obtained as part of this study may be directly beneficial in terms of choosing appropriate antibiotic therapy for pulmonary infections. From a longer term perspective, the results of this study may lead to discovery of important information regarding the pathogenesis of CF lung disease, potentially altering therapeutic strategies in early life.

9. **Inducements for participation:** If monetary, specify the amount and how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it.

Subjects will receive $100 for each bronchoscopy completed, plus another $50 for completing the entire series of 3 procedures, for a total of $350 for completion of the study. Parents of subjects will receive $50 for each bronchoscopy visit completed (including completion of questionnaire), and an additional $50 for completion of the entire series of 3 procedures, for a total of $200 for the study. Travel expenses will be reimbursed at a rate of $0.33 per mile, and parking will be provided free of charge in the parking deck. For subjects who do not complete the study, compensation will be prorated as indicated above.

10. **Costs to be borne by subjects:** Include clinic fees, diagnostic and laboratory studies, drugs, devices, transportation, all professional fees, etc. If there are no costs to subjects, indicate this.

None

11. **Statistical analysis:** If this is a single-center clinical trial, provide evidence that the sample size is sufficient to achieve the study aims and tell how the data will be analyzed. If a multicenter trial, indicate where and by whom statistical analysis will be performed.

Statistical comparisons of BALF mucin quantities (CF pre-infection vs. CF post-infection) will be done using paired tests (nonparametric vs parametric depending on distribution of data); (CF vs nonCF) will be compared using unpaired tests. Correlation between mucin and other BALF parameters such as neutrophils,

cytokines, or bacterial quantity will be done using linear regression (with transformed data if appropriate). Using preliminary data from the laboratory of Dr. C. William Davis and the formula $N \approx [2(SD)^2 (z_\alpha + z_\beta)^2]/\Delta^2$ [Mitulsky, H. 1995. Intuitive Biostatistics. Oxford University Press, New York, p. 198], an N of 5 CF patients should be sufficient to detect a 2-fold difference between CF and nonCF BALF mucin levels, and an N of 8 would detect a 1.6-fold difference, with 90% power and a significance level of $P < .05$.

12. **Methods of recruiting:** Tell how prospective subjects are contacted. If they are UNC Hospital patients, initial contact should be made by their treating physician, or by someone whom the patients know to have legitimate access to their medical records (for example, a clinical director). This may be accomplished by means of a letter from that individual to prospective subjects, requesting the patient's permission to be contacted by the investigator.

All infants with CF or with meconium ileus referred to UNCH are seen by a member of the Pediatric Pulmonary Medicine division for initial workup, either in the outpatient clinics or on the inpatient services. Recruitment will occur by surveillance at the weekly division clinical meetings. Investigators will ask the phyisican primarily responsible for care of the patient to introduce the study to parents.

13. **How will informed consent be obtained?** Describe the process. When the consent of a legally authorized representative is substituted for consent of the adult subject, explain why this is necessary. If non-English-speaking subjects will be enrolled, a consent form should be prepared in their foreign language. Someone who is fluent in the subjects' language must be available to interpret.

If parents are interested, one of the clinicians on the investigation team will obtain informed consent from parents via direct interview. This will most likely occur in the inpatient setting but could be during a scheduled clinic visit.

Submit the following to: IRB Office, Building 52, CB# 7097, UNC-CH

Original and 2 copies of this completed, signed application;
3 copies of each consent and assent form;

If applicable, submit also:
- **3 copies of the Master Protocol; represented by the full application if NIH/DHHS grant**
- **1 copy of the Investigator's Brochure;**
- **3 copies of questionnaires or survey instruments;**
- **3 copies of recruitment materials (letters, ads, posters, TV or radio scripts).**

For addition information call the IRB Office at xxx-xxxx or consult the IRB website at http://www.med.unc.edu/irb/