# *TITLE: PREMATURITY AND RESPIRATORY OUTCOMES PROGRAM (PROP)*

# *CORE DATABASE PROTOCOL*

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This document was prepared by the University of Pennsylvania, School of Medicine,
Clinical Research Computing Unit, in the PROP Data Coordinating Center.

# *STUDY SUMMARY*

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| Title | Prematurity and Respiratory Outcomes Program (PROP) Core Database Protocol |
| Study Design | Observational prospective cohort |
| Study Centers (6) | Cincinnati University Hospital, Washington University, University of California at San Francisco, Vanderbilt University, University of Rochester & University at Buffalo, and Duke University & Indiana University |
| Primary Hypothesis | In survivors of extreme prematurity to 36 weeks PMA, specific biologic, physiologic and clinical data obtained during the initial hospitalization will predict respiratory morbidity as defined by respiratory health care utilization and respiratory symptoms, between discharge and 1 year corrected age. |
| Significance | PROP will identify suitable predictors of respiratory outcome that may serve as surrogate endpoints in future trials of prevention and therapy of respiratory diseases in preterm infants. |
| Objectives | 1. To evaluate if a series of quantitative respiratory assessments performed prior to NICU discharge in extremely preterm infants will predict symptomatic respiratory disease and health care utilization during the first year of life more accurately than the current clinical or physiological diagnoses of BPD.<br>2. To create a biospecimen repository for preterm infants, by collecting DNA from PROP study participants and their parents and by obtaining tracheal aspirate and urine samples from infants at pre-specified postnatal ages, that can be used to stratify patient populations based on molecular as well as clinical phenotypes.<br>3. To collect detailed descriptive data on respiratory medication exposures in extremely preterm infants from birth through 1 year of age in order to understand the variability of current prescribing practice and its relationship to respiratory morbidity.<br>4. To create a multi-center core database containing prospectively collected, standardized, clinical data and to test for associations between these clinical parameters and the novel, putative biomarkers with the goals of quantifying severity, refining diagnosis and prognosis, and identifying mechanisms of causation of respiratory disease in preterm infants.<br>5. To assess pulmonary physiologic outcomes at 1 year of age by infant pulmonary function testing (iPFT) in a subset of infants to identify associations between standard measurements of lung function at 1 year and the quantitative and qualitative assessments of respiratory function and morbidity between 36 weeks PMA and 1 year of age. |
| Number of Infants | 750 survivors to a postmenstrual age of 36 weeks or discharge |
| Main Inclusion Criteria | Gestational Age 23 0/7 – 28 6/7 weeks |
| Duration of Study | 4 Years |
| Statistical Methodology | Model based and non-model based analyses, including descriptive analyses, and generalized linear mixed models (GLMM) |

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# 1. Introduction and Background

Acute and chronic respiratory morbidities are common in extremely small and preterm infants.[1] Increased survival of very premature infants is increasing the numbers of children with chronic lung disease.

The preterm birth rate in the US in 2008 was 12.3%.[2] Since 1990, preterm births < 34 weeks gestation have increased by 10% and late preterm births (34 to 36 weeks gestation) by 25%.[2] Between 20 and 35% of all extremely low birth weight infants die before their first discharge home.[1, 3] Risk-adjusted mortality rates vary considerably between different hospitals.[4, 5] Of those who survive the immediate neonatal period, 35-45% develop bronchopulmonary dysplasia (BPD), (Vermont Oxford Network data, 2009), when defined as need for oxygen therapy at 36 weeks postmenstrual age. The majority of infants with birth weights less than 1 kg will have a diagnosis of BPD by the Consensus Conference definition of 28 days in oxygen.[6] BPD results from abnormal repair and impaired lung development after acute lung injury. Infants with BPD are more likely to die than those without chronic lung disease even if they survive the initial hospitalization. The odds ratio for BPD as a predictor of post-discharge mortality among 10,602 VLBW infants in the Israeli Neonatal Network was 2.4 (95% CI: 1.4–4.2).[7]

However, the traditional categorical approach of classifying BPD as absent or present is likely an oversimplification. It is far more likely that very preterm infants develop respiratory disease across a continuous spectrum of illness severity ranging from mild to severe.[8] Importantly, very preterm infants without a categorical diagnosis of BPD have a fairly high rate of respiratory morbidity at a corrected age of 18 to 22 months.[1] Ex-preterm infants with and without a categorical diagnosis of BPD return frequently to pediatricians, emergency rooms and pulmonologists with symptoms and signs of post-prematurity respiratory disease (PRD): intermittent or chronic wheezing, cough without cold, poor growth, apnea and cyanosis, and lower respiratory tract infections.[9-13] More than 50% of all survivors after very preterm birth are readmitted to hospital at least once during their first 2 years of life.[14] Respiratory causes account for the majority of hospital readmissions and include lower respiratory tract infection, apparent life threatening events, bacterial pneumonia and respiratory viral illness such as infection with the respiratory syncytial virus.[15]

Although pulmonary compliance improves over time after discharge from the NICU, very preterm infants have chronic airflow limitations that may result in airway obstruction and gas trapping throughout early childhood. Impaired lung function in premature infants can persist into adulthood, contributing to chronic respiratory diseases including asthma and emphysema. The effects of extreme prematurity on the aging lung are unknown.[16]

Efforts to improve the respiratory outcomes of low birth weight, fragile infants are an important public health goal. There are currently no objective measures to predict which preterm infants will have persistent respiratory problems after discharge from the hospital. The understanding of the anatomy of BPD relies on a few anatomic studies of autopsy tissues, which may not be representative of the lungs of infants who survive.[17, 18] Furthermore, most of the available clinical and physiological information has been obtained in small series of patients who were cared for before the widespread use of antenatal steroids, postnatal surfactant, and gentle approaches to respiratory support, all of which may change the clinical spectrum of chronic respiratory disease.[19, 20] Given the high prevalence of lasting respiratory morbidities in ex-preterm infants, strategies and tools to identify newborns at risk of low lung function and to reduce

acute and chronic respiratory morbidity are urgently needed. The intensive characterization of a large cohort of extremely preterm infants may provide the insights needed to develop a better clinical definition of BPD.

The Prematurity and Respiratory Outcomes Program (PROP) will investigate multiple research hypotheses on the molecular mechanisms that contribute to respiratory disease risk of premature neonates over the first year of life. A standardized bundle of clinical and non-invasive respiratory assessments that is tailored to the respiratory status of the infant at the time of testing will be performed near the estimated due date of very preterm infants to describe the cohort and to predict the severity of respiratory outcomes in the first year of life. It is hoped that these respiratory assessments may be sufficiently predictive to serve as surrogate endpoints in future trials of prevention and therapy.

This protocol describes a collaboratively developed multicenter prospective cohort study of very preterm infants from birth through the time of discharge from the NICU and up to 1 year of age, corrected for the degree of prematurity. Each of the 5 clinical research centers (CRC) will participate in a cooperative and interactive manner with all other CRCs, leveraging local resources and sharing biospecimens and patient data with all other collaborating sites.

The program will use the cooperative agreement mechanism and require multidisciplinary expertise including neonatologists, pediatric pulmonologists, pharmacists, and physiological, molecular, biological, biostatistical and bioinformatics scientists. Participating sites will plan and coordinate their own single-site biomarker research and will develop and implement a shared protocol for respiratory phenotyping and respiratory outcomes of extremely preterm infants. The Data Coordinating Center (DCC) will collaborate on study design, manage data collection and data monitoring and provide support for standardization of definitions, clinical report forms, and analysis.

Successful multi-center research networks require collaborative efforts, precise planning and documentation of all procedures, regular communication among all participants, and uniform adherence to the research objectives and protocol. The DCC will cultivate an atmosphere of scientific collaboration and cooperation, establish and promote effective communication, and coordinate the design, development and conduct of all multi-center protocols within the PROP. The DCC will coordinate protocol and consent form development, utilizing an administrative structure that facilitates these processes.

# 2. Study Hypothesis, Objectives and Design

Primary Hypothesis:

In survivors of extreme prematurity to 36 weeks PMA, specific biologic, physiologic and clinical data obtained during the initial hospitalization will predict respiratory morbidity as defined by respiratory health care utilization and respiratory symptoms, between discharge and 1 year corrected age.

## 2.1. Primary Outcome: Post-prematurity Respiratory Disease

The primary goal of the PROP studies (single center and multicenter protocols) is to identify biomarkers (biochemical, physiological and genetic) and clinical variables that are associated with and thus potentially predictive of pulmonary status in preterm infants up to 1 year corrected age. An objective and validated measure of pulmonary outcome at 1 year does not currently exist. Some promising measures are in development but not yet ready for use in a multi-center large

clinical study. For example, reduced alveolar number is a key pathologic feature of BPD .[21] Physiologically, this is reflected in decreased diffusion capacity of the lung to carbon monoxide (DLCO), and infants with BPD have lower DLCO compared to healthy full term controls.[22] However, measurement of infant DLCO in a multi-center study is not feasible in PROP because of the lack of standardized equipment, the need for $C^{18}O$ instead of standard CO, and the need for a mass spectrometer to measure CO instead of a standard infrared sensor.[23]

Moreover, the PROP investigators firmly believe that the burden of chronic respiratory illness on the infants and their families is of utmost importance. Therefore, we propose a composite primary outcome of morbidity that is based on serial parental reports of respiratory symptoms, medications, hospitalizations and dependence on technology during the first year of life.

Data collection for the outcome assessment will be based on interviews conducted with the infant's main caregiver at 3, 6, 9 and 12 months corrected age. The time frame for data collection is based on questions "since last contact." Numerous epidemiological studies of asthma have used parental or self report of symptoms, physician-diagnosed asthma and allergies, or the use of medications (which may abrogate symptoms) as critical outcomes.[16, 24-26] Recent studies have most commonly employed the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire.[27] These studies have collected data regarding 1 year old children, although not children before one year of age. The questionnaire to be utilized in PROP has been modified from the Tucson Children's Respiratory Study (CRS)[20, 28-31] and the Breathing Outcomes Study. The latter is an ancillary study for the NICHD Neonatal Research Network (NRN) Surfactant Positive Airway Pressure and Pulse Oximetry Trial (SUPPORT).

Survey items selected for the determination of the primary outcome will be focused on the following four domains, with any positive response to any element identifying morbidity:

1. **Respiratory medications**: inhaled bronchodilators, inhaled steroids, systemic steroids, methylxanthines, diuretics, pulmonary vasodilators
2. **Hospitalizations for cardiopulmonary causes**: any hospitalization regardless of duration
3. **Symptoms:** any wheeze, cough without cold
4. **Home technology dependence**: use of home oxygen, ventilator or CPAP/BiPAP of any durations since last contact

A late death after a postmenstrual age of 36 weeks from cardio-respiratory failure will also meet the definition of PRD.

The primary outcome will be dichotomous, and defined as "No substantial post-prematurity respiratory disease" or "Post-prematurity respiratory disease." To be classified as having post-prematurity respiratory disease, infants must have a positive response in at least 1 of 4 morbidity domains during at least 2 separate parental interviews. Quarterly data collection up to 1 year corrected age will allow us to identify phenotypes based on the trajectory of post-prematurity respiratory disease and how these different trajectories predict later lung function and the diagnosis of asthma, if we continue to follow this cohort of children.

Follow up data from recently published randomized controlled trials and observational studies in similar but not identical populations of very preterm infants suggest that the incidence of this primary outcome will be at least 30% and possibly as high as 60%.[32-35]

## 2.2. Specific Aims

The specific aims of the PROP research program are:

1. To evaluate if a series of quantitative respiratory assessments performed prior to NICU discharge in extremely preterm infants will predict symptomatic respiratory disease and health care utilization during the first year of life more accurately than the current clinical or physiological diagnoses of BPD.
2. To create a biospecimen repository for preterm infants, by collecting DNA from PROP study participants and their parents and by obtaining tracheal aspirate and urine samples from infants at pre-specified postnatal ages, that can be used to stratify patient populations based upon molecular as well as clinical phenotypes.
3. To collect detailed descriptive data on respiratory medication exposures in extremely preterm infants from birth through 1 year of age in order to understand the variability of current prescribing practice and its relationship to respiratory morbidity.
4. To create a multi-center core database containing prospectively collected, standardized, clinical data and to test for associations between these clinical parameters and the novel, putative biomarkers with the goals of quantifying severity, refining diagnosis and prognosis, and identifying mechanisms of causation of respiratory disease in preterm infants.
5. To assess pulmonary physiologic outcomes at 1 year of age by infant pulmonary function testing (iPFT) in a subset of infants to identify associations between standard measurements of lung function at 1 year and the quantitative and qualitative assessments of respiratory function and morbidity between 36 weeks PMA and 1 year of age.

## 2.3. Study Population

The risk of short and long-term respiratory morbidity in preterm infants is inversely related to gestational age at birth. This is why the PROP investigators will study the most immature infants <29 weeks of gestation in this multi-center protocol. Importantly, infants who die early during their NICU stay will not contribute any data towards the major goal of this project, which is the prediction of post-prematurity respiratory disease up to one year of age. It is readily seen by comparing Tables 2.3.1 and 2.3.2 that one approach might have been to wait until the infants have survived at least 7 days before enrolling them in PROP. This would clearly lessen the loss of study participants due to early mortality from the 1 year cohort of survivors. However, several sites have parallel evaluations of the PROP study participants for their single center protocols that require the collection of biospecimens shortly after birth.

**Table 2.3.1 Gestational age distribution of recent admissions at Vanderbilt University, with mortality through the 1st year of life, among infants who survived at least 2 days**

| | | |
|---|---|---|
| **Gestational Age** | Percent of total admissions <28 weeks excluding nonviable infants | Mortality to 1 year |
| 23 | 8% | 44% |
| 24 | 20% | 28% |
| 25 | 22% | 15% |
| 26 | 26% | 19% |
| 27 | 24% | 7% |
| | | |

**Table 2.3.2 Gestational age distribution of recent admissions at Vanderbilt University, with mortality through the 1st year of life, among infants who survived at least 7 days**

| | | |
|---|---|---|
| **Gestational Age** | Percent of total admissions <28 weeks excluding nonviable infants | Mortality to 1 year |
| 23 | 5% | 17% |
| 24 | 19% | 18% |
| 25 | 22% | 16% |
| 26 | 28% | 13% |
| 27 | 26% | 3% |
| | | |

An alternative approach is to have few restrictions on the probability of the initial survival but to "replace" children who die with new recruits. Therefore, PROP infants who do not survive to 36 weeks PMA will be "replaced" by new participants to achieve the desired target sample size of 750 infants who are likely to survive to one year. The early deaths will be used in the assessment of total mortality as a secondary outcome.

Moreover, early mortality will be strongly correlated with the degree of immaturity (Tables 2.3.1 and 2.3.2). To optimize the numbers of infants who will be available for follow up through the first year of life at each week of gestational age between 23 and 28 weeks, we propose to enroll the PROP study participants in the following 5 strata:

i. 23 0/7 to 24 6/7 weeks GA
ii. 25 0/7 to 25 6/7 weeks GA
iii. 26 0/7 to 26 6/7 weeks GA
iv. 27 0/7 to 27 6/7 weeks GA
v. 28 0/7 to 28 6/7 weeks GA

Without this stratification, the final cohort of survivors at 1 year would contain only a small minority of the most immature infants who are born at the margin of viability. One of the greatest benefits of identifying additional biomarkers for respiratory morbidity in the first year of life would be the stratification by risk within gestational age groups with the potential to identify phenotypic subgroups, patient-specific mechanisms and therapeutic targets. It is therefore important to enroll sufficient participants to allow data analysis within gestational age subgroups.

The DCC will monitor the number of infants who survive to 36 weeks PMA in each of the 5 strata. Enrollment into a particular stratum will end as soon as 150 survivors to 36 weeks PMA have been documented.

The most immature infants born at 23 and 24 weeks gestation have been combined into one stratum because the investigators in the 6 clinical sites are concerned that they will not be able to enroll 300 survivors to 36 weeks PMA who are born at these very low gestational ages. However, in the event that enrollment and/or survival at these low gestational ages is higher than anticipated, we will not limit the number of infants in stratum 1 to a total of 150 infants.

#### 2.3.1. Inclusion Criteria

a. Infants who are less than or equal to 7 days old
b. Gestational Age (GA) between 23 weeks and 0/7 days and 28 weeks and 6/7 days

#### 2.3.2. Exclusion Criteria

Infants who meet any of the following conditions will be excluded from the PROP cohort:

a. The infant is not considered to be viable (decision made not to provide life-saving therapies)
b. Congenital heart disease (not including PDA and hemodynamically insignificant VSD or ASD)
c. Structural abnormalities of the upper airway, lungs or chest wall
d. Other congenital malformations or syndromes that adversely affect life expectancy or cardio-pulmonary development
e. Family is unlikely to be available for long-term follow-up

We recognize that extremely preterm infants who are born outside of the tertiary care centers and transported into the PROP clinical centers soon after birth have an increased risk of adverse pulmonary outcomes compared with infants who are inborn. However, we intend to study the entire spectrum of respiratory morbidity in the target population and will therefore enroll outborn infants provided the data that are needed for the PROP multi-center core database can be obtained.

# 3. Study Measurements and Procedures

## 3.1. Assessments During Hospitalization in the Neonatal Intensive Care Unit

Standardized data collection will include documentation of screening for eligibility and consent as well as pertinent maternal and infant baseline characteristics. Acquired co-morbidities of prematurity will be recorded at a postmenstrual age (PMA) of 40 weeks, or discharge if earlier. Co-morbidity data will include diagnosis and treatment of patent ductus arteriosus (PDA), pulmonary air leak, acquired airway anomalies, culture-proven infections, necrotizing enterocolitis, evidence of brain injury on neuroimaging tests, surgical procedures, and retinopathy of prematurity. In addition, detailed respiratory, growth, nutrition and medication data will be collected daily as described in sections 3.1.1 and 3.1.2. All of these data are routinely recorded in the medical charts of all eligible infants and will be extracted and transmitted to the DCC by the research coordinator

at each clinical site. In addition, a discharge interview will be conducted, where data will be collected with regard to family history of respiratory illness, allergy and atopy, potential environmental exposures and contact information.

### 3.1.1. Daily Respiratory, Growth, and Nutrition Data

During the infant's hospitalization, clinical data will be collected on a daily basis until 40 weeks PMA, or discharge if earlier that describe use of respiratory supports, duration and amount of exposure to supplemental oxygen and nitric oxide, growth parameters, and feeding status . Respiratory data will be collected on a weekly basis from infants who remain hospitalized beyond 40 weeks PMA.

### 3.1.2. Daily Medication Data

Data on all commonly used classes of drugs in the neonatal intensive care unit will be collected. For most drugs, the research coordinator will simply record whether or not a particular drug was administered.

However, with additional resources obtained as a supplement from the Eunice Kennedy Shriver National Institute of Child Health and Development (NICHD) under the Best Pharmaceuticals for Children Act, four classes of commonly used respiratory drugs will be studied more extensively with respect to dose and administration, duration of use and adverse effects: diuretics, inhaled bronchodilators, methylxanthines, inhaled and systemic corticosteroids. The PROP investigators will collect detailed data on these specific respiratory medication exposures from birth to one year corrected age in this prospective multi-center cohort of very preterm infants. In the NICU, the daily data collected from the medical record and will include name of medication, route of administration, dose and frequency. This will provide much needed insights into the current use of respiratory drugs in this high-risk population. There are no current evidence-based practice guidelines for most of the respiratory medications that are used in preterm infants. Moreover, the great majority of these respiratory medications are prescribed for this population "off-label". Understanding current drug prescribing practice and its potential variability in this vulnerable population of children will have important implications for the design of future clinical research studies in sick and preterm newborns who are at risk of developing chronic respiratory disease.

### 3.1.3. Collection of Biospecimens for a PROP Repository

The overriding hypothesis for this project is that measurement of selected biomarkers and DNA polymorphisms in this repository of specimens, combined with the robust clinical database, will provide new information related to biological events associated with the pathogenesis and occurrence of post-prematurity respiratory disease (PRD). Although diverse mechanisms contribute to the etiology of chronic lung disease in preterm infants, interactions between developmental and genetic influences are likely to play a role in determining the respiratory outcomes of prematurity.[36-38] Disruptive genetic variants in pathways mediating structural development, functional development of fluid fluxes, surfactant metabolism, anti-oxidant capacity, or regulation of inflammation and repair, may provide susceptibility to adverse pulmonary outcomes in the face of a developmentally immature lung.

Tracheal aspirate and urine specimens provide a source of potential biomarkers related to pulmonary outcome, and a majority of clinical sites have proposed single-center studies with these samples. Availability of samples from other sites via the repository will allow expanded studies of selected biomarkers of interest. The repositories of tracheal aspirate and urine samples will be available to all PROP investigators for validation of individual site biomarker results in a larger cohort of infants. The repositories will also permit studies of new biomarkers of interest or utilization of new or improved technologies such as proteomics for future biomarker discovery. The repository of DNA from infants and parents will also be available to all investigators for future genotyping studies.

Four centers have proposed projects that involve tracheal aspirate assays or genotyping as part of either their single center or multi-center proposal. Four centers have proposed studies of urinary biomarkers. The rapid changes in sequencing technology will permit acquisition of significantly more sequence data at less cost within the next 3-4 years; the DNA repository is therefore viewed as a resource for PROP investigators to develop additional proposals in later years that utilize the power of this large number of samples and extensive sequencing capacity to address more specific mechanistic/genetic questions. Thus, expected use of the repositories is substantial and the biospecimen along with extensive clinical data provide a novel resource for PROP as well as future investigators. In addition, collection of these three types of biospecimen is relatively straightforward and non-invasive without a major impact on PROP personnel, families, or funding.

All centers will obtain samples of tracheal aspirate, urine and saliva (for DNA extraction) from enrolled infants. In addition, saliva will be obtained from mothers, and fathers when possible, of enrolled infants. Tracheal aspirate and urine samples will be archived in investigator-led laboratories at UCSF and Vanderbilt, respectively. The Center for Human Genetics Research (http://chgr.mc.vanderbilt.edu) at Vanderbilt University will serve as the repository for the DNA specimens. The following samples will be collected from study participants using standardized methods:

a. Saliva for DNA extraction will be collected preferably during the first week or alternatively later during hospitalization or at the 1-year follow-up visit
b. Tracheal aspirate samples will be obtained during routine suctioning of intubated patients for clinical purpose
c. Urine samples will be collected from cotton balls that are placed in the diaper
d. Tracheal aspirates and urine samples will be collected on the following schedule:
    - 2 samples at 2-4 days in the first week after enrollment;
    - 1 sample 7 days later;
    - 1 sample at 28 days postnatal

## 3.2. Respiratory Assessments at 36 Weeks PMA

A unique and important feature of the PROP protocol is the inclusion of physiologic biomarkers as potential predictors of respiratory morbidity up to 1 year of age. We have selected a set of respiratory assessments that evaluate different potential mechanisms of respiratory disease. Respiratory inductive plethysmography (RIP) assesses alterations in tidal breathing resulting from reduced lung compliance and airway obstruction.[39, 40] Since infants with BPD and a history of

recurrent wheezing demonstrate airway reactivity in response to a bronchodilator,[41] we will assess changes in tidal breathing in response to bronchodilator using RIP. Continuous pulse oximetry during RIP will enable us to detect mild oxyhemoglobin desaturations associated with brief central apneas during sleep, which are associated with reduced functional residual capacity.[42] [42] To assess respiratory insufficiency under conditions of physical stress, continuous pulse oximetry will be performed during and shortly after oral feeding.

The above assessments will evaluate respiratory mechanics in the PROP cohort. However, some aspects of preterm respiratory disease may be more dependent on other alterations in respiratory function, such as ventilation/perfusion mismatch.[43] .[44] Infants who receive supplemental oxygen and/or flow via nasal cannula will undergo a standardized room air challenge to enable us to classify study participants according to the "physiologic" definition of BPD. Although the PROP investigators hope to improve upon the current definitions of BPD, we have designed our data collection tools and our respiratory assessments at 36 weeks PMA such that they will allow us to classify study participants according to all currently available definitions of BPD: (i) use of supplemental oxygen at 36 weeks PMA; (ii) NIH consensus definition; (iii) "physiologic definition.

Infants will receive these respiratory assessments dependent upon their respiratory status and on their ability to feed orally as shown in Figure 3.2.

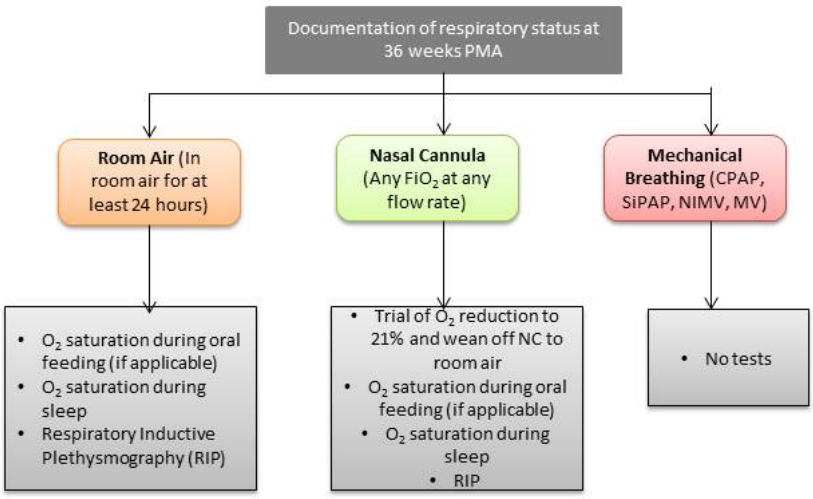


**Figure 3.2. Respiratory assessments at 36 weeks PMA:** The RIP with its associated tests of oxygen saturations during sleep and oral feeding will be done on separate days.

Infants whose respiratory and/or feeding status made them ineligible for some or all of the 5 respiratory tests may become eligible if they are still in hospital at 40 ± 1 weeks PMA. Eligibility criteria will be the same as in Figure 3.2. In addition, infants who received RIP testing at around 36 weeks PMA while on nasal cannula will undergo repeat testing at 40 weeks PMA if they are in room air for at least 24 hours and still in hospital..

The permissible window for all tests is 34 to 41 weeks PMA with one exception: the permissible window for the trial of flow and oxygen reduction (room air challenge) is 35 to 37 weeks PMA. However, an infant who was not eligible for this test at 36 weeks PMA, or who received it but failed, should be re-tested at 40+/- 1 week PMA if eligible and still in hospital.

### 3.2.1. Trial of Flow and $FiO_2$ Reduction to 0.21 in Infants Receiving Nasal Cannula Flow With or Without Supplemental Oxygen (Room Air Challenge)

A consensus definition of BPD severity at 36 weeks PMA was developed during an NICHD/NHLBI Workshop held in June 2000.[8] Mild BPD was diagnosed in preterm infants who are breathing room air at 36 weeks, after receiving at least 28 d of supplemental oxygen during their stay in the neonatal ICU. Infants receiving < 0.30 FiO2 at 36 weeks PMA were considered to have moderate BPD while severe BPD was defined as the need for ≥ 0.30 FiO2 or assisted ventilation (CPAP or mechanical ventilation). This clinical definition did not account for the variability in target SpO2 levels between clinicians and between hospitals. Walsh et al have evaluated a room air challenge test that aims to standardize the oxygen saturation target level on which a classification of oxygen dependency at 36 weeks PMA is based. Infants who were receiving low effective FiO2 < 0.30 by nasal cannula or hood (based on assumptions put forth by Benaron and Benitz and adapted by the STOP-ROP Trial[53]) and who met specific SpO2 criteria, were challenged with an oxygen and flow reduction test.[54] The initial oxygen and flow reduction procedure included a stepwise reduction in oxygen by 2%, followed by a reduction in flow by 0.1 LPM increments. Following each reduction, infants were monitored for 10 minutes before the next weaning step. Cannula were removed when flow was discontinued and infants were monitored for 1 hour. Infants failed the challenge if SpO2 fell to < 88% for 5 mins, or < 80% for 1 minute. Twenty-four infants were studied and the diagnosis of BPD decreased from 36% to 24%, using the physiological definition instead of the clinical definition.[54] Infant SpO2 consistently reached equilibrium after 5 minutes of each wean and all infants who failed during the room air observation period did so in the first 30 minutes.

A follow up multicenter study used a slightly modified protocol.[55] Flow was weaned first, by 0.5 LPM increments (for flows 1.0-2.0 LPM), and then by 0.1 LPM increments down to 0.1 LPM. Then FiO2 was reduced in 20% increments, until room air was reached. Wean steps were shortened to 5 minutes and the room air observation period was reduced to 30 minutes. The SpO2 threshold for failure was increased to 90%. The test procedure was changed again from weaning FiO2 first and flow second to weaning flow first because this was thought to shorten

the duration of the test in infants who failed (M. Walsh, personal communication). The testing procedure was safe (227 infants were studied), except for an increase in the number of infants with desaturation episodes (SpO2 < 88% for > 5 min) following the challenge test compared to the observation period prior to the test (3 vs. 15, P=0.0012).

It is presently not sufficiently understood how nasal cannula flow alone influences oxygenation in preterm infants. The application of nasal cannula flow may provide some positive airway pressure, with heated, humidified devices able to provide higher flows with less airway irritation than standard nasal cannula devices.[56] When studied with variable methodologies, the level of positive airway pressure delivered is influenced by the infant's weight, the nasal cannula flow and the outer diameter of the cannula.[56] Of 22 infants who underwent physiological reduction challenge while receiving FiO2 0.21 via nasal cannula 7 (32%) infants failed the challenge.[57] Thus, nasal cannula flow with FiO2 0.21 appears to influence oxygenation in these infants. However, when flow is weaned prior to FiO2, it is not possible to assess the effect of flow on oxygenation, since failures could result from either the reduction in the positive pressure due to the delivered flow, or from the reduction in effective FiO2.

In the PROP study, we will perform an oxygen and flow reduction challenge test in all infants who receive continuous respiratory support via nasal cannula, regardless of flow or effective FiO2, to assess the influence of nasal cannula support on oxygenation and to identify the need for ongoing respiratory support delivered by nasal cannula in a consistent manner. We will use an adaptation of the original protocol employed by Walsh et al, where FiO2 will be weaned prior to flow, in 20% increments and 5 min intervals, and then flow will be decreased in 10 min intervals, initially in 1 LPM increments until nasal cannula flow is ≤ 1.0 LPM, and then decreased by 50% increments to a minimum of 0.125 LPM. After a 10 minute observation period, the cannula will be removed. Infants will be monitored in room air for 1 hour, to allow for better detection of changes in oxygenation due to alterations in the delivery of positive pressure.

A detailed protocol and data collection instrument will outline the sequence of steps to safely conduct the test. Infants will be monitored continuously by pulse oximetry and vital signs before, during and after the test. If oxygen saturations are not maintained ≥ 90%, the test will not progress and the infant will be returned to their baseline level of respiratory support, or support as needed.

### 3.2.2. Respiratory Inductive Plethysmography (RIP)

Physiologic measures of respiratory function such as forced expiratory flows and measurement of lung volume can be obtained using infant pulmonary function tests (PFTs), but the techniques require invasive procedures or sedation. Because one of the goals of PROP is to identify biomarkers that can easily be applied to clinical practice, we will use less invasive respiratory physiologic measures that do not require sedation .

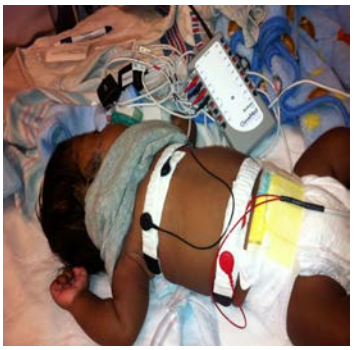


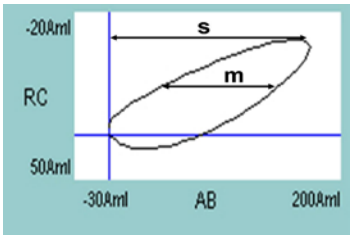
**Figure 3.2.3.A.** 4 month old former 26 week GA preterm infant wearing RIP equipment. Flexible inductance bands are placed around the chest and abdomen and attached to a wireless transmitter. The receiver for the RIP signals is connected to a notebook computer [not shown] and data are recorded for analysis. The infant's shirt has been pulled up to allow visualization of the bands.

RIP is based on the principle that the output of the respiratory system can be assessed by measuring the excursion of the rib cage and abdomen and relies on the assumption that there are two degrees of freedom of the respiratory system.[39] This assumes that motion during respiration is only from inward and outward motion of the rib cage or abdomen, and not from motion in other directions or from rotation about the spine. Therefore, the change in volume of the rib cage and abdomen together should equal the change of volume of air in the lungs, or the volume of air moved during respiration.

Rib cage and abdominal motion are recorded by measuring the change in impedance with bands that are placed around the rib cage and abdomen (Figure 3.2.3.A). The bands consist of a wire coiled in a sinusoidal pattern through the length of the band. There is an electrical connection point at each end of the band through which a low voltage current is passed through the wires during recording. Changes in the volume of the rib cage and abdomen result in stretching of the bands and subsequent changes in inductance, which are stored in an external recording device.

RIP can be used to measure a variety of respiratory parameters.[39] Comparing the relative timing of maximal excursion of the rib cage and abdomen produces a measurement of thoraco-abdominal asynchrony (TAA). Using a pneumotachograph, RIP signals can be calibrated to provide volume and flow values. However, because volume calibration is not necessary to measure phase angle or the ratio of time to peak tidal flow versus total expiratory time, volume measurements will not be part of the PROP study. Flow measurements can be made by calculating the derivative of volume over time, and the ratio of the time to peak tidal expiratory flow to expiratory time (Tpef/Te) can be determined without volume calibration. Tpef/Te has been found to be lower in infants with airway obstruction, and lower Tpef/Te is a risk factor for wheezing in the first year of life.[20, 58]

**Figure 3.2.3.B.** Calculation of the phase angle. RC excursion is plotted against AB excursion.




Respiratory disease can result in TAA through a variety of mechanisms, including increased negative intrapleural pressure due to increased airway resistance or increased chest wall compliance. The phase angle

(ϕ) is a quantitative measure of TAA that is defined by the relationship $\phi=\sin^{-1}(m/s)$, where m is the abdominal (AB) excursion at median rib cage (RC) excursion and s is the maximal AB excursion.[39] A plot of RC excursion vs. AB excursion (also known as a Konno-Mead plot) allows determination of m and s (Figure 3.2.3.B). In the absence of TAA, ϕ is zero, while paradoxical breathing represents maximal TAA, where ϕ=180°.

Although RIP was originally developed for adults with lung disease, it may be better suited for the study of infants. Infant chest wall compliance is much higher than that of older children and adults and therefore more easily deformed in the setting of lung disease.[59] This may make it easier to detect TAA. RIP has been used to demonstrate TAA in infants with BPD and airflow obstruction and in young children with acute upper airway obstruction.[60, 61] There is a correlation between ϕ and direct measures of lung function, such as airway resistance. In young children with acute upper airway obstruction ϕ fell after administration of inhaled racemic epinephrine,[61] suggesting that RIP can be used as an objective outcome measure of the effect of therapeutic interventions.

### Bronchodilator Response

Bronchodilator response (BDR) is present in some preterm infants. In one small study, 6 of 10 infants with BPD demonstrated a decrease in ϕ after inhalation of albuterol,[62] and in another larger study 67% of preterm infants had a fall in specific airway conductance after inhalation of a bronchodilator.[63] BDR in infants with BPD at 1 year of age is associated with a history of recurrent wheezing and lower lung function.[41] These observations suggest some preterm infants may have increased smooth muscle tone which may be a risk factor for lower lung function and/or increased symptomatic respiratory disease. For these reasons, we will assess BDR at 36 weeks PMA using RIP. BDR will also be assessed at 1 year of age in a subset of infants; see section 3.3.3.

## 3.2.3. Oxygen Saturation During Oral Feeding

The assessment of oxyhemoglobin desaturation during feeding is designed to test the hypothesis that infants who have ventilatory instability manifested by lower oxygen saturations with feeding at 36 weeks PMA, corrected for change in minute ventilation with feeding, will be more likely to have clinical respiratory disease over the first year of life. This test will be done in conjunction with the RIP and sleep tests at 36 weeks PMA.

Preterm infants are at increased risk of desaturation with feeding. This is especially true in preterm infants with BPD,[64, 65] bottle-fed infants,[66] infants with a nasogastric tube in place,[67] and in those infants with a lower baseline saturation before feeding.[68, 69] The mechanisms of desaturation in these infants is unclear, but may be related to underlying cardiopulmonary disease, uncoordinated feeding patterns, gastroesophageal reflux, upper airway obstruction, or to impaired central control of respiration. Cardiopulmonary disease in preterm infants may be due to impaired alveolarization and abnormal pulmonary microvascular development resulting in decreased surface area for gas exchange,[8, 17, 70] thickening of the gas exchange membrane as in fibrosis or edema,[21] or ventilation and perfusion mismatch induced by fibrotic obstruction and narrowing of airway and vascular structures promoting pulmonary vascular shunting, both physiologic and pathologic from collateral circulation.[71] Mechanisms of intermittent

hypoxemia with physical stress, such as with feeding or crying, include decreased ventilation[72] or increased pulmonary blood flow. Decreased minute ventilation, due to decreased tidal volume, respiratory rate, or both, diminishes oxygen delivery to the alveoli. Increased blood flow with exertion decreases transit time of blood through the pulmonary capillary beds, thereby decreasing time of oxygen uptake. Normal pulmonary and cardiovascular function should provide adequate reserve to maintain normal oxygenation even under these conditions of stress. The physical stress of feeding may unmask otherwise clinically silent cardiopulmonary disease. We hypothesize that feeding preterm infants with diminished pulmonary and/or cardiovascular reserve will more easily and reproducibly quantify this diminished reserve than other unpredictable stressful events such as crying. The timing of feeding is predictable and the workload can be more easily quantified, e.g. as a volume of feeding for the infant's weight consumed over a defined period of time.

Previous studies have documented parameters of ventilation that may be abnormal during feeding in preterm infants; however, no attempts have been made to date to correlate the results of feeding studies with long-term respiratory outcome. A study of "healthy" preterm infants, born at mean 32.3 (+/- 0.4) wks CGA and studied at a mean 23 (+/- 3) days of life, demonstrated decreases in minute ventilation (Ve), respiratory rate (f), inspiratory time (Ti), and tidal volume (Vt), associated with the initial sucking burst during feedings, with recovery of all of these parameters, except for Ti, during the intermittent sucking phase of feeding.[72] In these infants, the mean "workload" was a volume of formula consumed of 14 ml/kg over 14 minutes for the 34-35-6/7 wk infants, and 18 ml/kg over 10 minutes for the 36-38 wk infants. Oxygen saturations were not recorded in this study, but this workload was associated with significant changes in minute ventilation, primarily due to a reduced respiratory rate. Another study of preterm infants demonstrated a correlation between the severity and duration of desaturation events with feedings and the duration of apneic pauses.[73] Infants with both mild and severe BPD may experience tachypnea between sucking bursts.[74] In a study of healthy term infants, induced changes in feeding stimulus (increased volume of feeding by gravity) resulted in decreases in minute ventilation by only inducing a decrease in respiratory frequency, without a change in tidal volume.[75] Although it may be sufficient to measure changes in respiratory frequency during feeding to assess the relationship of ventilation to oxygen saturation, we will be able to measure relative changes in minute volume using the RIP equipment.[76] Utilizing the RIP equipment, we will calculate relative changes in minute ventilation from baseline and during feedings as a measure of change in the ventilatory pattern. Changes in oxygen saturation will be correlated with changes in minute ventilation from baseline, during and after feeding.

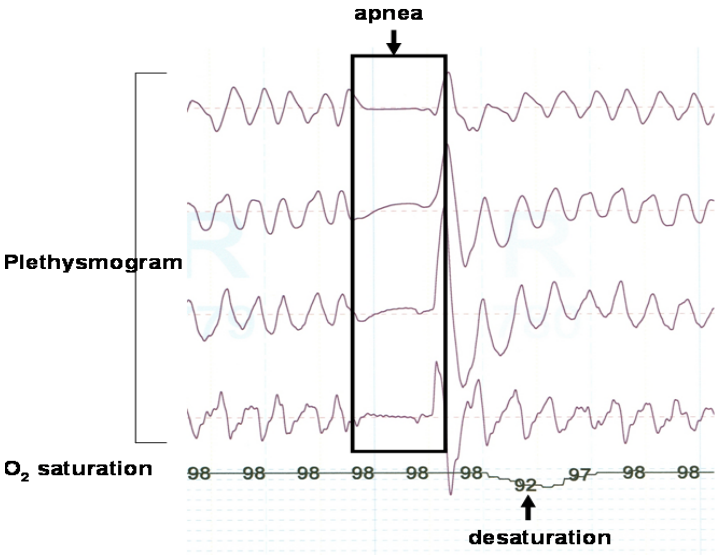
A detailed protocol and data collection instrument will outline the sequence of steps to conduct the Oxygenation with Feeding test. The study will be done with RIP impedance bands, electrocardiogram leads, and pulse oximeter in place. Data will be collected before feeding (2-3 minutes baseline in bed and then semi-upright in a standardized feeding position (approx 30-45 degree angle), continuously during PO feeding of a usual volume for the infant (minimal oral feeding volume criteria will determine eligibility for this test), and following feeding (2-3 minutes in semi-upright feeding position and then in the position in which the infant is normally placed after feeding). During each epoch, data from the recording oximeter will be

used to calculate mean SpO2, number of desaturations < 90% lasting for > 1 second duration, number of desaturations > 4% from baseline, lasting for > 1 second duration. The occurrence of clinically significant events that interrupt feeding will be recorded.

The proposed protocol, a noninvasive monitoring of feeding infants, poses minimal risk to the infants while having the potential benefit of identifying infants who are at greatest risk of developing post-prematurity respiratory disease.

### 3.2.4. Spontaneous Oxygen Desaturation During Sleep

Functional residual capacity (FRC) is dynamically determined during the first year of life,[77] and the volume of O2 available in the lung will be at its nadir at the end of tidal exhalation. Whether or not pauses in breathing, with stable or falling end-expiratory volume, cause desaturation will be closely linked to the volume of the dynamically determined FRC. Even brief pauses in breathing may result in small drops in oxyhemoglobin saturation (Figure 3.2.5). This critical relationship was shown in a recent study measuring FRC by washout techniques and analyzing central apneas in 36-week PMA infants.[42] With apneas as short as 6 to 7 seconds, some infants had falls in SpO2% of ~ 7%. The short apneas did not cause desaturation events in all infants, but the lower the FRC, the higher the frequency of apneas with desaturation ($P < 0.001$). The effects of smaller FRC, or end-expiratory volume (EEV), were greater during active sleep, and 28 of 29 infants had apnea with desaturation events > 5% during active sleep (33.3 + 29.2 desaturations / hr). These observations suggest that mild oxyhemoglobin desaturations




resulting from brief apneas may be a marker for impaired respiratory function and lower FRC.

**Figure 3.2.5. Recording of respiratory effort, airflow, and $SpO_2$ in a sleeping infant who was born at 28 weeks. Child is breathing room air at a rate of 20 breaths per minute . A 9 second apnea, the equivalent of 3 respiratory cycles, is followed by a fall in $SpO_2$% from 98% to 92%.**

Oxyhemoglobin desaturation events during sleep will be studied during RIP, and the same eligibility criteria and risks apply as for RIP. After placing RIP bands and study oximeter measurements will be taken during feeding (see section 3.2.4). The infant is allowed to fall

asleep after the feeding, and recordings of oxygen saturations and RIP measurements are performed one hour after feeding. The feasibility of this approach has been pre-tested at URUB.

## 3.3. Assessments During Follow-up to 1 Year Corrected Age

Research staff at the clinical PROP centers will obtain follow-up data from the infant's primary caregiver after the infant has been discharged from the hospital. This will require a significant investment of time and energy and is essential to characterize the respiratory status of study participants during the first year of life. We aim to achieve a very high retention rate of at least 90% and ideally > 95% to minimize ascertainment bias.

### 3.3.1. Data Collection at Months 3, 6, 9 and 12

At age 3, 6, 9, and 12 months corrected age (± 1 month corrected age), the research staff at the clinical centers will contact the family to schedule and conduct an interview with the primary caregiver about the infant's health status. Contact information will be verified and revised as needed. A focused questionnaire will be administered over the telephone or in person at 3, 6, 9 and 12 months corrected age by the research staff to gather detailed data about hospitalizations for cardiopulmonary causes as well as for other medical reasons, respiratory medication use, respiratory symptoms and home technology dependence. For further details please refer to section 2.1

Medication used during the previous 3 months will be reported by parents to the site staff by listing them in advance of each follow-up call and reading them to the interviewer or by reading the labels to the interviewer at the time of the encounter. Exposure to environmental risk factors (young siblings, smoking, kerosene heater, pets) will also be recorded. Lastly, we will administer a symptom survey for gastroesophageal reflux disease (GERD) that was developed and validated for infants and children < 18 months by Orenstein and colleagues.[78] The 12-item questionnaire assesses symptoms associated with reflux over the past week. .

### 3.3.2. Physical Examination at Month 12

During the in-person visit at 1 year corrected age, all infants will receive a standardized physical examination. A clinical scoring system was designed using measurements that (i) are commonly employed by practitioners and require no specialized equipment and that (ii) represent the underlying physiology and potential pathophysiology of the respiratory system. Scores like the Respiratory Distress Assessment Instrument (RDAI) have typically been used to demonstrate responses to interventions in wheezy infants.[79-81] Such scoring systems have been shown to be valid, responsive and have good inter-observer reliability. No such score, however, has been previously developed to track the respiratory courses of infants born prematurely. The PROP Physical Examination Score will use some elements of the RDAI, but incorporate other elements that reflect the effects of chronic respiratory impairment, such as growth and the development of digital clubbing. It has long been recognized that respiratory impairment and hypoxemia result in growth failure among infants with BPD.[82, 83] In contrast, rapid weight gain has been associated with lower lung function and increased risk of wheezing illness over the first three years of life in healthy term infants.[84, 85]

The PROP Physical Examination Score will be assessed at 36 or 40 weeks PMA, and again at 1 year corrected age. The infant will be evaluated during a period of quiet wakefulness. Weight will be measured with the infant unclothed, and length will be determined using an infant length board. Measurements of weight and length will be compared with published reference values and Z scores will be calculated. The respiratory rate will be counted over a full minute while the infant is breathing quietly. Assessment of retractions, accessory muscle use, and of thoraco-abdominal synchrony will be made with the infant in a recumbent or semi-recumbent position. Presence and distribution of crackles (none, isolated to 1 region, isolated to 2 regions, or diffuse) will be made by auscultation. Similarly, presence, timing (expiratory, inspiratory or both) and character of wheezing (none, monophonic, polyphonic, or mixed) will be made by auscultation under resting conditions, and again when the thorax and upper abdomen are gently manually compressed during exhalation by the examiner. The point of maximal cardiac impulse will be palpated, and absence or presence of digital clubbing will be visually assessed.

### 3.3.3. Infant Pulmonary Function Testing (iPFT)

Objective measures of lung function are critical for achieving the PROP goal of identifying novel mechanisms and associated functional and molecular biomarkers of respiratory disease risk in preterm infants. Diminished lung function is a risk factor for symptomatic respiratory disease, such as wheezing.[20, 29] Although a history of BPD is associated with lower lung function in infancy and childhood,[15] preterm infants without a history of BPD demonstrate airflow obstruction and gas trapping compared to normal controls.[86] These observations suggest factors other than BPD, or not captured by the current definitions of BPD, are involved in lung growth and repair following preterm birth. Previous studies of infant lung function after discharge from the NICU have involved small numbers of infants from single centers,[41, 87] PROP will study a large cohort of neonates from multiple centers, and infant PFTs will provide crucial objective physiologic outcome measures for this unique study population.

Measurement of forced expiratory flows in infants began with the pioneering work of Wohl, Taussig, Landau, and others in the 1970's.[88, 89] In these early studies, partial flow volume curves were generated at end expiration during tidal breathing by rapid thoracoabdominal compression (RTC) using a rapidly inflating jacket that surrounded the infant's chest and abdomen. The primary measure used was the maximal flow at functional residual capacity ($V'_{maxFRC}$). This technique was successfully used to demonstrate reduced expiratory flows in infants with BPD and cystic fibrosis.[90, 91] Young infants with low $V'_{maxFRC}$ prior to the onset of any respiratory illness are at increased risk for wheezing in the first 3 years of life.[92]

Despite the insights gained from the RTC technique, there are some potential limitations with this method. FRC in infants is dynamically maintained and also affected by underlying lung disease.[93-95] Hence, there may be some variability and instability in measuring flows around this landmark. Furthermore, the RTC technique does not allow measurement of fractional lung volume measures such as residual volume (RV) and total lung capacity (TLC). To overcome these limitations, investigators in the 1990's developed the raised volume rapid thoracoabdominal compression technique (RVRTC).[96-98] In RVRTC, the infant's lungs are inflated to a pressure of 20-30 $cmH_2O$ using a facemask sealed with therapeutic putty. This

allows measurements of forced expiratory flows starting at near TLC down to RV. RVRTC has been shown to be more sensitive than RTC alone in detecting airflow obstruction in infants with cystic fibrosis.[21] Limited normal reference data are available.[22, 23]

Two major developments have facilitated the use of RVRTC in a multicenter research setting. The first was the introduction of a commercially available device (nSpire Infant Pulmonary Lab (IPL), nSpire, Inc, Longmont, CO) which allows standardized equipment to be used at all study sites. The second was the development by the CF Foundation Therapeutics Development Network of a protocol for performing RVRTC and fractional lung volume measurements with the nSpire IPL and a quality control system to ensure capture of high-quality data. This system was recently applied to a multicenter study of lung function in infants with CF.[92] Bronchodilator response in both normal infants and those with a history of BPD has been successfully measured using the RVRTC method.[41, 98]

In PROP, we will use a standardized method of performing infant pulmonary function tests (PFTs) using the raised volume rapid thoracoabdominal compression (RVRTC) technique. Five sites have the capability to implement this protocol: Cincinnati Children's Hospital, UCSF, University of Rochester/University of Buffalo, Washington University and Duke/Indiana University.

Although our primary PFT measures will be derived from RVRTC, we will also measure $V'_{maxFRC}$ and respiratory system compliance (Crs) and resistance (Rrs) because these measures are easier to obtain. Crs and Rrs will be obtained using the single breath occlusion method.

Our target sample size of at least 180 studies will represent the largest number of RVRTC PFTs in the preterm population and will allow us to study the relationship between lung function at 1 year of age and clinical and biologic factors associated with respiratory disease.

Infant pulmonary function testing for PROP will proceed in the following order:

1. Tidal breathing analysis (which includes Tpef/Te)
2. Crs and Rrs (compliance and resistance)
3. FRC measurement using whole body plethysmography
4. Forced expiratory flows from raised volumes (FVC, FEV0.5, FEF25-75, FEF75)
5. Fractional lung volumes (ERV, TLC, RV/TLC, FRC/TLC)
6. Bronchodilator response (Forced expiratory flows from raised volumes)

Parents will be approached for the iPFT before the infant reaches 12 months corrected age. An infant will be excluded from PF Testing for the following reasons:

1. History of adverse reaction or allergy to chloral hydrate sedation
2. Current symptoms of nasal obstruction or discharge
3. Clinically significant upper airway obstruction as determined by the Site Investigator (e.g. severe laryngomalacia, markedly enlarged tonsils, significant snoring, diagnosed obstructive sleep apnea)
4. Severe gastroesophageal reflux, defined as persistent frequent emesis despite anti-reflux therapy
5. Acute intercurrent respiratory infection, defined as an increase in cough, wheezing, or respiratory rate with onset in 2 weeks preceding visit
6. Hydrocephalus
7. Congenital heart disease

8. Severe neuromuscular disease
9. Any physical findings or conditions that would compromise the safety of the infant or the quality of the study data as determined by site investigator.

The parents of all eligible PROP participants will be asked to consider PF Testing. A separate consent form will be administered at the time of the test to explain the risks and benefits.

### 3.3.4. Risks of iPFT

Performing infant PFTs includes the use of moderate sedation with oral or rectal chloral hydrate (CH). Potential side effects of CH include respiratory depression, gastritis, and paradoxical agitation. All CH sedation will be performed under local institutional sedation policies, which include having appropriate monitoring and the presence of appropriately trained individuals present in the event of an untoward event.

The RVRTC technique itself is safe, with no adverse events reported in hundreds of tests. Although the maximum jacket pressure can be up to 120 $cmH_2O$, almost all of this pressure is absorbed by the chest wall. The device is FDA approved for use in infant PFTs, and the manufacturer's instruction for use will be strictly followed. In the recently completed CF infant PFT study,[92] adverse events occurred in 44 out of 342 studies (12.8%), but only 3 events were severe. The most common adverse event was vomiting (29 events).

The 5 PROP clinical centers that will be performing IPFTs are very experienced in this technique and the overall serious adverse event rate (SAE) rate has been 1%. All SAEs have been related to sedation, and in most cases the infants had underlying conditions (e.g., obstructive apnea or neuromuscular disease) that increased their risk for respiratory compromise with CH therapy. Such infants would be excluded from iPFTs in PROP.

To assess bronchodilator response, infants will receive inhaled racemic albuterol. Albuterol is widely used in the clinical care of infants with a variety of respiratory diseases. Its safety profile is well known and well established. The common potential physical risks involved with albuterol therapy are mild, transient and easily reversed when the medication is discontinued. These include tachycardia, decreased serum potassium, decreased oxygen concentration, flushing, hyperactivity, prolonged cough and tremor. These risks are both dose dependent and cumulative. The risks during sedation may also include upper airway obstruction and the rare need for bag and mask ventilation due to reduced oxygen levels in the blood. Because our study design involves only receiving one dose of albuterol, these risks are lessened.

### 3.3.5. Longitudinal Measures of Tidal Breathing Indices Using RIP

The ideal approach to obtaining longitudinal measure of tidal breathing would be to perform RIP at 1 year of age. However, there are several obstacles to acquiring these data. Tidal breathing patterns vary with state of consciousness and sleep state. Criteria for clinically determined quiet sleep have been validated in newborn infants, but not at 1 year of age. Thus we would have to document sleep state with electroencephalogram, greatly increasing the complexity of the study. There would also be challenges in being able to reliably time study visits with nap times at 1 year of age. These factors make it challenging to perform RIP at 1 year. However, a subset of infants in PROP will be undergoing IPFTs. The IPFT device is capable

of measuring Tpef/Te via a mask with pneumotachometer. Previous studies have shown that Tpef/Te measured by RIP is equivalent to that of a mask,[99] and sedation with chloral hydrate does not affect this parameter.[100] Therefore we plan to obtain Tpef/Te in the cohort of infants undergoing IPFT at 1 year of age to assess longitudinal changes that may reflect changes in respiratory function.

### 3.3.6. RIP and iPFT Data Quality

The Infant PFT Core Laboratory at the University of North Carolina will serve as a consultant and service provider to the PROP.

We will incorporate several measures to ensure that respiratory function data will be obtained in a consistent fashion and be of research quality. Prior to initiation of the study, each of the PROP pulmonology site PIs with expertise in their respective respiratory assessment have refined the technique. We will develop a detailed manual of operations. In April 2011, we will have a training workshop with all of the site PIs and research coordinators to review the entire study protocol and provide hands on experience with the RIP equipment. During the validation phase of the study, each site's data will be reviewed to ensure adherence to procedure protocols.

Rochester, Buffalo, St. Louis, and Cincinnati have already been qualified by the CF Foundation in performing research quality iPFTs. The remaining iPFT site (UCSF) can receive qualification by sending 10 deidentified iPFTs for review by the CF Foundation Therapeutics Development Network Infant PFT Core Center. During the study, data from RIP, oximetry, and iPFT will be sent to the CF Foundation Therapeutics Development Network Infant PFT Core Center where they will be over-read by two blinded, independent overreaders. Only data that are certified as research quality by both overreaders will be used for analysis. The Core Center has extensive experience with these procedures, having successfully used them in the CF Foundation infant PFT study[101] and the preschool PFT study; the Core Center is also performing iPFT data management for the NIH/CFF funded Infant Study of Inhaled Saline. The physician director is Stephanie Davis, MD. The lab will work with the DCC to establish and test the data transfer protocol and then schedule routine data transfers. The iPFT Core Laboratory will participate in evaluating test data quality from clinical sites and advise the DCC regarding the need for intervention or retraining

# 4. Statistical Considerations

## 4.1. Global Objectives

The global objectives of PROP are the investigation of hypotheses on the molecular mechanisms that contribute to respiratory disease risk of the premature newborn with the long-term goal of improving outcomes in the first year of life. More specifically the study design allows for the assessment of hypotheses of pathophysiological mechanisms and biomarkers that will characterize preterm infants and predict respiratory morbidity in several domains including medical resource utilization and respiratory symptoms over time and physiological and other measures at one year. PROP is a hypothesis generating multicenter study with myriad candidate predictors with the

longitudinal nature of the study allowing for the assessment of risk modification over time. Our development of the Statistical Considerations section will start with an examination of the primary outcome and main secondary outcomes. These sections will be followed by discussion of general power considerations, analytic approaches and more general statistical issues such as causal pathways, multiple comparisons, selection bias, and issues in generalization.

## 4.2. Primary Outcome

PROP has chosen an outcome that incorporates the elements currently considered as hallmarks of respiratory morbidity (Section 2.1). The primary outcome is dichotomous, and defined as "No substantial post-prematurity respiratory disease" or PRD. The definition of PRD for PROP requires that infants must have at least one morbidity element reported in at least 2 time frames (approximately 3 month intervals). Survey items selected for the determination of the primary outcome will be focused on four domains, with any positive response to any element identifying morbidity: respiratory medications; hospitalizations for cardiopulmonary cause; respiratory symptoms; home technology dependence; and death.

Follow up data from recently published randomized controlled trials and observational studies in similar but not identical populations of very preterm infants suggest that the incidence of this primary outcome will be at least 30% and possibly as high as 60%.[32-35]

### 4.2.1. Predictors of Outcome

The primary focus of PROP is on the delineation of early and later biomarkers during the NICU stay that predict *respiratory morbidity post discharge through 1 year of age.* The later predictive biomarkers will include various physiologic respiratory assessments described in section 3.2. Some infants will be ineligible for any of these tests, e.g. those on mechanical ventilation at 36 and 40 weeks PMA. The values for these ineligible infants for these examinations will generally be assumed to have a failure in the respective test.

## 4.3. Secondary Outcomes

### 4.3.1. Mortality

Mortality as an outcome will be described as a dichotomous outcome as well as the time to death. Logistic regression will be used to predict mortality using covariates from the NICU period and Cox Regression will be used in examining time to death, also using the covariates from the NICU period.

### 4.3.2. Physical Examination Score

Physical Examination Score will be a composite of the weighted results of a 12 month battery of physical measurements including weight, Z-score, respiratory rate (awake, w/ RIP), SpO2 in RA, retractions, thoraco-abdominal movement, accessory muscle use, wheeze, wheeze character, crackles, digital clubbing , done at one year visit on all eligible infants.

#### 4.3.3. Respiratory Morbidity Severity Score

The respiratory morbidity severity score will be used to quantify the level of morbidity in each of the domains of resource utilization and symptoms. The outcome would be based on a scoring system involving the elements already described in the four domains of the primary composite outcome (Section 1.0).

#### 4.3.4. Refined Assessment of the Primary Outcome PRD

In secondary analyses, PRD will be defined in various ways to assess the sensitivity of the outcome to the chosen definition described in Section 4.2. These variations will include but not be limited to:

- The predictive ability of specific biologic, physiologic and clinical data obtained during the initial hospitalization will be compared to the predictive ability of the currently available definitions of BPD: (i) use of supplemental oxygen at 36 weeks PMA; (ii) NIH consensus definition that distinguishes between mild, moderate and severe BPD; (iii) "physiologic" definition.
- Repeated measures of morbidity at each visit defined as a binary indicator of at least one element out of four morbidity elements. Though different from what is measured in the primary outcome, this approach defines persistence of respiratory disease without specifying a particular PRD element or pattern and will utilize all complete surveys at any time point, and thus can be more robust when there is missing data (e.g. 3 month survey not done).
- Changing the frequency requirement of 2 time intervals to 3 time intervals, to create an ordinal outcome such as mild (< 2 time intervals), moderate (only 2 intervals) and severe (3 or more time intervals).
- Weighting the various components, such as hospitalizations having more weight than medications alone.

### 4.4. Power Considerations

Since the sample size of 750 participants was established by the RFA, discussion of power will be in the context of this fixed sample size for the primary outcome. We have made assumptions concerning loss to follow-up due to death and non illness related departures. As will be seen, the sample size is adequate to provide appropriate power for all of the examples presented below, which should account for reasonable departures from our assumptions, without losses of sensitivity to detect meaningful differences.

#### 4.4.1. Primary Outcome

Our primary outcome PRD is binary and we will use logistic regression models to test the correlation between a biomarker and the outcome. With current sample size, we consider a range of PRD rates from 30% to 50%, allowing us to conceive a possible range of power. We also let the odds ratios (OR) for the change of one standard deviation in the biomarker vary from 1.2 (small effect) to 1.6 (moderate effect). Assuming no measurement errors and no correlation between the biomarker and other covariates, Figure 4.4.1 shows the power to detect various odds ratios for the possible outcome with PRD rates of 30%, 40% and 50% at $\alpha = 0.05$ using a two-tailed test.

Because the power is the same for any two PRD rates symmetric around 50% (e.g., 40% and 60%), we only show the power for PRD rates up to 50%. Power increases as the PRD rate and the minimum detectable odds ratio increase. For a PRD rate of 40%, given a sample size of 750 and a conservative missing rate of 10% (5% due to late deaths and 5% due to loss to follow up), we will have sufficient power (>80%) to detect a significant biomarker-PRD association at OR of 1.25 or higher for one S.D. increase in the biomarker.

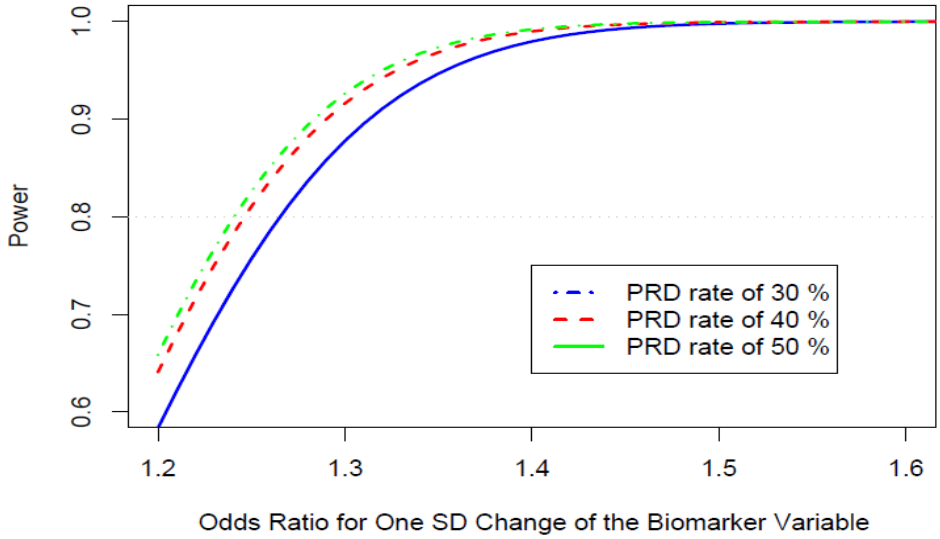



**Figure 4.4.1: Power of detecting a significant association between a biomarker and the binary outcomes for various odds ratios at a significance level of $\alpha$=0.05 assuming a missing rate of 10%.**

### 4.4.2. Secondary Outcomes

There will be several outcomes and subgroup analyses, including outcomes that are individual components of the broad domains already described. We consider the general question of power with a cohort of 750 as well as smaller sample sizes, by examining the relevant parameters for sample size/power assessment for a hypothesis test of a correlation between a continuous prognostic biomarker and a dichotomous or continuous one year outcome of interest using a logistic or linear regression.

For scenarios where the outcome is determined to be binary (e.g. respiratory re-hospitalization or not), we will use logistic regression to test the correlation between a biomarker and the binary outcome. If we consider a range of the outcome event rates from 30% to 50%[1, 32, 102] and examine the same missing rates as above, we will have the same power estimate as shown in Figure 4.4.1. Please note that respiratory re-hospitalization takes discrete non-negative ordinal values and will be analyzed using the proportional-odds (or ordinal logistic) regression models to test the correlation between a biomarker and the outcome. Such models allow unequal spaced differences among ordinal scales and yet estimate the common effect of predictors on the increasing scale of the outcome. Our power estimate based on the dichotomized variable is conservative and our planned proportional odds models generally have higher power than the logistic regression for a dichotomized outcome.[103] For other secondary outcomes that might be described as approximately continuous, we can assess the value of a biomarker as the

proportion of the outcome variance explained by the biomarker ($R^2$). One outcome that will be considered is a one year physical exam score.

Figure 4.4.2 shows the power to detect a significant association between a biomarker and a continuous outcome. For example, for the sample size of 750 and $R^2$ of 0.5% to 2% (equivalent effect sizes of 0.071 SD to 0.143 SD, which are generally considered as small effects) the power was estimated assuming conservative missing rates of 5% and 10% (as in Section 4.4.1 above) using $\alpha = 0.05$.[104, 105] Clearly for a continuous outcome, the PROP cohort will provide sufficient power to detect significance for even a small effect size.

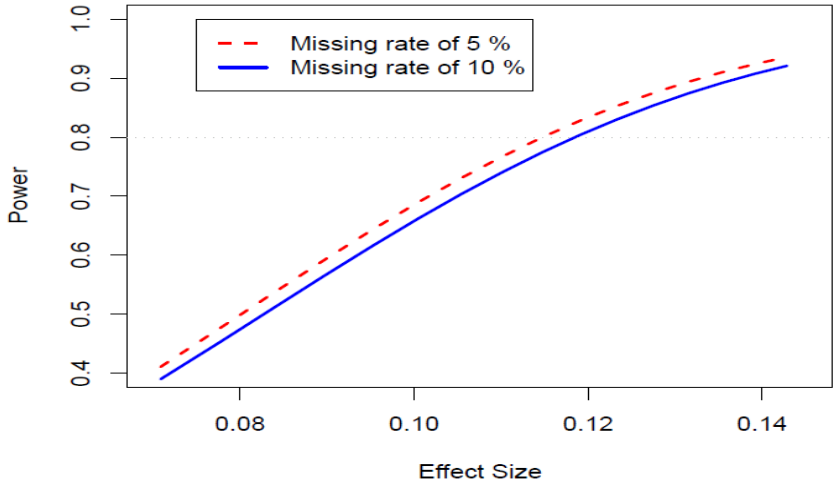



**Figure 4.4.2: Power of detecting a significant association between a biomarker and a continuous outcome for various effect size at a significance level of $\alpha$=0.05.**

Since the analysis will be adjusted for covariates including gender, birth weight, and gestational age at birth, the sensitivity of the analysis may be affected by the actual correlation between the biomarker and the covariate and the effect of the covariate. If multiple independent biomarkers can jointly explain the outcome, we are likely to have higher power to detect at least one of these biomarkers.

We may investigate the biomarker-outcome association analysis in a certain gestational age group. In the power assessment to follow, since the distribution of the numbers of infants born in 23-24, 25, 26, 27, and 28 weeks is close to uniform from sites' preliminary data, we assume there are 150 infants in each group, for which the power for detecting a significant association between biomarker and continuous outcome at $\alpha = 0.05$ is shown in Figure 4.4.3. Also in the same figure, we labeled the x-axis with the corresponding odds ratios assuming the power to detect the association between a biomarker and dichotomized outcome with an event rate of 40%. Given the subgroup sample size of 150, we will have 80% power to detect a significant association for an effect size of at least 0.24 (generally considered a small effect size) for a continuous outcome or an odds ratio of 1.64 or larger (a moderate OR) for a dichotomized outcome. Note that the minimum detectable effect size (Figure 4.4.3) required for subgroup analysis is more than twice that required using the full cohort (Figure 4.4.1, 4.4.2).

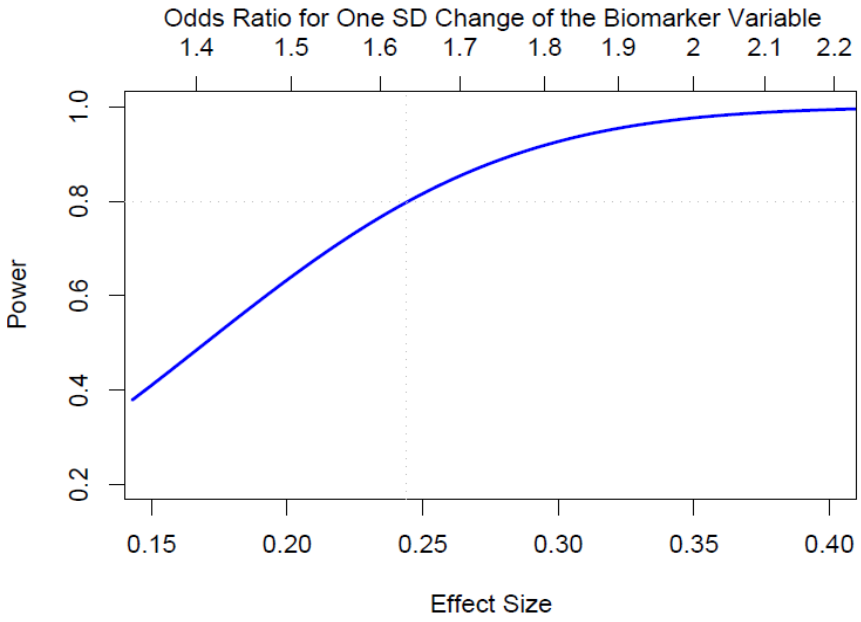



**Figure 4.4.3: Power of detecting a significant association between a biomarker and the dichotomized/continuous outcome for various odds ratio/effect size (top/bottom x labels) in a subgroup at a significance level of $\alpha$=0.05 assuming a missing rate of 10%.**

## 4.5. Descriptive Analysis

Before proceeding with the analysis of the PROP primary research questions, data will be fully described, including aspects of data quality. For both predictor variables and outcomes, a summary of each variable, or group of variables, will be produced. Graphical methods including histograms, scatter plots, and box plots, will be used at this stage in order to understand aspects of data quality and examine assumptions underlying statistical models. Means, standard deviations, medians, and ranges will be computed for measured continuous variables; marginal distributions will be used for categorical factors. The amount and patterns of missing data, if any, will also be characterized. Before specific research questions are addressed, several types of data manipulation may be considered. Transformations will be used if needed to produce variables that conform to the distributional assumptions underlying the analytic techniques that will be employed. For instance, some variables may be transformed to log scales, as needed to reduce any marked positive skew. Exploratory analyses and careful collaboration with investigators will be used to guide in the selection and creation of summary variables.

## 4.6. General Statistical Analytic Approaches

Most standard hypothesis tests will be specified as using a two-sided significance level (Type I error) of $\alpha$ = 0.05, although actual p-values will be reported whenever possible. For measured continuous variables, two-group comparisons will generally employ Wilcoxon rank-sum tests to protect against violations of normality assumptions. The Wilcoxon test affords little loss of power, as it is more than 95% efficient with respect to the two-sample t-test when normality holds. Similarly, Wilcoxon signed-rank tests will be used for paired data and Kruskal-Wallis tests will be used for k-group comparisons. In some instances, t-tests and analysis of variance (ANOVA) methods may be used to facilitate group comparisons when the appropriate assumptions are met. Categorical variables,

including dichotomous factors, will be summarized by proportions and compared among groups using standard chi-square tests of association and generalized Mantel-Haenszel (MH) methods, as described in Landis et al[106] to accommodate both nominal and ordinal measurement scales. These MH methods are useful for adjusting primary associations for potential confounders. Whenever possible, exact p-values from Exact Conditional Tests, such as Fisher's exact test and its multi-degree of freedom extensions, will be produced for these tests.

### 4.6.1. Univariate Models

Candidate biomarkers for inclusion in multivariate modeling will be assessed using marginal models or simple two way analyses, depending on the structure of the biomarker, to ascertain predictive value, if any. At this stage of model selection, factors will be moved forward for further evaluation if either their predictive value produces a p value of ≤ 20% or they are biomarkers supported in the literature.

### 4.6.2. Multivariable Models

Multivariate models will be used to both simultaneously assess the contributions of the many biomarker predictors as well as to control for factors such as gender, race, products of multiple gestation, birth weight, gestational age at birth, site differences and between hospital variation, in the evaluation of potentially complex associations. Models specified in the protocol will include linear regression for continuous outcomes and logistic regression for binary or ordinal responses. We will provide in the analyses, in addition to tests of significance, confidence intervals for parameters of risk factors and biomarkers, and various approaches to assess the robustness of the estimates to nuisance factors. Particular attention will be paid to unusual observations (outliers) that may have undue influence on the analytical results. Standard regression diagnostics, including residual plots, and influence statistics will be used to identify such observations and examine their effect on analyses.

### 4.6.3. Repeated Measurements

For repeated measures, we will use generalized linear mixed models (GLMM[107]) to account for dependence of the measures within subjects. We will first check for patterns in the residuals from the model without any random effect to determine plausible covariance structures that may account for the temporal correlation within subject[108] and then select the best one using routine likelihood-based criteria: maximized likelihood, largest Akaike information criteria (AIC[109]), Schwarz information criterion (SBC[110]). We will use maximum likelihood method (ML) and residual maximum likelihood method (REML) for parameter estimates and significance testing.

### 4.6.4. Multiple Comparisons

The analysis approaches described will likely involve a great number of individual statistical tests at both the univariate and multivariate levels. In some cases we utilize global statistical testing (e.g. omnibus contingency table assessments prior to marginal assessments). In other cases it will be appropriate to provide some protection against detecting false positive results by using a stepwise modeling procedure which enters and removes covariates based on

threshold values of p<0.15 and p<0.10 (or otherwise specified in the protocol), respectively, to initially reduce the number of candidate factors.[111] We are choosing to generally avoid severe adjustments of significance levels using multiple comparison techniques, which would lessen the chance of detecting potentially important biomarkers and other risk factors for severity of respiratory disease.[112] We take these more liberal approaches to dealing with multiple comparisons because PROP, an observational study, is considered a hypothesis generating rather than hypothesis testing study. Thus we will generally report significance values related to odds ratios for biomarker prediction of one year outcome as indicators for risk factors that are candidates for confirmation in future studies.

## 4.7. Specific Analytic Approaches

The specific analytic approaches to the assessment of which biomarkers play an important role in the prediction of one-year outcome will be discussed. The following sections provide a brief overview of some of the statistical methodologies and considerations that will be used at the time of analysis, both for descriptive purposes, and univariate and multivariate analyses relating biomarkers to one-year outcome(s). For a large study with many exposures or predictors, several outcome variables, and many scientific questions of interest, some of which will evolve with analysis itself, it is not possible to detail analyses of each question. Nonetheless, the types of analyses described here are likely to be used to answer many of the questions that arise. We consider here the principal methodological issues and approaches for dealing with those issues.

### 4.7.1. Prediction of Outcome

The primary focus of PROP is on the delineation of early biomarkers during the NICU hospitalization period that predict *respiratory morbidity through 1 year of age.* We have chosen to approach the assessment of this hypothesis using mixed effect logistic regression, Preliminary analyses will provide a parsimonious set of predictors to be considered in multivariate analyses. A full model will include all main effects (fixed) of parsimonious predictors and a random effect reflecting ICN variation and we will use variable selection techniques such as AIC, BIC and LASSO to select a subset of predictors as a candidate prediction model. All higher-order and interaction terms will be considered as well if there is any indication of effect modifier or non additive effects. For repeated measures, we will use generalized estimating equations (GEE[113]) for prediction of the outcome that can be implemented in SAS GENMOD procedure.

#### Center and Site Variability

The PROP is composed 6 designated clinical research centers (CRC), affiliated with 13 intensive care nurseries (ICN) and we will address both the CRC variation and the within CRC ICN variation. All testing models will be fitted separately for each CRC to see whether there are systematic differences among the five CRCs. Then, we can make explicit comparisons of one CRC to another by including the CRC differences as fixed effects in the regression model.
For the second layer of variability, the within CRC ICN variability, we will treat the ICN effect as a "random" effect in the mixed effect linear or logistic models. This can be done because our

primary interest is in the effects of biomarkers across multiple ICNs and the number of ICNs is relatively large.

### 4.7.2. ROC Analysis

Biomarkers can be evaluated as diagnostic tests for both primary and secondary dichotomous outcomes. The sensitivity of the predictive function is defined as the proportion of participants with the primary outcome PRD, who have a positive test (e.g., duration of oxygen therapy); the specificity as the proportion of participants without the target condition who have a negative test. Multiple test cutoff points can be defined using different probabilities of the outcome derived from the logistic regression model. Each of these cutoffs is associated with a true positive rate and a false positive rate based on actual outcome. Optimal cutoffs can then be chosen based on their relative costs and benefits of the various types of errors.[114] Analysis of an ROC curve, in which the sensitivity and (1-specificity) of the rule for different cutoff criteria are plotted[115] will be used to graphically describe the relationship between chosen cutoff values of the logistic regression analysis and the associated test characteristics. The ability of the biomarker to discriminate between outcome groups will be reflected in the shape of this curve. The greater the integrated area beneath the curve, the greater the ability of the rule to differentiate individuals with/without the target condition.[116] The ROC curve can be developed and tested using the algorithm of Hanley and McNeil[116], adapted for use on a microcomputer by Centor and Schwartz.[117]

### 4.7.3. Missing Data

The optimal approach to missing data is assiduously avoiding it. It is likely that missing outcome data will be at or below an assumed 10% value, primarily because of mortality after 36 weeks PMA and some attrition. Nevertheless, extensive efforts will be made to collect complete one year data, with care taken to record participant primary and secondary participant contact telephone numbers and addresses, as well as reports of rehabilitation and nursing home visits, and follow-up for deaths, to death registries if necessary. If there is more than a trivial amount of missing outcome data, in secondary analyses, we will assess the impact of missing one year data. First, we will compare participants with substantially complete follow-up to those with missing one year data, with respect to observed baseline characteristics, and will pursue discrepancies to shed some light on the reasons for missing data in participants with incomplete follow-up. Second, we will investigate the impact of missingness on the estimates of effect levels and variability of effect estimates using multiple imputations.[118]

We will consider performing sensitivity analyses to assess the potential impact of missing data by fitting shared parameter models that relax the missing at random assumptions of the proposed random effects models. In addition, we shall also use inverse probability of censoring weighted methods of analysis to adjust for nonignorable dropout; when there are repeated measures of the outcome, this approach will be used in conjunction with generalized estimating equations to account for nonindependence of observations within participant.

## 4.8. Predictors of Specific Pulmonary Outcomes

The PROP investigators will attempt to perform RIP in all eligible infants at 36 weeks PMA. However, some infants will not be eligible, and others will not have recordings of sufficient quality. In considering sample size estimation below, we use one component of the composite dichotomized primary outcome, namely post-discharge rehospitalization.

### 4.8.1. Thoracoabdominal Asynchrony (Φ) as a Predictor of Post-discharge Hospitalization

We want to predict respiratory hospitalization using the phase angle Φ as measured during RIP testing. In order to estimate the sample size to achieve adequate power, we consider the hospitalization rate ranging from 30% to 50%. We also let the odds ratios (OR) for one standard deviation (SD) change in the Φ (1SD is about 40°)[119] vary from 1.5 to 2.0 (both moderate effects). If we assume a conservative attrition rate of 10% (5% due to death and 5% due to loss to follow up), the total sample size required at the entry of the study in order to have 80% power to detect various odds ratios for hospitalization with a rate of 30-50% is shown in Figure 4.8.1. The required sample size decreases as either the hospitalization rate or the odds ratio to be detected increase. For example, to obtain 80% power to detect a significant association with OR of 1.5 (for one SD change of Φ) between Φ and hospitalization, we will require an initial sample size of 222, which after allowing for 5% post discharge mortality and 5% post discharge loss to follow-up will be an effective sample size of 200, at α = 0.05 using a two-tailed test when the hospitalization rate is 40%. We have chosen to attempt 253 evaluations of $\phi$ to be conservative (Table 4.8.1, hospitalization rate 30%, OR = 1.5).

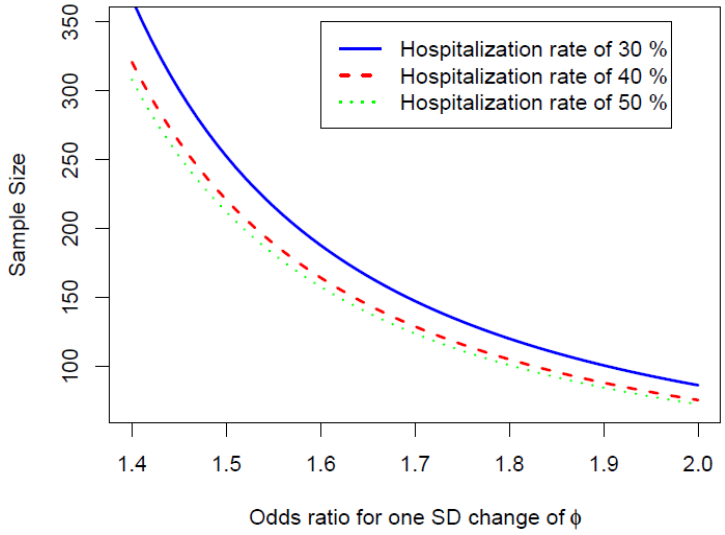



**Figure 4.8.1: Sample size required to obtain 80% power of detecting an association between Φ and respiratory hospitalization at a significance level of α = 0.05 (two tailed).**

**Table 4.8.1: Examples of sample sizes required to obtain 80% power of detecting a significant association between Φ and respiratory hospitalization**

| Hospitalization Rate | OR | Sample Size |
|---|---|---|

| | | |
|---|---|---|
| 30% | 1.5 | 253 |
| | 1.7 | 248 |
| 40% | 1.5 | 222 |
| | 1.7 | 130 |
| 50% | 1.5 | 213 |
| | 1.7 | 124 |

### 4.8.2. BPD as a Predictor of Forced Expiratory Flow (FEF-75) by iPFT

Our primary lung function outcome is the FEF75 measured at one year by RVRTC, in the unit of ml/s. The sample size for iPFTs is estimated based on this primary outcome, although we will also examine the relationship of various predictors and other lung function measures. Although factors other than a history of BPD may contribute to lower lung function at one year, we have chosen BPD for our sample size estimates because it is a well documented risk factor for lower lung function.[15] Linear regression will be used to test the difference in FEF75 between PROP premature infants who have a history of BPD and those without, adjusting for weight, length, and other possible covariates.

Because there is no existing study that has investigated the difference in FEF75 between the infants with and without BPD, we will estimate our effect size from a cohort study that examined lung function and bronchodilator responsiveness between full term infants and preterm infants with a history of BPD.[41] The time range (outcome measured at an average 68 week for preterm infants and 57 week for full term infants) and comparison groups (infants with and without BPD) are similar to this study. But in contrast to full or close to full term infants in that study, our comparison will be in preterm infants, which may lead to smaller differences.

In the Robin et al.'s study, the mean difference in FEF75 between two groups was 34.8% and the observed s.d. of FEF75 was about 30.7% and thus the estimated effect size is 1.1 SD (a moderate size). The prevalence of BPD is estimated to be 40% in infants born between 24 to 28 gestational weeks (SUPPORT Study Group[120]). To judge the impact on sample size in the face of an observed prevalence different in our PROP cohort, we examined potential prevalence rates of BPD at 30%, 40%, and 50%. In addition to our conservative estimated dropout of 5% of the cohort (Section 4.4) from discharge, we will need to consider the ability to obtain high quality data for this outcome measure. The success rate in obtaining high quality research data is variable, ranging from 70-90%, which is highly associated with experience.[92] Although all of the PROP sites that will be performing iPFTs are highly experienced, we will use a conservative 75% success rate. We have assumed a combined loss of data from both infant dropout and failure of testing that gives a cohort available and completing exams of 71.3%. It is estimated that approximately 50% of the parents will consent for their child to have iPFTs. In addition, only 5 of the sites will be performing iPFTs. The potential available sample size may be 600 (4 x 150) with little further reduction based on the exclusions outlined in Section 3.3.3.

The sample size required to obtain 80% power to detect a significant difference in the primary outcome between two groups for effect sizes ranging from 0.5SD (relative small effect) to 1.2SD (moderate effect) is shown in Figure 4.8.2. Sample size decreases dramatically with

decreasing OR, but is not highly sensitive to changes in the prevalence of BPD. If the prevalence of BPD is 40%[107] then with a size of 164, we will have 80% power of detecting a significant difference in FEF75 between two groups with an effect size 0.75 SD or more (a significant level of α= 0.05 using a two-tailed test (Table 4.8.2). A 0.75 SD difference between those infants with and without BPD would correspond to 23.0 (%) differences between two groups if we assume the same s.d. in our sample as in Robin et al.'s paper. For a smaller effect size of 0.5 SD, we will need a sample size of 368 to have 80% power. The above sample sizes are the initial sample sizes required at discharge and after considering the 5% post discharge mortality, the 50% consent rate, the 5% loss to follow-up rate and a 25% test failure rate, the effective sample sizes would be 56 and 125 for detecting a difference of 0.75 SD and 0.5SD, respectively. We have chosen to attempt 368 evaluations of FEF75 to be conservative. The potential for selection bias is discussed in Section 4.10.1.

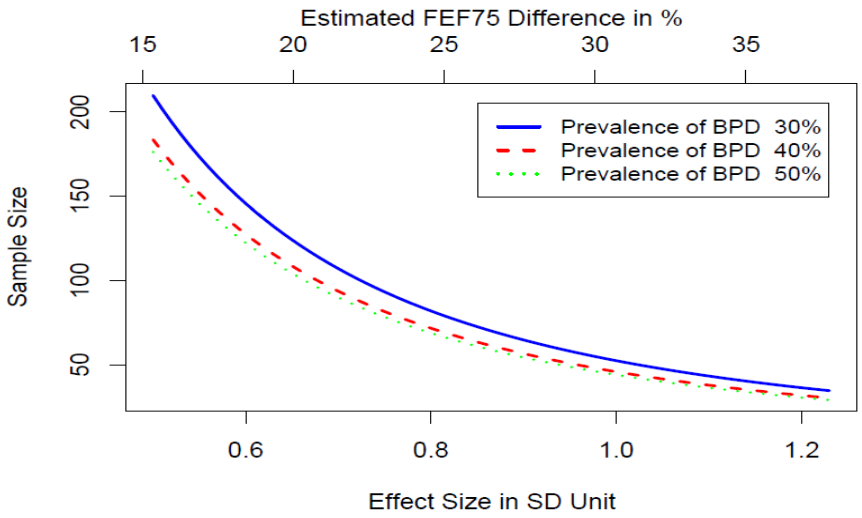



**Figure 4.8.2: Sample size required to obtain 80% power of detecting a difference in FEF75 between infants with and without BPD at a significance level of α = 0.05 (two tailed).**

**Table 4.8.2 Examples of sample sizes required to obtain 80% power of detecting a difference in FEF 75 between infants with and without BPD.**

| **Effect Size (Group Difference)** | | **BPD Prevalence** | | |
|---|---|---|---|---|
| In SD | In % | 30% | 40% | 50% |
| 0.5 SD | 15.4 | 420 | **368** | 354 |
| 0.75 SD | 23.0 | 188 | 164 | 158 |
| 1 SD | 30.7 | 106 | 92 | 90 |

## 4.9. Validation of Models and Biomarkers

It is generally the case that model building within a data set (derivation set) will produce results superior to those when the model is applied to an independent data set (test set), one not used in building the original model. It is expected that model building in the PROP cohort will involve the assessment of one or more markers independently and in conjunction with each other and with

consideration of other factors such as gender, birth weight, and gestational age at birth. While these complex models can be assessed in a sample held back from the original sample for use as a test sample, this approach will reduce the reliability of our statistical testing procedures due to the decrease in sample size in the sample used to derive the model. After deriving the primary base model, we will then use 10-fold cross validation to evaluate the predictive accuracy of the candidate final model.

## 4.10. Causal Pathways

Prediction of one-year outcome will benefit from other analyses dealing with the mechanism or pathway by which a baseline predictor is associated with outcome. It will also be informative to determine NICU observed measurements that predict BPD at 36 weeks. In previous work with the outcome of cerebral palsy, original estimates of the impact of low Apgar on the risk of Cerebral Palsy (CP) were considerably attenuated when it was discovered that low Apgar not sequentially followed by newborn signs and other neurologic outcomes did not appreciably increase the risk of CP.[121] As an example, in the PROP setting one might envision in the schematic in Figure 4.10. that the estimates of risk of PRD related to initial baseline variables such as birth weight might be greatly enhanced or diminished dependent on the participant's course over time (e.g. with or w/o BPD at 36 weeks). Simple tree approaches will be utilized to assess the sequences of events over time that distinguish levels of risk of respiratory morbidity.

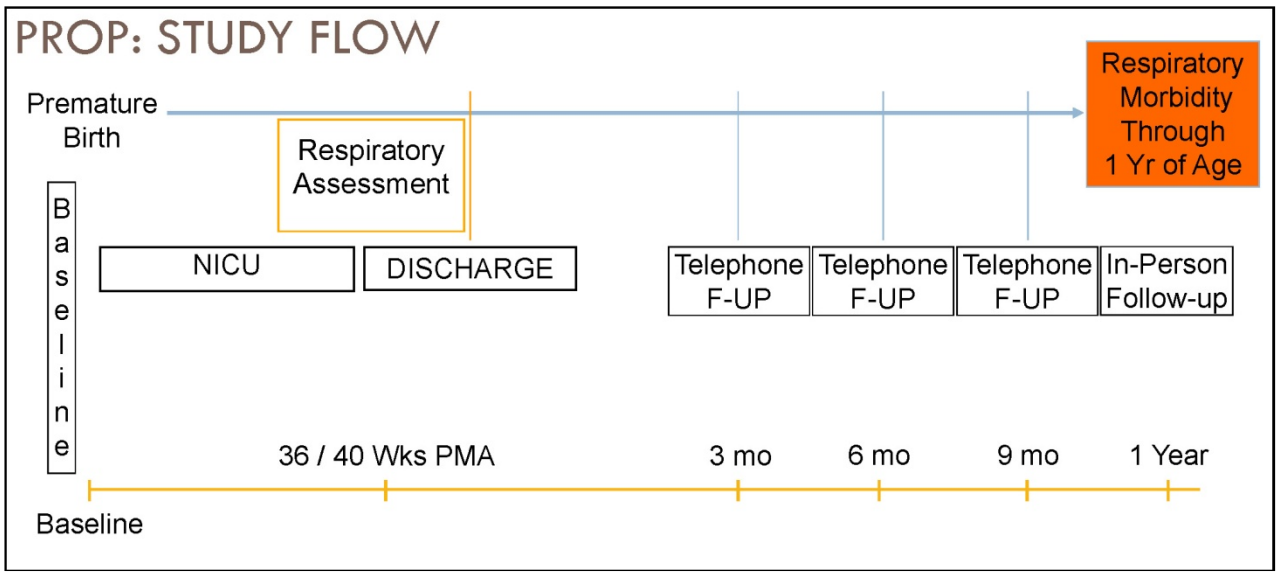



**Figure 4.10. Schematic of PROP data collection and pathways to One Year outcome.**

### 4.10.1. Selection Bias and Inference

The PROP cohort is a consortium of five university-based Neonatal Intensive Care Units that applied for and secured funding under an NHLBI federal grant announcement (Prematurity and Respiratory Outcomes Program (PROP) RFA-HL-10-007). As such, these centers of excellence are not necessarily representative of the NICUs throughout any defined geographic area. At issue is whether these centers are likely to or in fact will enroll premature infants that are skewed in such a manner that the results from PROP will not be generalizable to other NICUs around the country or even to other NICU centers of excellence. For example, will the

race/ethnicity make up of the PROP centers' catchment areas be different from those encountered in the general population and will such factors have an impact on the association between the specified biomarkers and the respiratory outcome at one year? We will assess known and measured factors that might impact on the results, and that are measured in the cohort to determine if there are imbalances not only within the PROP cohort and between centers and also whether they reflect these factors in the general population. If these differences do exist, we will attempt to evaluate the sensitivity of our approaches to changes in these factors and adjust or at least make known such discrepancies in any statements or inferences from PROP. With regard to factors unknown or unmeasured that might impact the analytic results, we will not be able to counteract the impact of such factors.

The generalization of results must be limited by the replacement sampling discussed in Section 2.3. Since all infants who do not survive to 36 weeks PMA will be replaced, the results will only be applicable to survivors to 36 weeks PMA. This is more of a de juro rather than a de facto consideration, since most deaths occur early in this group of infants and very little data would be available to assess respiratory morbidity over the first 12 months of life in this cohort.

Section 4.8.2 covers a substudy that will evaluate BPD as a predictor of FEF75 measured at one year by RVRTC. A series of restrictions including the loss of infants who are ineligible to take this test, the difficulty of obtaining universal consent from parents to do the procedure (perhaps as high as 50%), the 20-25% failure rate of the test itself, and the limited resources to complete these test on all eligible children will potentially lead to a subsample that is biased in a way that is related to the outcome. We will try to decrease some of the potential bias by making sure that the clinical centers attempt to enroll eligible children consecutively and use standard approaches to all children so as not to influence consent in a systematic way. Along with analytic assessments of the ability of BPD to predict FEF, we will assess the differences between those infants who did and those who did not receive the examination to gain some information on those factors that are known to impact on the outcome and that have been measured in the study. Finally we will limit the generalizability of our inference appropriately.

# 5. Study Management and Administration

## 5.1. Screening and Enrollment

All infants who meet the eligibility criteria will be considered for enrollment in PROP. Parents will be asked to sign the informed consent form after admission to the NICU after the investigator or clinical site staff have explained the study and all of its procedures including the one year follow up.

The reason for non-enrollment of eligible infants will be recorded.

### 5.1.1. Participant Retention

Retention of participants is central to the internal validity of the study and will be a high priority of the investigators and staff. The clinical site staff will strive to maintain relationships with parents and provide a flexible schedule in conducting follow-up interviews.

### 5.1.2. Participant Withdrawal

It is anticipated that participants may withdraw over the course of the study. This may occur officially by formal notification from the family/caregiver to the investigator, or unofficially when a participant cannot be reached via the usual methods of contact. Every effort will be made to acquire complete data on all infants. However, a participant may withdraw consent for use of his or her infant's data at any time.

Participants who relocate to an area from which it is no longer feasible to travel to one of the PROP centers for the 12-month in-clinic visits will be asked to continue participation in the study by providing crucial outcome data through phone interviews and access to medical records.

### 5.1.3. Potential Risks to Participants

The potential risks to infants are minimal as the protocol is predominantly based on routinely collected clinical data.

During the conduct of respiratory inductive plethysmography (RIP) tests, infants may be tested for airway responsiveness to a bronchodilator. The bronchodilator (Albuterol) is administered as an aerosol by a mask held in front of the infant's nose and mouth. Albuterol may cause tachycardia in infants, elevating the heart rate to slightly above normal during and for several minutes after the treatment is administered. The heart rate typically returns to normal within 15 to 20 minutes after treatment.

The primary risk of the trial of $FiO_2$ reduction to 0.15 is a transient decrease in the infant's oxygen saturation. Infants who meet criteria for failure (SpO2 < 85% x 1 min or < 80% x 15 seconds) will be immediately returned to room air. A source of supplemental oxygen will be available for the infant, if she or he fails to recover after returning to room air.

The conduct of infant pulmonary function tests (iPFT) may require a short acting sedative (chloral hydrate), administered as a liquid. As discussed in section 3.3, families will be consented separately for this aspect of the PROP protocol.

Collection of saliva for DNA analysis poses a risk of exposure of confidential genetic information due to an inadvertent or malicious data security violation. Expert data security measures, de-identification procedures, and limited access to this information will minimize this risk.

### 5.1.4. Adverse Event Reporting

The site staff will document adverse events associated with research tests such as the trial of oxygen reduction to 15%, the RIP test and infant PFTs. These events will be summarized in standard AE reports and presented to the OSMB for review on a regular basis.

Serious Adverse Events (SAE) will be reported according to standard definitions to the DCC which will facilitate reporting to the clinical sites, the sponsor and the OSMB. Included in the definition of an SAE is the need for ventilatory support or increase in supplemental oxygen support of at least 10% for a sustained period after the or bronchodilator challenge during respiratory inductive plethysmography (RIP) testing.

All SAEs possibly or probably related to a study procedure will be reported to the OSMB promptly by the DCC, within 48 hours of first knowledge of the event.

### 5.1.5. Informed Consent

Interested parents will be asked to sign the informed consent form approved by the local Institutional Review Board (IRB). This form will provide consent to collect in-hospital data as well as permission to contact them in the future.

The DCC will provide an informed consent template for use at all participating clinical centers. Each clinical site will prepare an informed consent form following the guidelines of their local IRB, and applicable regulations for Informed Consent. This form will be reviewed by the DCC before submission to the local IRB. The form will, at a minimum, contain a description of the procedures, potential risks, and benefits. Prior to signing the informed consent, the Research Coordinator will review the details of the consent form orally with the participant, and answer any questions that the participant has concerning participation in the study. The original signed consent form will be kept in the infant's study file at the clinical center; a copy of the signed consent form will be given to the participant. Specifically, the following must be accomplished during the informed consent process:

- The participant must be informed that participation in the study is **voluntary** and that refusal to participate will involve no penalty or loss of benefits or negative impact on their medical care
- The participant must be informed of the **purpose** of the study and that it involves **research**
- The participant must be informed of any **alternative procedures**, if applicable
- The participant must be informed of any reasonably foreseeable **risks**
- The participant must be informed of any **benefits** from the research
- An outline of safeguards to protect participant **confidentiality** must be included as well as an indication of the participant's right to withdraw without penalty. This should be balanced with a discussion of the effect withdrawals have on the study, and the responsibility a participant has, within limits, to continue in the study if he or she decides to enroll
- The participant must be informed **whom to contact** for information about research participants' rights, information about the research study, and in the event of research-related injury
- The participant must be informed as to whether or not **compensation** is offered for participation in the study and/or in the event of a medical injury
- The participant must be informed that he/she will be notified of any significant **changes** in the protocol that might affect their willingness to continue in the study

The consent process may differ somewhat by clinical center according to local IRB guidelines and single center study procedures. The informed consent document will be structured such that it enables potential participants to indicate which aspects of study they may not be willing to engage in.

### 5.1.6. Consent for Genetic Testing and DNA Storage

A separate section and signature page will be required for consent to collect a saliva sample for genetic testing and storage of DNA. A parent may refuse to sign the separate consent for use

of DNA without consequence to study eligibility. Specimens will be stored at The Center for Human Genetics Research at Vanderbilt University. At a later date, the specimens will be submitted to the NHLBI BioLINCC Repository (https://biolincc.nhlbi.nih.gov/home/).

A separate section for indicating the choice to contribute other biospecimens specific to the studies at each site, which will include the collection, for the PROP biorepositories, of 4 tracheal aspirate samples (if the infant is intubated) and 4 urine samples at pre-specified postnatal ages.

Participants will be informed that DNA and other biological samples may be used for many types of genetic and biomarker analyses, but that the confidentiality of this information will be ensured by (1) data security measures, at the participating sites and DCC, and (2) at the point that their clinical information is combined with biological data (e.g., genetic studies) where these datasets will be de-identified. Information generated from these samples will not be reported to study participants. Paternity will not be determined.

### 5.1.7. HIPAA Authorization

In accordance with the mandated Federal HIPPA regulations, authorizations will be provided to all research participants at the time of presentation of consent that detail all potential risks of disclosure and individuals and organizations who may have access to participant research data.

### 5.1.8. Participant Confidentiality

Procedures to assure confidentiality will be strictly observed. All identifiable personal health information data will be (1) kept in confidential locked files; (2) identified by participant number only; and (3) kept separately from identifying information used for participant tracking and follow-up contacts. Identifying information will be kept in separate locked files. No identifying information will be disclosed in reports, publications or presentations.

Protection of participants depends on the joint activities of all Clinical Centers as well as the DCC. Extensive efforts will be made to ensure that participants' confidentiality is maintained. Each participant is assigned a unique study identification number and is never tracked through the study by name, social security number, medical record number, or other personal identifier. A log of the participant names, participant ID numbers, and pertinent registration information (e.g., home address, telephone number, and emergency contact information) is maintained in a locked area at each clinical site. The staff at the DCC does not have access to this log. Only the participant ID number and initials are given to the DCC staff and entered into the study database. Any communication between DCC and clinical sites regarding participant data occurs via the participant ID number. Any forms or documents sent to DCC, IRB or other regulatory authorities will have all personal information removed.

Authorized representatives of the National Heart, Lung and Blood Institute (NHLBI), National Institutes of Health (NIH), participating clinical institutions as well as the IRB have access to and may copy both medical records and records from participation in this study. Such access is necessary to ensure the accuracy of the findings, the safety and welfare of participants. If any publication or presentation results from this research, participants will not be identified by

name or other personal identifier. All research reports, articles, and presentations will report only aggregate findings.

## 5.2. Study Organization and Oversight

### 5.2.1. Clinical Centers

The clinical sites participating in the PROP will have primary responsibility for developing the study protocol, recruiting a sufficient number of infants, maintaining high rates of follow-up and data collection, obtaining data of high quality, and interpreting, presenting, and publishing findings from the study. The 6 clinical centers are as follows:

1. Cincinnati Childrens's Hospital and Medical Center (CCHMC), Cincinnati, OH
   *Principal Investigators*: Alan H. Jobe, MD, PhD, Claire Chougnet, PhD
   *Co-Investigators:* Paul Kingma, MD, PhD, Lisa Young, MD, James M. Greenberg, MD, Kurt Schibler, MD
2. Washington University, St. Louis, MI
   *Principal Investigators:* Aaron Hamvas, MD and Thomas Ferkol ,MD
   *Co-Investigator:* James Kemp, MD
3. University of California at San Francisco, (UCSF), San Francisco, CA
   *Principal Investigators:* Roberta L. Keller, MD and Phil Ballard MD, PhD
   *Co-Investigators:* Roberta Ballard, MD, Dennis Nielson, MD, PhD
4. Vanderbilt University, Nashville, TN
   *Principal Investigator:* Judy L. Aschner, MD, Paul Moore, MD,
   Marshall Summar, MD, Tina Hartert, MD
   *Co-Investigator:* Lance Prince, MD, PhD
5. University of Rochester and University of Buffalo, Rochester and Buffalo, NY
   *Principal Investigators:* Gloria S. Pryhuber, MD, Thomas Mariani, PhD, Rita M. Ryan, MD
   *Co-Investigators:* Tim Stevens, MD, MPH, Clement Ren, MD, Carl D'Angio, MD,
   Anne Marie Reynolds, MD, MPH, Jack Sharp, MD
6. Duke University, Durham, NC and Indiana University, Indianapolis, IN
   *Co -Principal Investigators:* Judith A. Voynow, MD and Michael Cotten, MD, [Duke] and Stephanie D. Davis, MD and Brenda Poindexter, MD [Indiana]

**Table 5.2.1: Clinical center affiliates.**

| Center # | Site # | Site ID# | Name |
|---|---|---|---|
| 01 Cincinnati | 1 University | 011 | Cincinnati University Hospital |
| | 2 Children's | 012 | Cincinnati Children's Hospital |
| | 3 Good Samaritan | 013 | Cincinnati Good Samaritan |
| 02 Washington U | 1 Washington U | 021 | Washington University |
| 03 UCSF | 1 UCSF | 031 | University of California, San Francisco |
| | 2 AB/CHO | 032 | Alta Bates Summit Medical Center/CHO |
| | 3 UT Houston | 033 | University of Texas, Houston |
| 04 Vanderbilt | 1 MCJCHV | 041 | Monroe Carell Jr. Children's Hospital at Vanderbilt |
| | 2 Jackson | 042 | Jackson-Madison County General Hospital |
| 05 Rochester and Buffalo | 1 Rochester | 051 | University of Rochester |
| | 2 Buffalo | 052 | University of Buffalo |
| 06 Duke and Indiana | 1 Duke | 061 | Duke University |
| | 2 Indiana | 062 | Indiana University |

### 5.2.2. Data Coordinating Core (DCC)

The Data Coordinating Core (DCC) for the PROP is located at the University of Pennsylvania School of Medicine, Philadelphia, PA. The Principal Investigator is Barbara Schmidt, MD, University of Pennsylvania and the Co-Principal Investigator is Jonas H. Ellenberg, PhD, University of Pennsylvania.

**The DCC is responsible for the following:**

- Providing comprehensive data management services in support of multi-center studies; providing site management in the conduct of multi-center protocol activities
- Promoting program-wide quality assurance standards, practices and tools, including a comprehensive, secure www-based data management system (DMS) for collection and centralized storage of all multi-site study data
- Providing methodological and biostatistical expertise in research design, outcome measures and analytic strategies for translational and clinical investigations
- Guiding and implementing statistical analyses, interpretation of findings, and contributing to presentations and publication of results
- Collaborating with the laboratories on best practices for data collection, specimen tracking and storage, as well as support technical processes between the DCC and laboratories
- Providing Data Coordinating Core administrative support for the PROP, promoting effective communications, coordinating meetings, working groups, document development and management, and distribution of study proceedings
- Supporting the Ancillary studies of PROP investigators by assisting in their design, as well as implementing a process for the submission, review, and development of ancillary studies
- Establishment and maintenance of the PROP website

### 5.2.3. PROP Biorepositories

The Center for Human Genetics Research (CHGR) will be responsible for the following:

- Providing DNA specimen collection, banking, annotation/blinding, distribution services across the PROP.
- Providing genomic analyses and generate assay platforms, on a per analysis cost basis, for multi-site efforts or single site requests.
- Providing selection, aliquoting and shipment of specific DNA repository samples to requesting PROP sites and future transfer of all samples to the NHLBI Biorepository on a per request basis.

The laboratory at Vanderbilt as the urine biorepository:

- Providing for urine samples aliquoting, labeling and banking of specimens submitted by each site.
- Providing selection and shipping of requested infant urine samples to sites after approved requests.
- Providing regular summaries of sample acquisition and remaining amounts.

The laboratory at UCSF as the tracheal aspirate biorepository:

- Providing collection kits for both urine and tracheal aspirate collection and shipping batches of kits to each site
- Providing for tracheal aspirate samples aliquoting, labeling and banking of specimens submitted by each site
- Providing selection and shipping of requested infant tracheal aspirate samples to sites after approved requests
- Providing regular summaries of sample acquisition and remaining amounts

### 5.2.4. Steering Committees and Subcommittees

The primary governing body of the study is the Steering Committee, which is comprised of each of the principal Investigators at the Clinical sites, DCC and the NHLBI Project Officer. Dr. Lynn Taussig from the University of Denver is the Chair of the Steering Committee. The Steering Committee develops policies for the study pertaining to access to data and specimens, ancillary studies, performance standards, publications and presentations. They develop the study protocol and meet to discuss the progress of the study and resolve problems that arise.

A subset of the Steering Committee membership makes up the Executive Committee. This includes the NHLBI Project Officer, Dr. Taussig, the DCC investigators and a representative investigator from each clinical site. The Executive Committee communicates regularly and makes the day-to-day decisions of the PROP, consulting the larger Steering Committee or specific members where necessary.

In addition to the Steering and Executive Committees, committees and working groups may be established to focus on instrument development, quality control, publications, and ancillary studies. Working groups may be established to prepare manuscripts and presentations. The following subcommittees have been established to address specific study issues:

- Baseline Respiratory Status to 36 weeks PMA or Discharge Working Group
- Discharge to 1 Year Corrected Age Working Group

- Biospecimen Working Group
- Pulmonary Outcomes Working Group
- Best Pharmaceutical for Children Act Working Group
- Training Initiative Working Group

#### 5.2.5. Observational Study Monitoring Board (OSMB)

An Observational Study Monitoring Board (OSMB) has been appointed to review protocols and consent form templates and advise the program in the overall conduct of the program activities. The members have been named by the NHLBI and represent subject matter expertise in neonatology, pediatric pulmonology and biostatistics.

## 5.3. Study Management

#### 5.3.1. Clinical Site Responsibilities

Conduct of particular aspects of the study may be delegated to qualified personnel; however, it is the responsibility of each Clinical site Principal Investigator to oversee the overall study management within their site. The Clinical site staff must be trained in all study procedures.

Each Clinical site is responsible to enroll and retain a designated number of infants. It is the responsibility of the Clinical site study staff to assess their accrual, ensure participant confidentiality, maintain appropriate study documentation, enter and transfer data in a timely manner, and participate in the PROP study meetings and conference calls.

#### 5.3.2. Institutional Review Board

It is the responsibility of each clinical site to conduct the study according to the protocol, to adhere to all applicable regulatory guidelines, and to provide the appropriate IRB with all pertinent material. Approval of the protocol and the informed consent form must be obtained, and forwarded to the DCC before screening or enrolling participants. The Investigator also maintains the responsibility of initiating protocol re-approval, notification of protocol and/or consent form changes, notification of unanticipated events, and termination of the study according to the appropriate IRB requirements.

#### 5.3.3. Record Retention

Investigators must maintain study documents on-site and in an orderly and secure fashion for a minimum of 2 years after the close of the study, and make available to the sponsor or the sponsor's representative: signature pages, amendments, informed consent documents, and approval letters from the IRB, CRFs, all primary source documentation, and all letters of correspondence. The DCC maintains all study records for a period in accordance with their internal SOPs and applicable regulations.

## 5.4. Data Coordinating Core Responsibilities

#### 5.4.1. Quality Assurance

The DCC has developed written standard operating procedures (SOPs) to ensure that all aspects of the study are conducted in a standard and uniform manner. These procedures are

organized into a Manual of Procedures (MOP), which is in alignment with the protocol, Good Clinical Practice (GCP), and applicable regulatory requirements. The DCC will include a Quality Assurance (QA) Plan in the MOP that will consist of the following activities:

**PERSONNEL TRAINING AND CERTIFICATION:** Prior to the PROP initiating patient enrollment, a comprehensive training session will be conducted with all study personnel that will encompass all aspects of the study, including communication, data security, principles of GCP, study implementation and procedures, data entry and verification, test and specimen collection and transfer.

**CLINICAL PROTOCOL, MOP ADHERENCE AND AUDITING ACTIVITIES:** The DCC will request and verify specific information from clinical centers, to ensure the application of study procedures as they apply to participant safety, required intervals for timely conduct of procedures, appropriate documentation of data and specimens, and compliance with SOPs. This information will take the form of a written report.

**DATABASE AUDITING:** The DCC will periodically request source documentation to conduct a remote data audit. A comparison of a certain percentage of data written on source documents compared to that entered into the electronic database provides information that quantifies the accuracy of the data entry process and use of the data management system by clinical site personnel. This information will take the form of a written report.

**DATABASE ADMINISTRATION AND NETWORK SECURITY:** The DCC has SOPs established for authorizing and documenting secure access to the study website, study documents and the electronic Data Management System (DMS). These procedures ensure that only authorized personnel are able to view, access, and modify study data.

**DATA REPORTING:** A set of standard reports will be developed to describe study activities that include accrual, study progress, and data quality. These reports will be provided to investigators, NHLBI and designated committees as appropriate.

**PREPARATION AND INTEGRITY OF ANALYSIS DATASETS:** The DCC Biostatistical core will create a set of standard data access descriptor/view files, which will be used in the generation of SAS analysis datasets. As datasets are extracted from the main study database, they can be utilized separately from direct database processing, thereby, safeguarding the integrity of the data.

**DATA MANAGEMENT:** The DCC provides overall coordination, logistical support, and implementation for all aspects of the study protocol including data collection, data processing, tracking of participant recruitment, training, quality assurance, and statistical analysis. The Clinical Research Computing Unit (CRCU), through its clinical data management, project management, and software systems developments, places into the field and maintains a state-of-the-art www-based data system that accommodates all scientific study data, and permits tracking and coordination of all PROP activities within the framework of multidisciplinary project teams.

### 5.4.2. Website

The DCC has developed a PROP website (http://www.propstudy.org/) for study-wide communication management, data and document management, and activity management and

coordination. The website is restricted to study personnel by secure username and password, issued by the DCC.

### 5.4.3. Data Security

The research computing environment for the PROP DCC is supported by a Biomedical Research Computing (BRC) group within the Clinical Research Computing Unit (CRCU) of the Center for Clinical Epidemiology and Biostatistics (CCEB) at the University of Pennsylvania, School of Medicine. The BRC group is responsible to provide an integrated research computing and storage environment in a manner that supports the required confidentiality, integrity, and access of a common set of research data through all stages of its use. The PROP project is maintained within this compliant environment.

The CRCU database environment for PROP utilizes Oracle's Advanced Security Option (ASO) with two primary foci: 1.) Strong encryption of the database transmissions to protect data traversing the data networks to and from the CRCU databases; and 2.) Internal database encryption of individual sensitive data elements, thus protecting electronic Protected Health Information (ePHI) data if present in the database. Both of these features are in use with the PROP database. The CRCU further utilizes a database monitoring tool that maintains an audit of all user session activities that occur in the protected PROP databases. This tool is able to then recreate requested past user sessions to track all changes that occurred to data in the database.

## 6. Visit Schedule

| Form Name | Form Code | Screening | Baseline | | | Follow-Up | |
|---|---|---|---|---|---|---|---|
| | | Birth | Daily | W36 or Discharge | **W40*** | M3, M6, M9 Phone Contact | M12 Visit |
| **Data Collection Instruments** | | | | | | | |
| Screening for Eligibility and Consent | ELIG | X | | | | | |
| Maternal Baseline Data | MABASE | X | | | | | |
| Baby's Baseline Data | BABASE | X | | | | | |
| Daily Growth and Nutrition/Daily Medication Data | GNMDAY | X | X | X | X | | |
| Daily Respiratory Data | RESDAY | X | X | X | X | | |
| Brain Imaging Data | BRAIN | | W01, W04 | | | | |
| Co-morbidities of Prematurity | COMORB | | | X | X | | |
| Discharge Form | DISC | | | X | X | | |
| Specimen Collection | SPEC | X | PRN | | | | |
| Contact Form | CONTACT | | | X | PRN | PRN | |
| Standard Visit | STV | | | | | X | X |
| Follow-up Interview (includes medication use) | FUP | | | | | X | X |
| Reflux Questionnaire | I-GERQ-R | | | | | X (M6) | X |
| **Tests** | | | | | | | |
| Respiratory Assessments if applicable: | | | | | | | |
| -----Oxygen Desaturation w/Feeding | | | | X | X | | |
| -----Oxygen Desaturation. w/Sleep | | | | X | X | | |
| -----RIP | | | | X | X | | X [w/iPFT] |
| -----Bronchodilator Response | | | | X | X | | X [w/iPFT] |
| iPFT (RVRTC) | PFT | | | | | | X |
| Physical Examination | EXAM | | | X | X | | X |
| Oxygen and Flow Reduction to room air (if applicable) | RAC | | | X | X | | |
| | | | | | | | |
| **Additional Forms** | | | | | | | |
| Maternal Medication Log | MAMED | PRN | | | | | |
| Adverse Event Log | AE | | PRN | PRN | PRN | PRN | PRN |
| Additional Medication Log | ADDMED | | PRN | PRN | PRN | | |
| Record of Death | DEATH | | PRN | PRN | PRN | PRN | PRN |

***Tests are conducted at 36 weeks PMA or discharge, whichever occurs first, and/or at 40 weeks PMA if still hospitalized, depending on respiratory status.**

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