# Extending CPAP Therapy in Stable Preterm Infants to Increase Lung Growth and Function: A Randomized Controlled Trial

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Version 2.0

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## PROTOCOL AMENDMENT HISTORY

| Version number, date | Alterations |
|---|---|
| Version 2.0, 3/9/2020 | Clarification of measurement of FRC before randomization, addition of PHSW site |
| | |
| | |

## 1. Objectives

The objective of this randomized clinical trial is to determine the efficacy of extended continuous positive airway pressure (eCPAP) to improve lung growth and lung function in preterm infants, measured at six months of age. Prematurity occurs in 10% of births and is the most common cause of impaired lung development and increased respiratory morbidity[1]. In the early 1970's, CPAP was used to acutely increase lung volumes and improved survival of preterm infants with respiratory distress. The subsequent addition of antenatal steroids, surfactant, and intubation with mechanical ventilation (MV) became the standard of care for several decades[2]. Despite these advances, prematurity still caused impaired lung development[3] with decreased airway[4] and parenchymal function[5], and increased respiratory morbidity lasting into adulthood. *No current therapies exist to stimulate lung growth, improve lung function, and thus decrease respiratory morbidity after preterm birth.*

Nasal CPAP at birth is now standard care to stabilize and maintain functional residual capacity (FRC), to avoid atelectrauma, volutrauma, intubation, and to reduce bronchopulmonary dysplasia (BPD)[2;6-9]. Although early CPAP use has been well studied, there are few studies defining the optimal time to discontinue CPAP in preterm infants[10]. Early CPAP use may benefit the preterm lung by minimizing injury; but, clinical and animal data indicate that mechanical stretch of the lung with CPAP may provide an important benefit of stimulating lung growth. Congenital diaphragmatic hernias (CDH) compress the lung in-utero causing lung hypoplasia[11;12], and increasing intra-tracheal pressure in-utero stimulates lung growth in CDH models[13]. We found 1-week of CPAP increased lung volume in ferrets[14]. We also reported in human preterm infants without BPD, those treated with CPAP had larger alveolar volumes and lung diffusion versus non-CPAP treated infants when studied after discharge[15]. These data suggest a physiologic rationale that CPAP may stimulate lung growth and development.

Our recent data[16] strongly suggest that CPAP in stable, convalescing preterm infants stimulates lung growth. Preterm infants receiving CPAP and meeting criteria for stopping CPAP were allocated to an extra 2-weeks of CPAP versus CPAP discontinuation (usual care). Both groups met CPAP stability criteria at ~ 32 wks of corrected gestational age (CGA). Infants assigned to the eCPAP demonstrated a greater increase in FRC compared to those who had CPAP stopped (**Fig. 1**). *Importantly, the larger FRC in the eCPAP group persisted at discharge (~36.1 wks in both groups),although CPAP had been off > 2 weeks.* There were no adverse events during treatment. This exciting data suggests that eCPAP in stable preterm infants is a novel non-pharmacologic therapeutic strategy to stimulate lung growth. In this study, lung growth and function were not measured after discharge.

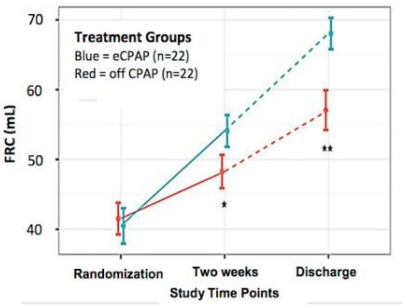



***Figure 1**. FRC in Randomized Patients; Values Mean ± SEM; *p<0.05 ΔFRC b/w groups during treatment; **p<0.05 ΔFRC b/w groups randomization to discharge*

***We hypothesize that eCPAP in stable preterm infants in the neonatal intensive care unit (NICU) will increase alveolar volume ($V_A$), lung diffusion ($D_L$), and forced expiratory flows (FEFs) measured at 6 months CGA compared to stable preterm infants who had CPAP discontinued by clinical criteria**.* Infants stratified by gestational age will be allocated to CPAP discontinuation (dCPAP, usual care) or eCPAP for 2-weeks to address the following Specific Aims (**Fig 2**):

Specific Aim 1 / Primary Outcome**:**

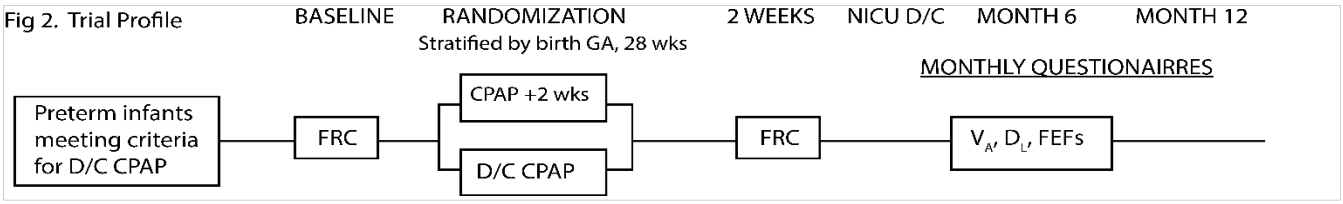
Establish that 2 additional weeks of CPAP in the NICU for stable preterm infants increases alveolar volume at 6 months of age compared to infants who have CPAP discontinued, usual care.

Specific Aim 2 / Secondary Outcomes**:**

Establish that 2 extra weeks of CPAP in stable preterm infants increases lung diffusion and forced expiratory flows at 6 months of age versus infants who have CPAP discontinued, usual care.

Specific Aim 3 / Exploratory Outcomes**:**

Evaluate whether 2 extra weeks of CPAP in stable preterm infants results in lower respiratory morbidity and/or improved neurodevelopmental outcomes through 12 months of age compared to infants who had CPAP discontinued, usual care. This will provide pilot data for a future multi-center randomized trial.




*Abbreviations: FRC= functional residual capacity; VA= alveolar volume; $D_L$= lung diffusion; FEFs= forced expiratory flows*

The clinical implications of our studies are highly significant as results could provide a non-pharmacologic approach to stimulate lung growth, improve lung function, and therefore decrease respiratory morbidity in preterm infants. Our proposal capitalizes on our novel hypothesis, strong pre-clinical and clinical pilot data, unique respiratory physiologic measurements to be obtained in infants, and clinical investigators with expertise in neonatology and pediatric pulmonary physiology and a record of productive collaboration.

## 2. Background

### a. Introduction

The primary function of the lung is gas exchange, which requires the parenchyma to develop a large alveolar surface area coordinated with pulmonary capillary blood volume. The lung must develop airways that conduct air between the environment and the lung parenchyma for gas exchange. These two different lung components have differing patterns of growth and development *in utero*. During fetal development, the airways are present at 16 weeks gestation and formation is nearly complete by 24 weeks gestation. However, development of a rudimentary gas exchanging region of the lung (saccules) does not begin until 23 weeks gestation, which defines the current limit for extra-uterine viability. In-utero lung parenchyma development occurs with an increase in capillary density and alveolar surface area, thinning of the interstitial space, and the presence of alveoli in late gestation[17-20]. Alveolar number and lung volume increase rapidly during the first 2-years of life and then continues at a far slower rate[17;21;22].

Preterm delivery is the most common cause of altered lung development with potential lifelong sequelae, and prematurity is a global health crisis[23;24]. The origins of adult respiratory disease can be traced back to the fetal/early childhood period when the lung undergoes rapid development. Our laboratory has shown that infants born at 30-35 weeks GA with no clinical respiratory disease after birth had decreased airway function with lower forced expiratory flows (FEFs) in the first year of life, which lasted into the second year of life[4]. This confirms findings of other birth cohorts that individuals track along the lung function percentile that is established early in life[25]. This emphasizes the need to optimize lung function/growth in fetal and early postnatal life. Multiple risk factors for prematurity have been identified, but little progress has been made to find interventions to stimulate lung growth[1]. ***We, and others[14-16;26-28] have strong pilot animal and human data that mechanical strain of the lung from continuous positive airway pressure (CPAP) may be a simple, safe, and non-pharmacologic stimulus to promote lung growth, improve lung function, and decrease airway reactivity***. Although CPAP is the standard of care for preterm infants with respiratory distress, there is little evidence defining the optimal time to discontinue CPAP in stable preterm infants[10]. We have promising data randomizing stable preterm infants (≤ 32 weeks GA treated with CPAP for respiratory distress) to extended CPAP [(eCPAP) extra 2 weeks] versus CPAP discontinuation. The eCPAP resulted in increased lung volumes at the end of treatment and at discharge[16]. This study did not perform pulmonary function tests (PFTs) after discharge. Our goals in this proposal are to test the hypothesis that eCPAP in stable premature infants will stimulate lung growth and improve lung function as measured at 6 months of age.

The incidence of prematurity is a health care crisis with significant economic and emotional costs in the United States (US) and worldwide. One of every 9-10 babies in the US[29], and an estimated 15 million babies worldwide[29;30] are born preterm each year. Complications from preterm births are the leading cause of neonatal mortality and an important contributor to child and adult morbidities. In the US, the yearly estimated costs associated with preterm births are $26.2 billion[31-33]. All preterm infants have altered lung development with a high incidence of wheezy respiratory illnesses, hospitalizations, and asthma compared to full term infants[34]. In preterm infants <37 weeks with and without BPD in the first year of life, the incidence of cough was reported to

be 79%, wheezing 44%, and respiratory re-hospitalizations 25%[35]. A recent study in 537 infants born at < 28 wks gestation reported that 65% had post discharge respiratory disease and 71% had frequent wheeze or cough for ≥ 6 months in the first year of life[36]. ***If eCPAP is demonstrated to stimulate lung growth and improve lung function, it could improve the respiratory health of up to 500,000 preterm infants yearly in the US and millions of preterm infants worldwide.***

Although CPAP is standard therapy for preterm infants with respiratory distress, it is not viewed as a potential therapeutic stimulus to promote lung growth and function. There is data that delivery room CPAP is feasible and effective in stabilizing preterm infants, even those very preterm[2;7-9]. Previously it was standard for very preterm infants to be intubated quickly after delivery and be given prophylactic surfactant. However, a 2016 Cochrane meta-analysis concluded that compared to mechanical ventilation, prophylactic CPAP in very preterm infants reduces the incidence of BPD and death or BPD[37]. The consensus is that BPD is decreased in part due to the avoidance of volutrauma from intubation and mechanical breaths. However we and others have shown that CPAP used in small animal models can stimulate lung growth and airway size[14;38], so the reduced BPD may be partially due to CPAP's ability to promote lung growth and function in preterm infants. This has been shown in large preterm animal models. Preterm baboons quickly weaned from mechanical ventilation to CPAP did not show an alveolar arrest and had a normal internal surface area and surface to volume ratio[39]. In a preterm BPD lamb model, Albertine et al showed that high frequency nasal ventilation (to mimic CPAP) is as effective as mechanical ventilation but led to better alveolarization[27;28]. Of note, Columbia University has been known for its low rate of BPD for decades and in addition to minimizing intubation, they continue CPAP until at least 34 weeks of gestation[40]. ***Our proposed study will provide physiologic evidence as to the optimal duration of CPAP therapy in preterm infants to stimulate lung growth and function.***

Our group has created two robust translational models for the fetal origins of childhood respiratory disease. The first is an ongoing trial in preterm primates with pilot data showing evidence of increased alveolar growth in the group randomized to CPAP versus sham CPAP[41]. This supports the findings of our pilot clinical eCPAP trial[16] and the importance of our proposed study. The second is a model of in-utero nicotine in which we demonstrated that nicotine crosses the placenta, up regulates nicotinic receptors in the fetal lung[42;43],and causes significant decreases in the offspring's FEFs[42-44]. In this model we demonstrated that vitamin C blocked some of the negative effects of prenatal nicotine[45]. We translated these findings in a randomized controlled trial (RCT) showing offspring of pregnant smokers randomized to vitamin C had significantly improved newborn PFTs and less wheeze compared to the infants of pregnant smokers randomized to placebo[46].

**b. Background Studies**

**b1. Mechanical Stretch and Lung Growth**

Congenital diaphragmatic hernias (CDH) encroach upon the lung during fetal development and prevent in-utero lung expansion, which results in a hypoplastic lung at birth with high neonatal morbidity and mortality[11;12]. In contrast, complete congenital airway obstruction by laryngeal or tracheal atresia prevents lung fluid from leaving the trachea and produces a large, hyperplastic lung[13]. As an approach to treating CDH in utero, animal models have shown that experimental occlusion of the fetal trachea increases the lungs distending pressure and results in increased fetal lung growth[47;48]. Increased fetal lung distension stimulates capillary growth and epithelial branching in the fetal lung[49-52].

While one approach to stimulate lung growth has focused on the fluid filled fetal lung, mechanical distention of air-filled lungs can also increase lung growth. Resection of lung tissue early in life can stimulate compensatory lung growth, which may occur due to the increased distending force in the remaining lung tissue[53-55]. Delivery of CPAP to young tracheostomized ferrets for 1– 2 weeks increased lung volume and DNA without altering the lung's elastic properties[38]. Using young tracheostomized ferrets, we showed that 2 weeks of CPAP at 6 cmH2O increased both lung volume and airway size compared to non-distended lungs at CPAP = 1 cmH2O[14]. Therefore, chronic lung distention (in-utero or extra-utero) stimulates lung growth in preterm and term lungs, which may occur due of mechanical strain stimulating mechano-sensitive angiogenic pathways.

### b2. Preclinical Preliminary Data

#### b2a. Chronic CPAP increases Lung Volume and Airway Size in Young Ferrets

Eight-week old ferrets were tracheostomized and maintained at High (H) CPAP of 5-6 cmH2O or Low (L) CPAP of 0-1 cmH20 for 2 weeks while mobile in their cages. Volumetric CT scans were obtained at distending airway pressures of 0, 10 and 20 cmH2O to measure lung volume and airway size, pre and post H vs L CPAP treatment (**Figures 3 A, B**). There were no differences between groups prior to treatment; however, H-CPAP treatment significantly increased lung volume and airway size at all distending pressures[14].

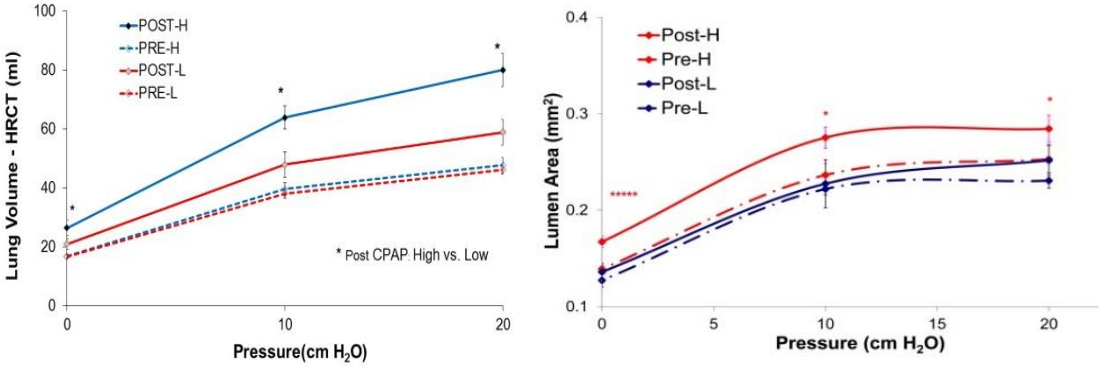



**Figure 3: A)** lung volumes and **B**) airway size, pre and post treatment with high vs. low CPAP

#### b2b. Chronic CPAP Decreases Airway Reactivity in Young Ferrets

Mechanical strain imposed on the lungs during breathing is an important modulator of airway responsiveness *in vivo*[56-60]. Our laboratory has demonstrated that chronic mechanical strain produced *in vivo* by imposing CPAP dramatically reduces airway reactivity *in vivo*[14;61] both in animal models and humans. Following CPAP treatment for 2 weeks, animals underwent in vivo bronchial challenge with increasing concentrations of acetylcholine. Animals treated with H-CPAP had markedly lower airway reactivity compared to animals treated with L-CPAP (**Figure 4**). We have also reported that adults with asthma treated with nocturnal CPAP (10 cmH2O) for 1-week resulted in a significant reduction in airway reactivity compared to adult asthmatics sham treated (CPAP = 0 cmH2O)[26]. As infants and children who were born preterm often have increased airway reactivity and are treated with asthma medications, extended use of CPAP very early in life to stimulate lung growth may suppress airway reactivity in premature infants[62-65].

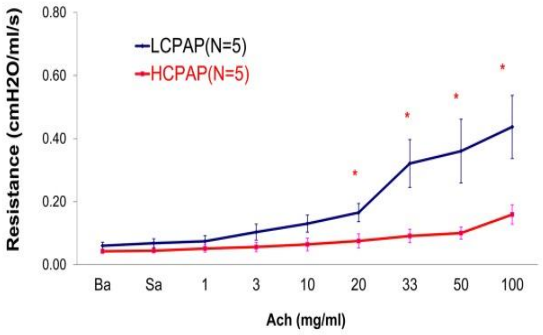



**Figure 4**. Airway reactivity after low versus high CPAP.

### b3. Clinical Preliminary Data

### b3a. Lung Growth and Development in Human Infants

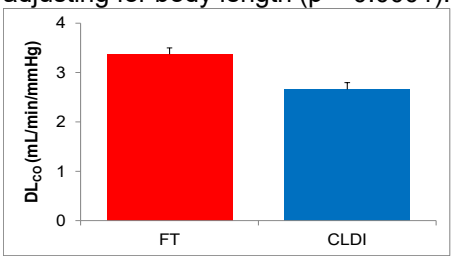



**Figure 5**: Pulmonary diffusion ($D_L$) was significantly lower for Infants with BPD than those born Full term (FT) adjusting for body length ($p < 0.0001$).

Lung Parenchyma: The primary function of the lung is gas exchange, which requires the parenchyma to develop a large alveolar surface area coordinated with pulmonary capillaries. This process begins during the saccular stage of gestation (≥23 weeks) with an increase in capillary density, an increase in lung volume, and a thinning of the interstitial space. Alveolarization, which dramatically increases the pulmonary surface area and capillary density, begins in late gestation (36-weeks) and continues until at least 2-years of age[5;66;67]. All preterm birth may impair ongoing parenchymal development. Our laboratory developed the methodology to assess alveolar volume ($V_A$) *and* $D_L$ in human infants[5;68]. We demonstrated that infants with BPD have significantly lower $D_L/V_A$, (**Figure 5**) which is consistent with morphometric findings from autopsy studies of infants born very preterm that indicates impaired alveolar development with fewer and larger alveoli[69-72].

While in human infants we are not able to demonstrate a direct relationship between alveolar structure and the physiologic measurement of $D_L$, we have done so using a murine hyperoxia model of BPD. **Figure 6** illustrates the impaired alveolar development following neonatal hyperoxia for 7 days, while **Figure 7** illustrates the very strong correlations between structural measures of impaired alveolar development (increased mean linear intercept and decreased vessel density) and in-vivo measurements of pulmonary diffusion (DF) prior to sacrifice[73]. ***Therefore, physiologic measurements of pulmonary diffusion provides an excellent in vivo assessment of parenchymal growth and development.***

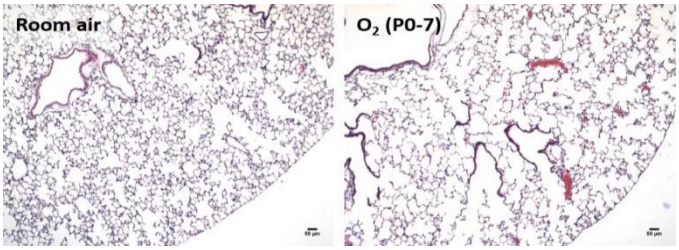



***Fig. 6.*** *Alveolar development after room air exposure and hyperoxia for 7 days.*

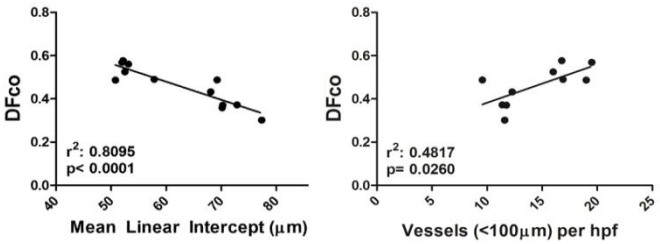



***Fig.7.*** *Alveolar development after room air exposure and hyperoxia for 7 days.*

*Airways*: Our laboratory also developed the methods to measure FEFs in infants using the raised volume rapid thoracic technique, which provides comparable information about airway function as maneuvers obtained in cooperative older children and adults[74;75]. We demonstrated that infants with BPD have decreased FEFs compared to infants born full term[76]. In addition, we have also reported that infants born preterm (30-35 weeks) and who didn't require significant respiratory support also have lower $FEF_{75}$ in the first year of life compared to full term infants, which persisted into the second year of life[4] (**Figure 8**). These findings demonstrate our ability to assess airway function in infants, and the significant and persistent effect of preterm birth upon airway function.

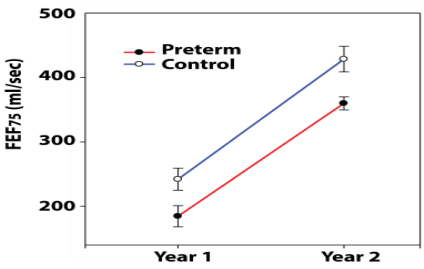



***Fig 8.*** *$FEF_{75}$ were significantly lower in preterm versus term infants when measured in both year 1 and 2 of life.*

### b3b. Preterm Human Infants treated with any CPAP vs no CPAP in NICU had Higher $V_A$ and $D_L$

Using measures of $V_A$ and $D_L$, we assessed lung growth and development of 48 stable outpatient infants who had been born preterm between 27 and 36 weeks gestational age (GA), but did not develop BPD versus 88 healthy full term infants[15]. The preterm infants had a mean GA at birth of 31.7 weeks, and were studied at a mean of 13.7 months of age. Term infants had a GA of 39.3 weeks and were studied at an average of 14.4

months. $V_A$ and $D_L$ were not associated with GA, mechanical ventilation or oxygen therapy suggesting that in the absence of extreme prematurity and BPD, prematurity itself does not impair lung parenchymal development. However, further analysis of the preterm infants demonstrated that both $V_A$ and $D_L$ were significantly higher (by about 16%) for infants who received any CPAP in the NICU compared to those who had not received any CPAP (**Table 1)**. ***This was not a controlled trial of CPAP, but our findings suggest that mechanical stretch from CPAP in preterm infants may produce larger, more functional lungs.***

**Table 1**. *$V_A$ and $D_L$ in Healthy Preterm Infants According to CPAP Treatment*

| Lung Function Measures | No CPAP (n=29) | CPAP (n=19) | P value | % difference |
|---|---|---|---|---|
| $V_A$ (mL) | 606.8 ± 171.5 | 694.6± 205.2 | 0.046 | 16% |
| $D_L$ (mL/min/mmHg) | 3.86 ± 0.97 | 4.49± 1.42 | 0.007 | 16% |

*Values are Mean ± SD; p values adjusted for length and gender*

### b3c. Pilot Study: The Effect of eCPAP on FRC through NICU discharge, a RCT

We recently published a pilot study randomizing stable preterm infants ≤ 32 weeks of gestation at birth and requiring ≥ 24 hours of CPAP for respiratory distress to 2 extra weeks of CPAP (eCPAP) versus CPAP discontinuation when CPAP stability criteria were met[16]. CPAP stability criteria included: on CPAP of 4-5 cm $H_2O$; on room air; respiratory rate ≤ 70 breaths/minute, retraction score ≤ 1; < 3 self resolving apneas (< 20 seconds) and/or bradycardia (< 100 bpm) and/or desaturations (pulse oximeter oxygen saturation, SpO2 <86%) in 1 hour for past 6 hours; average SpO2 >86% for 90% of the time over the past 24 hours, not being treated for a PDA or sepsis, and tolerating time off CPAP during routine care (up to 15 minutes).

Critical to the pilot's success was the extensive in- servicing with all caregivers on the rationale for the study prior to the study start, establishing bedside and parent advocates, and emphasis that participation in the study would not prolong the infant's hospitalization or increase patient costs. In the pilot, 66% of the families whose infant met all of the inclusion and exclusion criteria consented to the study. The primary reason parents refused consent was the desire to see their infant's face unobstructed by CPAP. Additional recruitment approaches developed during the pilot included: professional grade framed photo for the parent done during short periods infant was taken off CPAP for "CPAP cares"; emphasizing the ability for parents to hold their baby on their chest skin to skin while infant on CPAP. Only 1 parent out of 50 withdrew consent after randomization.

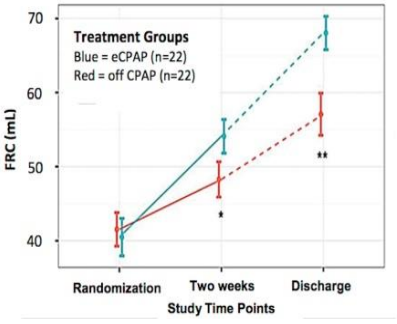



***Figure 1***. *FRC in Randomized Patients; Values Mean ± SEM; *p<0.05 ΔFRC b/w groups during treatment; **p<0.05 ΔFRC b/w groups randomization to discharge; solid line is treatment period and dotted is through discharge.*

As shown in Table 2, both groups of infants fulfilled CPAP stability criteria at ~32 wks CGA. Infants randomized to the eCPAP had a significantly increased change in FRC at the end of the 2-week treatment period and excitingly also at discharge (**Figure 1**, **Table 2**). Participation in the study did not extend the infant's duration of stay which is usually dictated by the infant's oral maturation to adequately nipple all of their feeds (usually occurs at 35-36 weeks CGA). The CGA at full nipple feeds and at discharge (35.8 ± 0.6 wks in the eCPAP and 36.0 ±1.0 wks in the CPAP discontinuation) was comparable between groups. ***This data supports the hypothesis that eCPAP in stable preterm infants will stimulate lung growth.***

**Table 2**. **Characteristics of Randomized Infants**

| | Birth GA(wks) | CGA at Rnd (wks) | Weight at Rnd (g) | FRC at Rnd (mL) | Avg FRCΔ, Rnd to 2 wks (mL) | Avg FRCΔ, Rnd to Discharge (mL) |
|---|---|---|---|---|---|---|
| eCPAP (n=22) | 29.5±1.9 | 32.2±0.7 | 1567±293 | 40.5± 11.8 | 12.6 ±11.4* | 27.2±12.5* |
| Off CPAP (n=22) | 28.7±2.2 | 32.0±0.8 | 1482±323 | 41.5±10.6 | 6.4 ± 10.1 | 17.1± 11.7 |

*Values are Mean ± SD; eCPAP= extended CPAP; CGA= corrected gestational age; RND= randomization; Avg=Average; *p<0.05 for the difference in the change in FRC between the CPAP and no CPAP infants by multiple linear regression with repeated measures and time treatment interaction adjusting for gender, twins, and weight at randomization.*

Extended CPAP may constrain developmental therapies[77] and may affect intermittent hypoxic episodes which are common in preterm infants, underestimated by clinically used pulse oximeter settings, but have been associated with long-term neurodevelopmental problems[78-80]. The pilot eCPAP study had limited funding for the collection of detailed clinical respiratory and neurodevelopmental outcomes after discharge from the NICU. These outcomes will be explored in greater detail in the proposed study to provide data for a planned multi-center trial. In the pilot eCPAP study, 89% of the infants had a respiratory questionnaire done at 12 months of age with wheeze or cough without cold as per parent report[35] occurring in 58% of the infants in the eCPAP arm versus 73% in the CPAP discontinuation. This will be further studied with monthly respiratory questionnaires in the proposed study. Available Bayley neurodevelopmental scores at 6 months CGA from 29 of the 44 infants in the pilot study demonstrated the infants in the eCPAP arm had an average cognitive percentile score of 61.5% versus 46.7% in the CPAP discontinuation arm.

#### b3d. Sedation with Chloral Hydrate

Sedation with chloral hydrate is the standard for infant PFTs after 44 weeks of CGA as per the American Thoracic Society[81;82]. Dr. McEvoy has IRB approval for sedation for PFTs at Oregon Health & Science University (OHSU) for term/preterm infants (current eIRB #386). In our recent RCT (Drs. McEvoy, Morris Co-PIs; Dr. Tepper Co-I) “Vitamin C to Decrease the Effects of Smoking in Pregnancy on Infant Lung Function (VCSIP)[83;84], chloral hydrate sedation was done in 220 infants at 3 and 12 months of age for a total of about 440 total pulmonary function tests. There were no serious adverse events due to sedation.

## 3. Rationale for a Randomized Clinical Trial

Our pilot study which showed a significantly increased change in FRC at the end of the two week treatment period and at discharge in the infants randomized to the additional two weeks of CPAP versus CPAP discontinuation is very encouraging[16]. However the measurements in the pilot study were limited to those done in the NICU and did not measure lung diffusion which currently cannot be measured in the NICU. This new randomized trial will allow us to measure the persistence of the effect of eCPAP on lung volumes and to measure the effect on lung diffusion at six months of age. It will also allow us to measure the effect of eCPAP on forced expiratory flows and other exploratory outcomes including clinical respiratory and neurodevelopmental outcomes.

## 4. Study Design

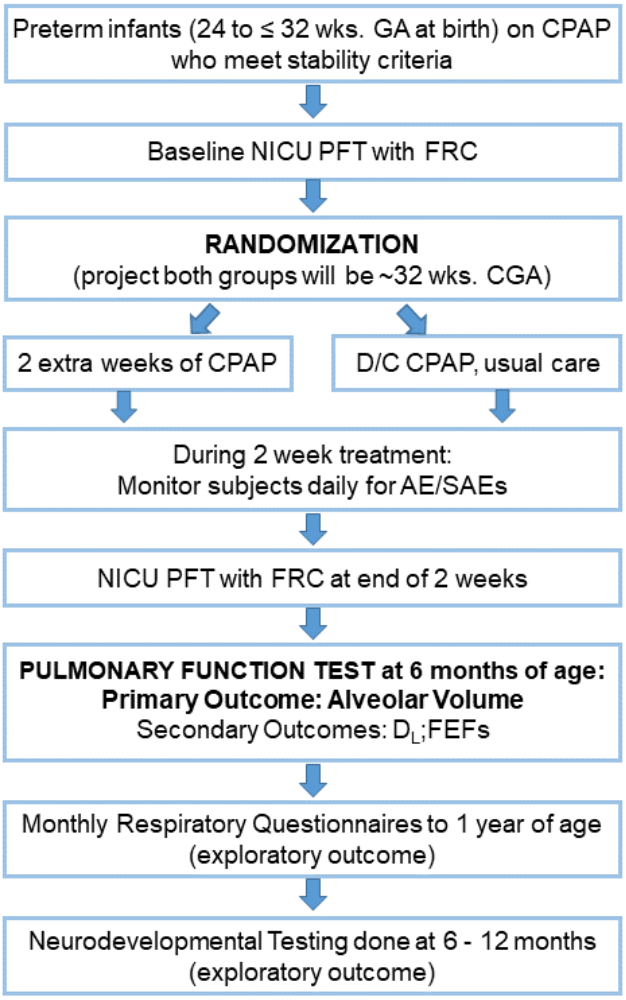
### a. Overview

This is a randomized single-blind Phase 2 trial of eCPAP in 100 stable preterm infants born at 24 to ≤ 32 weeks of gestational age (**Figure 9,** Study Overview). Once infants are consented and, meet CPAP stability criteria they will be randomized to one of two groups:

1. 2 additional weeks of CPAP **(eCPAP)**
2. CPAP discontinuation as per usual NICU care **(dCPAP)**

Prior to randomization and at the end of the 2 weeks of treatment, a pulmonary function test (PFT) will be performed. Infants will return to OHSU at approximately 6 months of corrected gestational age (CGA) for pulmonary function **(primary outcome of alveolar volume). N**eurodevelopmental testing will be done at 6-12 months of age. Infants will be followed up to 1 year of age with monthly respiratory questionnaires that will be completed via phone. The study will be performed at OHSU and at PeaceHealth SouthWest Washington Medical Center (PHSW) and build on Dr. McEvoy's strong pilot data[16]. It is not feasible or ethical to blind the caregivers and researchers to the randomized intervention in the NICU; however, the 6 month PFTs will be directed by personnel blinded to treatment allocation in the NICU and blinded personnel will administer the monthly respiratory questionnaires and neurodevelopmental assessments. The individual PFTs will be reviewed for acceptability, reproducibility, and completeness by Dr. Tepper, (Co-PI of study) and Christina Tiller, RRT, an experienced infant pulmonary function technologist. They will be blinded to the treatment allocation as will the statisticians analyzing the final data.

**Figure 9. Study Overview**




## 5. Study Population

### a. Number of Subjects

- We anticipate screening 300 infants to recruit the cohort
- Up to 130 infants will be consented once inclusion/exclusion criteria have been verified
- 100 infants will be randomized

### b. Inclusion Criteria

**1)** Infants born at >24 to ≤ 32 weeks gestation
**2)** Treated with CPAP for ≥ 24 hours for respiratory distress (either as initial therapy or following extubation)

### c. Exclusion Criteria

**1)** Significant congenital heart disease
**2)** Major malformations
**3)** Chromosomal anomalies
**4)** Culture proven sepsis at consent

5) Complex maternal medical conditions
6) Clinical instability
7) Multiple gestations > twins
8) <3rd or >97th percentile for weight[85]
9) Participating in another neonatal randomized clinical trial with a competing outcome
10) Mother/legal guardian without stable method of communication

Additional criteria that must be met **prior to randomization**:

1) CPAP stability criteria met for at least 12 hours

- The following CPAP stability criteria[16;86] has to be met for more than 12 hours by 34 weeks of CGA:
  - CPAP of 5 cmH2O
  - On room air
  - Respiratory rate ≤70 bpm without significant retractions
  - <3 self-resolving apneas or bradycardias or desaturations in 1 hour in last 6 hours
  - Average SpO2 >86% for 90% of the last 24 hours
  - Not being treated for a patent ductous or sepsis
  - Tolerating CPAP "cares"

### d. Vulnerable Populations

Neonates will be included in this research. The inclusion of neonates is critical to the research because we have pilot data in preterm infants demonstrating that eCPAP may stimulate lung growth. However, our initial trial did not include follow-up testing after discharge to determine the potential duration of the effect of eCPAP on lung volumes and did not include measurements of lung diffusing capacity. Parent or guardian permission will be obtained before the child will be enrolled in the study, as per IRB guidelines.

### e. Setting

Infants will be recruited in the 44 bed NICU at OHSU where Dr. McEvoy is the Director of Neonatal Research and the 20 bed NICU at PHSW where the OHSU neonatology division also provides NICU services. The OHSU Neonatal Division has extensive experience with bubble CPAP which has been the standard therapy of preterm infants for > 15 years at OHSU. The Neonatal Division follows extensive clinical consensus guidelines (CCG) with regards to important aspects of patient care including: enteral feeds, transfusions, septic workups among others. This will provide critical consistency to the proposed eCPAP study for the multiple aspects of patient care. Most importantly, the CPAP CCG since 2014 establishes bubble CPAP via Hudson prongs as the only CPAP delivery system used in the OHSU NICU and outlines the following practice as usual care at OHSU: when an infant meets CPAP stability criteria for at least 12 hours, the CPAP is discontinued and the infant is placed on room air. This CCG was established after review of limited published evidence to provide consistent weaning of preterm infants on CPAP. In NICUs worldwide, there are many different approaches to weaning patients off CPAP[87;88] even within the same NICU. The consistent approach at OHSU and PHSW is an exception and will minimize potential confounders to our results.

### f. Recruitment Methods

Recruitment will be maximized by having a specific NICU research coordinator screen daily for subjects, maintain a screening log, and consent subjects. Daily review of eligibility and CPAP stability criteria for individual patients will be performed with the neonatologist and bedside nurse. The study will be introduced to parents whose infant meets the screening criteria (24 to ≤ 32 weeks gestation and on CPAP). The infant will be

consented after inclusion and exclusion criteria are verified and randomized when CPAP stability criteria are met for at least 12 hours.

We will do extensive in-servicing with all caregivers working in the both NICUs prior to starting the study outlining the study's rationale. We will present data from our initial study[16] which demonstrated that the infant's participation in the study did not affect length of stay or the time taken to achieve full nipple feeds. We will also discuss the fact that participation in the study will not increase hospitalization costs for the infant (level of hospital charges are based on need for gavage feeds regardless of whether infant is on or off CPAP). These same discussions will be done with the parents, starting far in advance of the infant being consented and meeting randomization criteria. We will also assemble a patient advisory group of a projected four parents who will meet at least twice per year to give advice and feedback on the study elements. These parents will be recruited from the current OHSU NICU family advisory council that meets monthly.

**g. Consent Process**

Written informed consent will be obtained from each subject's parent/legal guardian prior to any study procedures taking place. Once an eligible infant is identified, individuals authorized to obtain written informed consent (PI, co-investigators, and study staff specifically designated by the PI) will begin the informed consent discussion with the subject's parent(s)/legal guardian. The informed consent process will take place in the NICU or in a quiet, private area.

The information provided in the consent will cover the elements in the CFR Part 50.25 and be approved by the site Institutional Review Board (IRB). This includes the investigational nature and objective of the trial; the procedures and treatments involved and their attendant risks, discomforts, and benefits; and the potential alternative therapies, alternative to not participate and right to withdraw without penalty, all of which will be explained to the parent/legal guardian in detail. All of the parent/legal guardian's questions will be answered before signing the consent form. If the parent/legal guardian wishes to take the consent form home to consult with other family members or health care providers, or to allow more time for consideration, they will be allowed to do so. A copy of the signed consent form will be given to the parent/legal guardian.

The informed consent process will be an ongoing active process of sharing information between the investigator and the parent/legal guardian(s). If a protocol change requires a change to the consent form, parents/legal guardian(s) will be notified in a timely manner and the new informed consent form will be signed. Parent(s)/guardian(s) can withdraw from the study at any time. The study PI can discontinue a subject from the study at any time for safety concerns. Assent will not be obtained as the subjects in this study are neonates.

Permission may be obtained from legally authorized representatives, described as an individual who is authorized under applicable state or local law to consent on behalf of a child to general medical care when general medical care includes participation in research. Non-English speaking subjects may be enrolled. We will utilize a translated consent in Spanish and the IRB approved short forms for other languages. We will obtain an "Investigator's Certification for Activities Preparatory to Research" from the OHSU IRB to examine records of infants preparatory to research that contain protected health information. The medical records will be reviewed to assess eligibility for this trial prior to approaching the parents to discuss consent.

**h. Randomization Method and Blinding**

Subjects will be randomized after meeting inclusion/exclusion criteria, being consented, and meeting the additional randomization criteria:

1) CPAP stability criteria met for at least 12 hours

The following CPAP stability criteria[16;86] has to be met for more than 12 hours:

- CPAP of 5 cmH2O
- On room air
- Respiratory rate ≤70 bpm without significant retractions

- <3 self-resolving apneas or bradycardias or desaturations in 1 hour in last 6 hours
- Average SpO2 >86% for 90% of the last 24 hours
- Not being treated for a patent ductous or sepsis
- Tolerating CPAP "cares"

Randomization will be done via REDCap by the data management team within strata defined by gestational age at delivery (28 and 6/7 and 29 and 0/7 weeks). If twins are both enrolled they will be allocated to the same treatment arm[89]. A computer generated randomization scheme with stratum using random block sizes of 2 or 4 will be uploaded into REDCap for retrieval. The NICU team will know the treatment allocation but the follow-up research team assessing outcomes will be blinded to the treatment allocation. The electronic medical record will note the infant was in the study, but will not state allocation arm.

### i. Assessment of Treatment Adherence

After randomization, the infant will be assessed daily by research staff for the 14 days on the assigned trial arm. As done in the pilot eCPAP study[16], failure criteria for the respective randomization arms will be as follows: Infants randomized to dCPAP (usual care) will be defined as failing if they demonstrate:

- significantly increased work of breathing with a persistent respiratory rate > 75 breaths/ minute,
- increased apnea and/or bradycardia and/or desaturations > 2 significant episodes in 1 hour in the past 6 hours,
- oxygen need to maintain oxygen saturations > 86%,
- a blood gas pH of < 7.2, $PaCO_2$ > 65 mm Hg, or
- major apnea or bradycardia needing resuscitation[16;86]

Subjects who meet the criteria for failing dCPAP will be resumed on their previous level of CPAP. Another attempt to discontinue the subject's CPAP will occur within the next 5 days when they again meet the predefined stability criteria. If the subject does not meet the stability criteria or fails a second attempt at discontinuation, further weaning/management will be by the clinical team. The infant will continue to be studied as intention to treat. In our pilot study[16], only 1 out of 26 subjects failed CPAP discontinuation.

Infants randomized to the eCPAP arm will have the CPAP stopped during the treatment period if there is significant abdominal distension with feeding intolerance or significant nasal breakdown (checked and documented every 3-4 hours as outlined in the OHSU NICU's CCG). The CPAP will be discontinued for up to 5 days and if symptoms resolve, the CPAP will be reapplied. If symptoms reoccur, the CPAP will be removed and further management will be by the clinical team. The infant will continued to be studied as intention to treat. In the pilot CPAP study[16], all 22 patients tolerated eCPAP without adverse events.

### j. Removal of Patients from Allocated Treatment Arm or from the Study

The investigator or the parents of the preterm infant may discontinue treatment arm allocation or withdraw the infant early from the study at any time for safety or administrative issues. The infant may be withdrawn from the treatment arm per parent request, protocol violation, worsening clinical condition, or investigator decision. Infants who discontinue study arm/treatment allocation will remain in the study for outcome measurements, unless the consent is withdrawn.

## 6. Study Procedures Involved

### a. Clinical Data Collection in NICU

Pertinent maternal and infant data through discharge from the NICU will be collected, including: maternal: age; parity; smoking history; antenatal steroid treatment; etiology of preterm delivery; birth weight and gestational age; surfactant dosing; duration of: oxygen, mechanical ventilation and CPAP (prior to study randomization); respiratory medications; gestational age at full nipple feeds and at discharge.

During the 2-week treatment period detailed data will include daily weights; weekly lengths; calories per day; apneic episodes; oxygenation needs; parental interactions; developmental interactions such as frequency and duration of time spent skin to skin with a parent.

In addition, downloads of continuous oxygen saturation will be done during the two week treatment period with a separate pulse oximeter (will not be used for clinical care so monitor will be covered from caretaker and alarms disabled) with a 2 second averaging time and a 2 second sample rate (Radical; Masimo Corp., Irvine, California) monitors. Intermittent hypoxemia (IH) events will be defined as an oxygen saturation ≤ 80% for ≥ 20 seconds and ≤ 180 seconds as previously described[78-80]. Episodes of IH and other patterns of oxygen saturation will be compared between the groups.

**b. Blood for Epigenetics and Proteomics**

Alterations in epigenetics including DNA methylation have been associated with perinatal origins of lung disease after different scenarios including in-utero smoke exposure[90] and in association with decreased lung disease in preterm infants managed on CPAP versus mechanical ventilation[91]. At the end of the two week treatment period of randomization to eCPAP versus dCPAP, about 2 mL of blood will be collected at the time of a blood draw for clinical care. Blood or DNA will be stored for future analysis of targeted and global DNA methylation and for proteins shown to be important in lung development in our primate model of CPAP such as YAP1, SCGB1A1, and STAT1[92]. Proteomic analysis will give a non-invasive assessment of the types and quantity of circulating proteins since samples of lung tissue and washings are not feasible. The targeted DNA methylation will be done using bisulfite amplicon sequencing or polymerase chain reaction based methods based on pre-existing knowledge from the literature such as IGF1 or TGFB pathways. Global methylation patterns will be investigated using ELISA based method (Imprint® Methylated DNA Quantification Kit, Sigma). This blood sampling and analyses are an optional part of the consent and will be stored in Dr. McEvoy's locked research freezer in the CDRC-P at OHSU.

**c. Inpatient Infant Pulmonary Function Tests (PFTs)**

Infant pulmonary function testing will be performed when CPAP stability criteria is met prior to randomization and at the end of the two week treatment period. Studies will be performed with the infant supine, quietly asleep and at least 30 minutes after a feed. All measurements will be obtained using a computerized infant pulmonary function cart (SensorMedics 2600; SensorMedics Inc, Yorba Linda, CA; Jaeger/Viasys Master Screen Baby Body) in the OHSU NICU at the infant's bedside. Standard testing criteria outlined by the American Thoracic Society / European Respiratory Society will be met [81;93,94]. Tidal breathing will be recorded with up to 50 flow-volume loops[46]. Passive respiratory mechanics (respiratory compliance [Crs] and respiratory resistance [Rrs]) will be obtained using the single breath occlusion technique[95]. Functional residual capacity (FRC) will be obtained with the nitrogen washout technique with acceptance criteria as per ATS/ERS guidelines[94]. Vital signs (respiratory rate, heart rate, oxygen saturation) will be continuously monitored during the testing. If a PFT does not meet the pre-specified technically acceptable criteria it can be reattempted in 24-48 hours.

**d. Outpatient Infant Pulmonary Function Tests**

Infant pulmonary function testing will be performed at 6 months of CGA (range of 5-8 months corrected age) at OHSU for all randomized patients. These will be primarily directed and performed by Dr. MacDonald who will be blinded to the NICU treatment allocation. Dr. MacDonald has experience with measurements of $V_A$, $D_L$,

and FEFs as these are the PFTs that were performed in the study at the Oregon National Primate Research Center examining the effect of CPAP on lung development. Keith Jackson RRT will also be a part of the research team performing the 6 month PFTs and he will be blinded as well. He was an integral part of the successful VCSIP study[83,84] which measured FEFs at 3 and 12 months of age in offspring of pregnant smokers randomized to vitamin C versus placebo during pregnancy. Standard acceptance criteria will be applied. The PFT results will be reviewed off site for acceptability, reproducibility, and completeness by Dr. Tepper and/or Christina Tiller, RRT who has over ten years of experience in Dr. Tepper's PFT laboratory performing and interpreting these tests. They will be blinded to the treatment allocation in the NICU as will the statisticians at Indiana University performing the data analysis.

No testing will be done within 3 weeks of a respiratory illness. A test may be repeated if the quality was not acceptable. This testing will include the measurement of alveolar volume (VA), pulmonary diffusing capacity (DI),[5;15] forced expiratory flows (FEFs),[84] and functional residual capacity[93;94] following the guidelines of the American Thoracic Society and European Respiratory Society which were co-written by Dr. Tepper [74;81]. The pulmonary function testing equipment and operational procedures will be rigorously calibrated according to the manufacturer's directions and outlined explicitly in the operations manual. The OHSU Pediatric Sedation Protocol will be followed using oral chloral hydrate.[74;81] The infant will be given 50 to 100 milligrams per kilogram of chloral hydrate by mouth, with a maximum dose of 1 gram. The parents will be counseled about possible side effects including temporary disorientation, nervousness, excitement, dizziness, coughing, nausea, mild desaturations, obstruction of airway, apnea and will be asked to sign a separate consent for sedation. They also will also be given patient education-discharge instructions. Adverse events will be reported as outlined below. For pulmonary function measurements with published normative data, the parents will be told whether the results of the pulmonary function test were within normal limits or outside of normal limits, and this report will be faxed to the patient's physician. The hemoglobin level will also be shared with the parent and if outside of normal limits for the CGA will be relayed to the infant's physician.

Alveolar Volume ($\mathbf{V_A}$ ) **(Primary Outcome of Study)** and: Pulmonary Diffusing Capacity ($\mathbf{D_L}$)**(Secondary Outcome):** Measurements will be obtained using an induced respiratory pause technique at an elevated lung volume (30 cm of $H_2O$) that we developed and have previously described[5;68] as approved in OHSU eIRB# 386. The inspiratory test gas contains 0.5% Ne (neon), 0.5% CO (carbon monoxide), 21% $O_2$ (oxygen) and balance $N_2$ (nitrogen). Concentrations of CO and Ne are measured with a gas chromatograph. During passive expiration following the 4-second induced respiratory pause for gas exchange, CO and Ne concentrations are used to calculate $\mathbf{V_A}$ and $\mathbf{D_L}$. Results are expressed as averages of 2-3 measurements within 10%, adjusting for Hemoglobin from a finger stick.

Forced Expiratory Flows ($\mathbf{FEF_{50}}$) **(Secondary Outcome)** Measurements in triplicate will be obtained using the raised volume thoracic compression technique that we developed, and previously described[74;75;83].

Chloral hydrate sedation: Infant PFTs will be performed at Doernbecher Children's Hospital in sleeping infants following chloral hydrate sedation, as previously described[68;74;84]. For testing, infants are examined by a physician and continuously monitored by a sedation nurse or health care provider certified in sedation and a respiratory therapist. The parents will be counseled about possible side effects, sign a separate sedation consent, and be given the patient education-discharge instructions. Safety will be monitored as outlined in the DSMB charter.

**e. Respiratory Questionnaires**

After discharge, a validated respiratory questionnaire will be administered monthly to the infant's primary caregiver [83;96] by research personnel blinded to the treatment allocation of eCPAP versus dCPAP in the NICU.. Caregivers are asked about new episodes of wheezing in the previous month, as well as administration of prescribed medications, other illnesses, trips to the emergency room, and hospitalizations. If an infant has wheezing associated with an illness lasting up to seven days, this will be counted as one episode. These questionnaires will be administered in person at the PFT done at approximately 6 months of CGA while the

remainder of the monthly questionnaires will be administered by phone. A small time reimbursement will be available for those patients after each questionnaire is completed.

### f. Neurodevelopmental Testing

All preterm infants born at <34 GA are examined in the OHSU Developmental Evaluation Clinic (DEC) if born at OHSU or the PHSW DEC clinic if born at PHSW.at 6 -12 months CGA. This assessment is the standard practice of their care. We will document the Bayley IV neurodevelopmental scores for cognitive, receptive language, expressive language, fine motor, gross motor, and audiology results from these visits[97;98]. The personnel performing this testing will be blinded to the treatment allocation of eCPAP versus dCPAP in the NICU.

### g. Cohort Retention Methods

Cohort retention will be maximized with the application of novel and evidence based retention techniques[83] as used in our previous VCSIP study which had a cohort retention of 93% at attempted 3 month PFTs[84] and 94% of infants with completion of respiratory questionnaires through 12 months of age as per protocol [84]. Techniques include regular multimodal contact such as phone calls and text messages, social media private messaging, and pre-arranged check-ins at mother or child's appointments. In instances where contact is difficult, alternative retention strategies are: searching databases for updated contact information; calling at different times of the day and on weekends; and contacting close friends and/or relatives who were listed by the participant as back-up contacts, or the participant's work if permission was obtained during consent.

### h. Parent Questionnaire and Future Contact

At the completion of the study the parent will be asked to complete a series of questions adapted from the standardized Research Participant Perception Survey [99] consisting of Likert scale items to assess their experience and satisfaction with the research study, team, and process.

We may contact subjects in the future if there are things we need to discuss or ask regarding this study or for future study opportunities, if they agreed on the consent form to this future contact. We will also review the subject's medical records in the future to look at breathing and behavior outcomes up to 24 months of age. We may also review and obtain other health information which may be important for the study. We may obtain this information so that we can be sure to have all of the important information we need for the study.

### i. Adverse Event and Protocol Violation Reporting

Safety events requiring expedited reporting will be sent to the DSMB chair within 7 calendar days of learning of the event. The DSMB chair will respond to Dr. Cindy McEvoy (study PI) with recommendations within 14 calendar days.

All adverse events after randomization through discharge from the NICU and any adverse events after discharge deemed related or possibly related to the study (performance of the pulmonary function test done at 6 months of age with required sedation) will be recorded on the Adverse Events Log. Events will be reported to the IRB as per IRB policy and procedures. Adverse events (AEs) will be identified by review of the subject's electronic medical record (EMR), and by physical exam / observations (including vital signs monitoring during sedation). AEs will be graded as to their expectedness and attribution (unrelated, possibly, probably or definitely related to the protocol). The following standard definitions will be used:

#### i1. Definitions

Adverse Event (AE): Any untoward or undesirable, although not necessarily unexpected, event experienced by a human subject that may be a result of:

- The interventions and interactions use in the research
- The collection of identifiable private information in the research
- An underlying disease, disorder, or condition of the subject
- Other circumstances unrelated to the research or any underlying disease, disorder, or condition of the subject

Serious Adverse Event (SAE): Any AE that:

- Is fatal
- Is life-threatening
- Is persistent or significantly disabling or incapacitating
- Results in inpatient hospitalization or prolongation of hospitalization
- Results in psychological or emotional harm requiring treatment
- Creates a persistent or significant disability
- Results in a significant medical incident (considered to be a serious study related event because, based upon appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition)

Unanticipated problems (UP): Events that are not expected given the nature of the research procedures and the subject population being studied and suggest that the research places subjects or others at a greater risk of harm or discomfort related to the research than was previously known or recognized. Harm to a subject need not occur for an event to be an unanticipated problem.

**i2. Reporting of Adverse Events**

All AEs, SAEs, and UPs should be documented on the appropriate adverse event logs/forms,(see Appendix B of DSMB charter) entered into the REDCap database, and reported to the study PI, Dr. McEvoy, as outlined below.

- All SAEs and UPs should be reported by to Dr. McEvoy within 1 business day.
- All other AEs should be entered into REDCap within 7 days.

Dr. McEvoy and her research team will evaluate each event and will determine reporting requirements. Dr. McEvoy and/or her research team will report events to the DSMB according to the following timeframes:

- All SAEs and UPs that require expedited reporting (SAEs that are deemed related, suspected to be related, and unexpected, all UPs) will be reported to the DSMB chair within 7 calendar days of the time the PI learns of the event.
- All other SAEs and AEs will be tabulated and reported to the DSMB at the next biannual meeting.

Additionally, the following events will also require expedited reporting:

- Neonatal or infant death through 1 year of age
- Any sedation related event requiring significant resuscitation and/or hospitalization

**i3.Periodic Summary Reporting**

All other AE's (that did not require expedited reporting as above) will be summarized biannually for the DSMB.

**i4.Expected Risks**

As detailed in the consent form, expected risks during the course of the study may include:

- Restlessness during PFTs in the NICU
- Pain during heel stick
- Embarrassing questions

- Skin breakdown around infant's nose or significant abdominal distension with feeding intolerance during the 2 weeks treatment period in infants randomized to eCPAP
- Increased respiratory distress with significant apnea during the 2 week treatment period in the infants randomized to dCPAP
- Sedation with chloral hydrate may include temporary disorientation, nervousness, excitement, dizziness, coughing, nausea, mild desaturations, obstruction of airway, apnea
- Breach of confidentiality

These risks are considered moderate and are addressed in the consent form.

The DSMB will meet twice per year by teleconference and review all other AEs/SAEs at that time. The DSMB will be composed of two neonatologists, a research ethicist (who may also serve as one of the neonatologists), a pediatric pulmonologist, and a statistician. Members of the NHLBI will also attend the DSMB meetings. See DSMB charter for further details. Minor protocol deviations will be documented in the subject's research chart. Major protocol violation will be reported to the IRB, per IRB guidelines.

## 7. Data Analysis

### a. Sample Size Estimation and Power Considerations

No studies have compared $V_A$ at 6 month of age in stable preterm infants randomized to eCPAP versus dCPAP in the NICU. Data from our pilot eCPAP study showed a 12% higher FRC in stable preterm infants randomized to eCPAP versus dCPAP at the end of the two week treatment period[16]. A study by Dr. Tepper et al reported a 16% difference in both $V_A$ and $D_L$ in preterm infants according to CPAP treatment in the NICU (Table 1)[15] and a 12% difference in $D_L$ between infants with BPD versus healthy term infants[69]. Therefore, we powered our randomized trial to detect a 12% difference in $V_A$ between the eCPAP and dCPAP groups.

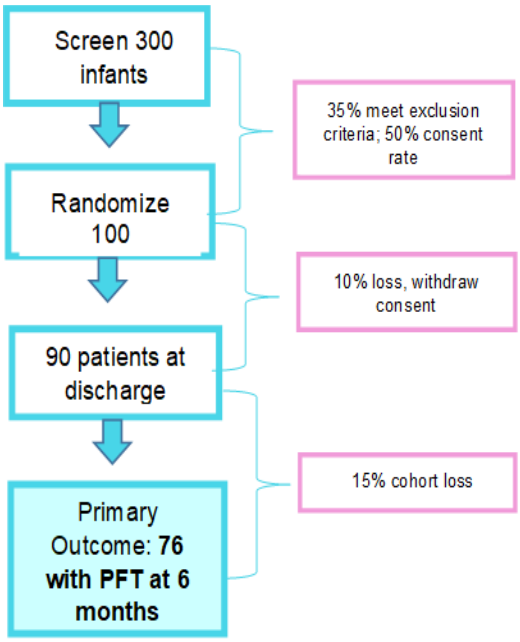
Using an ANCOVA model and assuming a mean $V_A$ of 642 mL (SD=189) based on data of the overall mean and SD from the control and CPAP groups reported in Assaf et al[15], a sample size of 34 infants per group is needed to yield 80% power for detecting a 12% difference (effect size=0.42) in $V_A$ in the eCPAP group at $\alpha$=0.05 (Table 3). This sample size also allows us to detect a 12% difference in $D_L$ with an effect size of 0.42. We estimate that 18% of the study samples will be twin pairs based on our pilot eCPAP study. Conservatively counting just one infant out of each twin pair for the sample size estimation, we will need 38 infants per group (76 total infants at 6 months) with completed $V_A$ outcomes. We further conservatively project a 10% loss in the NICU and a 15% loss from NICU discharge to the 6 month PFT (due to preterm population and need to be at least 3 weeks after a respiratory infection for the performance of the PFT) and therefore we project we will

**Table 3**. Estimated power for 34 infants per group with completed PFT data

| Outcome Measures | | Mean (SD) in Control Group | Estimated Power for Group Difference | | |
|---|---|---|---|---|---|
| | | | 12% | 15% | 17% |
| Alveolar volume ($V_A$) | Primary Outcome | 642 (189) | 80 | 92 | 97 |
| Lung diffusing capacity ($D_L$) | Secondary Outcome | 4.1 (1.19) | 80 | 92 | 98 |

need to randomize 50 infants per group (total n=100) into the trial. This sample size will provide 80% power for detecting an effect size difference of 0.42 in outcome measures between the two groups in Aim 1 (primary outcome of $V_A$) and Aim 2 for $D_L$. This sample size allows us to detect a 23% difference in $FEF_{50}$. Figure 12 shows the projected study recruitment to achieve the desired sample size based on recruitment and cohort loss data from the pilot eCPAP study and from the VCSIP[83,84] study. In the pilot eCPAP study 66% of parents whose infants met all inclusion and exclusion criteria consented to enrollment in the study. In this follow-up eCPAP study we are projecting that 50% of the parents whose infant meets all inclusion and exclusion criteria will consent due to the needed sedation at the 6 month PFT.

**Figure 12: Projected Study Recruitment and Follow-up**




With regards to the exploratory clinical respiratory outcome, published data in 48 preterm infants with an average gestational age of 28 weeks who did not develop BPD (similar to the group we project to recruit) reported a 48% incidence of wheeze and a 79% incidence of wheeze and cough in the first year of life [35]. These researchers also documented a 21% incidence of wheeze in 195 term infants during the first year of life. In our initial vitamin C study[46] using the same questionnaire we will use in this proposed eCPAP study, 76 term infants born to non-smokers had a 27% incidence of wheeze in the first year of life. Based on these data, we project that 48% of the infants who have CPAP discontinued by the CPAP stability criteria will have wheeze in the first year of life. With our projected sample size of 50 per group accounting for a 15% missing questionnaire rate, we will have 70% power to show a decrease in the incidence of wheeze from 48% to 21% and 90% power to show a decrease in wheeze and cough from 79% to 50%.

### b. Statistical Analysis

To verify the comparability of the randomized groups, infants' baseline characteristics will be compared using analysis of T-test or non-parametric Wilcoxon test for continuous variables and chi-squared test for categorical variables. We will examine the distributions of continuous variable and use alternative approaches such as transformation or nonparametric methods in cases of violation to the normal distribution assumption. We will examine the frequency distribution of all categorical variables and adopt exact inference procedures in cases of zero or small cell size. All analyses will be conducted using the SAS 9.4 (SAS Institute, Carey, NC).

### b1. Specific Aim #1 (Primary Outcome)

General linear mixed models (GLMMs) will be used to compare the primary outcomes of alveolar volume ($V_A$) between the eCPAP and dCPAP while adjusting for the design factor gestational age at delivery (28 and 6/7 and 29 and 0/7 weeks) and other baseline variables that are found to be statistically different between the two groups. Since the lung function measures are highly dependent on infant length and sex, we will further adjust for length and sex in the GLMMs. In order to adjust for intra-twin correlations, generalized Estimating Equations (GEEs) with the linear link function will be used to model $V_A$.. The GLMMs can be performed using the GEE estimation approach. Intention to treat analysis will be used for all aims as the primary analysis. The intention to treat population is defined as all randomized infants and the analysis uses the assigned randomization group even if infants did not complete the intervention. In addition, a per protocol analysis will be completed for all aims. The per protocol population is defined as all infants who successfully completed the treatment arm to which they were assigned.

### b2. Specific Aim 2 (Secondary Outcomes)

Lung diffusion ($D_L$) and $FEF_{50}$ will be will be analyzed with the same general approaches described for the primary outcome in SA #1.

### b3. Specific Aim 3 (Exploratory Outcomes)

Logistic regression models will be used to compare the incidence of wheeze and the incidence of wheeze and cough between the two groups adjusting for GA at delivery (<29 vs ≥ 29 weeks). Neurodevelopmental scores will be analyzed with the same general approaches described for the primary outcome in Specific Aim 1.

### c. Missing Data

We anticipate loss to follow-up for the lung function outcomes at 6 months and respiratory outcomes through 12 month of age. Our sample size estimation conservatively accounts for 10% cohort loss from randomization to NICU discharge and a 15% loss from discharge until the 6 month PFTs. However based on VCSIP, which had a 93% cohort retention for attempted of 3 month PFTs[84], coupled with our retention techniques, we suspect this will be overestimated but want to ensure an adequate sample size for analysis of the primary outcome. We will compare patients' characteristics at baseline between those with outcome measures and those lost to follow-up and determine whether the missing data differ between the groups. If the missing at random assumption is violated, we will conduct extensive sensitivity analyses to model various non-ignorable missing data patterns to examine the robustness of our findings to the various missing data assumption[100].

### d. Interim analysis and stopping rules

No interim analysis for efficacy or futility is planned. There will be no pre-specified stopping rules, but the DSMB will review SAEs as they occur. Although this is a fragile population, based on our pilot CPAP study, the fact that infants will be convalescing by the time they are enrolled in the study, and our experience with sedation in the VCSIP[84], we do not anticipate a large number of SAEs but will be vigilantly monitoring. The DSMB chair may call a DSMB meeting to specifically review safety concerns.

## 8. Data and Specimens

### a. Handling of Data and Specimens

All data will be stored in locking file cabinets and contained within a password protected database. Neonatal and follow-up data from all patients consented for participation into the study will be recorded on forms designed specifically for this study. A screening log of all preterm infants who qualify for randomization to eCPAP versus dCPAP will be kept to determine the generalizability of the population. All data will be entered electronically by the research staff into the secure REDCap system. Data will be store indefinitely.

### b. Sharing of Results with Subjects

For pulmonary function measurements with published normative data, the parents will be told whether the results of the pulmonary function test were within normal limits or outside of normal limits, and this report will be sent to the patient's physician. The hemoglobin level will also be shared with the parent and if outside of normal limits for CGA will be relayed to the infant's physician.

### c. Data and Specimen Banking

Data and blood specimen will be banked in a repository for future research as outlined in the separate repository protocol.

### d. Performance Monitoring

The data center will present regular reports to the eCPAP Steering Committee and the Data and Safety Monitoring Board. These will include:

- Monthly recruitment reports: reports of the number of preterm infants screened and enrolled by month will be provided weekly and monthly to the eCPAP Steering Committee.
- Quarterly Steering Committee reports: a report detailing recruitment, baseline patient characteristics, data quality, incidence of missing data and adherence to study protocol will be provided quarterly to the eCPAP Steering Committee.
- Data and Safety Monitoring Board reports: for every meeting of the DSMB, a report is prepared which includes adverse events, patient recruitment, treatment data, baseline patient characteristics, performance information with respect to data quality, timeliness of data submission and protocol adherence (in addition to safety and efficacy data).

## 9. Privacy, Confidentiality, and Data Security

Upon enrollment, subjects will be assigned a code that will be used instead of their name, medical record number or other personally identifying information. Electronic files for data analysis will contain only the subject code. Codes will not contain any part of the 18 HIPAA identifiers (initials, DOB, MRN). The key associating the codes and the subjects personally identifying information will be restricted to the PI and study staff. Data will be entered into the REDCap system by site study personnel.

Standard practices will be followed to maintain the confidentiality and security of data collected in this study. Study staff will be trained with regard to these procedures. Paper files will be stored in locked filing cabinets in restricted access locations. Electronic data will be stored in a web-accessible encrypted REDCap database housed on an OHSU secure server. Access to data/specimens is restricted to study personnel.

### a. Provisions to Monitor the Data to Ensure the Safety of Subjects

A locally appointed NIH approved DSMB will monitor the study. See attached DSMB charter

### b. Risks and Benefits

### b1. Risks to Subjects

See section i4 above.

### b2. Potential Benefits to Subjects

The subject may or may not benefit by taking part in this study. There is no guarantee that the infant will receive direct benefit from his/her participation in this study. The benefits of being in this study may be a chance that the additional 2 weeks of CPAP may improve lung function and possibly lower respiratory morbidity and/or improve neurodevelopmental outcomes. Participation may provide information that may benefit other premature babies by helping to determine what length of CPAP provides maximum benefits. Still, the infant may get no direct benefit from this study.

## 10. Study Administration

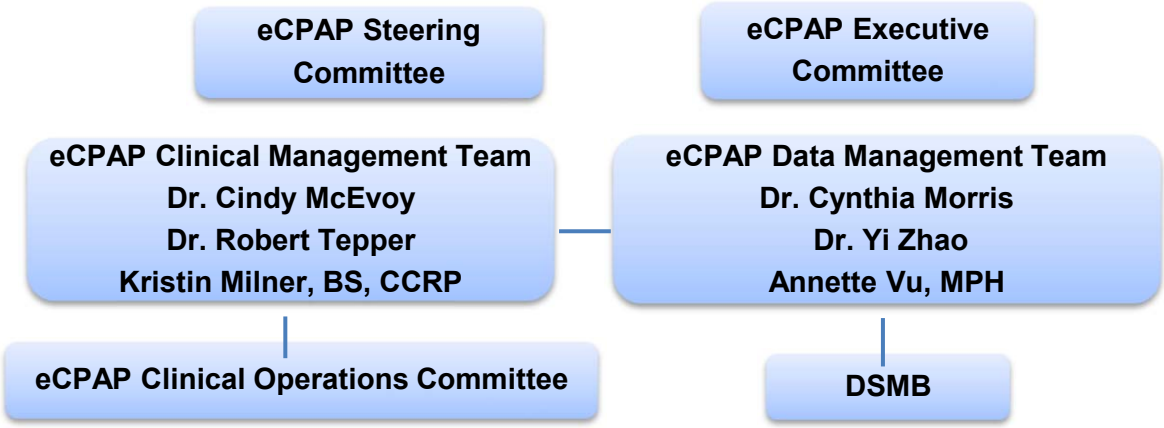
### a. Funding and Participating Center

The study is funded by the National Institute of Health's Heart, Lung and Blood Institute. The study is being conducted at one clinical center at Oregon Health & Science University in Portland, Oregon.

### b. Committees

The Principal Investigators (Drs. McEvoy and Tepper) of the study have agreed to abide by the study protocol. Execution and oversight of this trial will occur through a clinical coordinating team and a data coordinating team as detailed in Figure 13 along with a clear understanding of roles and responsibilities. The clinical team will be led by Dr. Cindy McEvoy (Co-PI of the proposed study) and the data team will be led by Dr. Cindy Morris. Kristin Milner is an experienced project manager who has worked with Dr. McEvoy for over 8 years and will be a crucial part of this administrative structure as the clinical project manager. Annette Vu will serve as the data team project manager.

Figure 13. eCPAP Study Team




### b1. eCPAP Steering Committee

This committee will provide oversight for protocol development and implementation as well as troubleshooting any issues that arise that may impact the overall success of the trial. The committee will meet quarterly in person and/or via teleconference. The Steering Committee will also act as the Publication Committee to review and approve all abstracts, presentations, and manuscripts prior to submission or presentation.

Dr. Cindy McEvoy and Dr. Cynthia Morris will lead the eCPAP Steering Committee which will include:

- Clinical management /center leader/PI, Dr. Cindy McEvoy
- Data management /center leader/PI, Dr. Cynthia Morris
- Dr. Robert Tepper, Co-PI, expert on measurements of infant alveolar volume and lung diffusing capacity
- Data statistician, James Slaven, MS (Indiana)
- Dr. Kelvin MacDonald, Co-Investigator
- NIH representative

### b2. eCPAP Executive Committee

Drs. McEvoy and Morris as well as the corresponding project managers Kristin Milner and Annette Vu will meet every two weeks as the executive committee to review progress in study start up during the R61 phase, and recruiting and retention during the R33 phase. Their role is to provide day-to-day management of the trial and to resolve operations issues as they arise. This group will review the study tracking as well as reports of recruiting, retention, data safety and data quality at every visit. In the event there is a disagreement or dispute between the clinical management team and the data management team, it will be resolve in conjunction with the NIH representative

### b3. Data and Safety Monitoring Board

The DSMB will be approved by the NHLBI and will be a group of individuals not affiliated with any of the institutions. Before the trial can begin, the protocol must be approved by the committee. During the conduct of the study, the committee is charged with monitoring the emerging results for efficacy and safety, in addition to center performance and protocol adherence. Recommendations by the committee can include protocol modification, early termination for efficacy, or for unexpected safety problems. Recommendations are made to the NHLBI and disseminated to the Steering Committee.

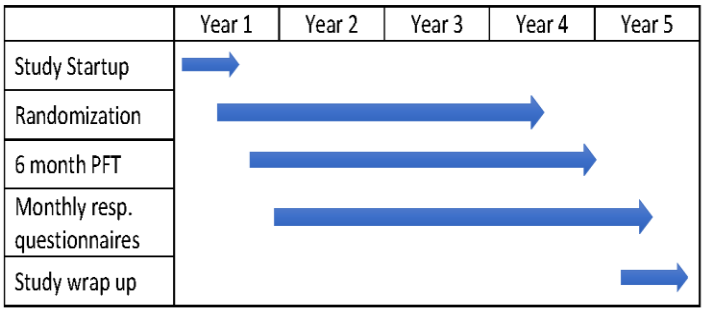
### c. Subject Stipend or Payments

The infant's legal guardian will receive the value of the following as a gift card, placed on a ClinCard, or as cash: $10 per monthly questionnaire completed, $200 for time and travel reimbursements for the pulmonary function test at about 6 months of age and $100 for time and travel to the neurodevelopmental testing.

### d. Study Timeline

We plan on a 6-month start-up period followed by patient randomization over 36 months (month 7 through month 42). This requires recruitment of 3 infants per month. Using an average randomization GA of 32 weeks, we project infants will have their 6 month PFT done during months 14 to 49 and 12 month respiratory questionnaires done during months 20 and 54, allowing 6 months to complete final data analysis, dissemination, publication, and resource sharing.

Figure 14. Study Timeline




### e. Training and Certification

Prior to the beginning of the study, research coordinators, respiratory therapists, and study staff will undergo training on all aspects of data collection and procedures at either OHSU in Portland, Oregon or Indiana University in Indianapolis. All personnel must be trained in all applicable study procedures before patient recruitment can begin.

**f. Recruitment and Data Collection Period**

As in the pilot CPAP study[16], infants will be recruited in the 44 bed NICU at OHSU where Dr. McEvoy is the Director of Neonatal Research. She has been performing infant PFTs here for 15 years as part of research studies[93;101;102], so the NICU is familiar with this testing. The number of patients admitted to the OHSU NICU meeting the gestational age inclusion criteria (>24 to ≤ 32 weeks gestation) is stable (**Table 4**) and enables the desired sample size to be randomized over 36 months. This will allow trial completion, including patient follow-up to finish within the 5 year grant period.

| **Table 4.** Year | 2014 | 2015 | 2016 | 2017 | 2018 | Number needed per year |
|---|---|---|---|---|---|---|
| Infants 24 to ≤32 wks admitted at OHSU/ year | 113 | 118 | 128 | 94 | 120 | 36 |

Recruitment will also occur at PHSW where Dr. McEvoy recruited patients for the VCSIP study. It is a 20 bed NICU that admits approximately 50 infants >24 to ≤ 32 weeks per year.

**g. Final Analysis**

After a two month period for completion of data entry for the primary outcome of the trial, the data set will be locked and available for analysis. Approximately six months will be required to complete the final report to the eCPAP Steering Committee and to prepare the study's primary paper for publication.

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## Appendix A

**Data Collection Schedule**

| | Study entry | Beginning of treatment period | After 2 week treatment period | 6 months of age | 6-12 months of age | Discharge through 12mo of age |
|---|---|---|---|---|---|---|
| Consent and medical history | X | | | | | |
| Breathing test* | | X | X | X | | |
| Blood sample** | | | X | | | |
| Monthly breathing questionnaire by phone | | | | | | X |
| Neurodevelopmental testing | | | | | X | |

*A syrup to make child sleepy will be given only at the 6 month breathing test
** Collected when a sample for clinical care is being done