# A Prospective, Randomized, Open-label Clinical Trial to Assess Apnea Following Administration of 13-valent Conjugate Pneumococcal Vaccine, Diphtheria Toxoid, Tetanus Toxoid, and Acellular Pertussis Vaccine, Inactivated Polio Vaccine, Hepatitis B Vaccine, and *Haemophilus influenzae* Type B Vaccine in Preterm Infants

Short Title: Apnea in Preterm Infants Following the Administration of Routine Childhood Vaccines

**Centers for Disease Control and Prevention**
**Clinical Immunization Safety Assessment (CISA) Project**

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## STATEMENT OF COMPLIANCE

- This trial will be conducted in compliance with the protocol, the International Conference on Harmonization (ICH) Guideline E6-Good Clinical Practice (GCP), and the applicable guidelines and regulatory requirements from the United States (US) Code of Federal Regulations (CFR), 45 CFR Part 46.
- All study personnel with subject contact have completed Human Subjects Protection Training.

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## PROTOCOL SUMMARY

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|---|---|
| **Title:** | A Prospective, Randomized, Open-label Clinical Trial to Assess Apnea Following Administration of 13-valent Conjugate Pneumococcal Vaccine, Diphtheria Toxoid, Tetanus Toxoid, and Acellular Pertussis Vaccine, Inactivated Polio Vaccine, Hepatitis B Vaccine, and *Haemophilus influenzae* Type B Vaccine in Preterm Infants |
| **Phase:** | Phase 4 |
| **Population:** | Up to 300 premature infants <33 weeks and 0 days gestational age and 6-12 weeks postnatal age who will receive 13-valent Conjugate Pneumococcal Vaccine (PCV13), Diphtheria Toxoid, Tetanus Toxoid, and Acellular Pertussis Vaccine (DTaP), Hepatitis B vaccine (HBV), and *Haemophilus influenzae* Type B Vaccine (Hib) |
| **Clinical Sites:** | Three: Duke University (Lead); University of North Carolina (Lead Subcontractor); Cincinnati Children's (Contributing) |
| **Study Duration:** | 28 months |
| **Participant Duration:** | Up to 14 days |
| **Description of Study Procedures:** | This is a prospective, randomized, open-label clinical trial. Infants will be randomized (1:1) to receive either simultaneous administration of PCV13, DTaP, HBV, IPV, and Hib vaccines or no vaccination. Unvaccinated infants will be followed for 48 hours after randomization to capture episodes of apnea. Vaccinated infants will be followed for 48 hours after vaccination for apnea. In addition, vaccinated infants will be followed for 14 days after vaccination for adverse events using information documented in the medical record or obtained by phone call to the parent/legally authorized representative (LAR) on day 14. |
| **Objectives:** | **Primary Objective (PO):**<br>PO 1: To compare proportions of preterm infants with apnea in a 48-hour monitoring period after vaccination in the "vaccinated" group versus a 48-hour monitoring period after randomization in the "unvaccinated" group.<br><br>*The primary hypothesis is that the proportion of infants with apnea will be higher in the "vaccinated" group compared to the "unvaccinated" group.*<br><br>**Secondary Objectives (SO):**<br>SO 1: To compare the clinical importance of apneic events in a 48-hour monitoring period after vaccination in the "vaccinated" group versus a 48-hour monitoring period after randomization in the "unvaccinated" group.<br>SO 2: To compare proportions of preterm infants with severe cardiorespiratory events in a 48-hour monitoring period after vaccination in the "vaccinated" group versus a 48-hour monitoring period after randomization in the "unvaccinated" group.<br><br>**Exploratory Objectives (EO):**<br>EO 1: To compare proportions of preterm infants with temperature instability in a 48-hour monitoring period after vaccination in the "vaccinated" group versus a 48-hour monitoring period after randomization in the "unvaccinated" group. |

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| | EO 2: To compare proportions of preterm infants with other clinically important adverse events in a 48-hour monitoring period after vaccination in the “vaccinated” group versus a 48-hour monitoring period after randomization in the “unvaccinated” group.<br>EO 3: To compare clinically important adverse events occurring between 48 hours and 14 days after vaccination among infants in the vaccinated group who do, and do not have apnea in a 48-hour monitoring period after vaccination. |
| **Outcome Measures:** | **Primary Outcome Measure (POM):**<br>POM 1.1: Proportion of infants with ≥ 1 apneic event in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.<br>**Secondary Outcome Measures (SOM):**<br>SOM 1.1: Average number of apneic episodes in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.<br>SOM 1.2: Average duration of apneic episodes in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.<br>SOM 1.3: Proportion of infants requiring any increase in respiratory support in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.<br>SOM 2.1: Proportion of infants with ≥1 severe cardiorespiratory event in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.<br>SOM 2.2: Proportion of infants requiring positive pressure ventilation in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.<br>**Exploratory Outcome Measures (EOM):**<br>EOM 1.1: Proportion of infants with fever in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.<br>EOM 1.2: Proportion of infants with hypothermia in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.<br>EOM 2.1: Average number of oxygen desaturation events in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.<br>EOM 2.2: Average number of bradycardia events in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.<br>EOM 2.3: Proportion of infants requiring blood culture for and/or antibiotics (intravenously or intramuscularly) in the setting of blood culture for sepsis evaluation in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group. |

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| | EOM 2.4: Proportion of infants with a serious adverse event in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.<br>EOM 3.1: Proportion of hospitalized infants with ≥ 1 episode of clinical apnea between 48 hours and 14 days after vaccination.<br>EOM 3.2: Proportion of hospitalized infants requiring any increase in respiratory support between 48 hours and 14 days after vaccination.<br>EOM 3.3. Proportion of hospitalized infants requiring blood culture and/or antibiotics (intravenously or intramuscularly) in the setting of blood culture for sepsis evaluation in between 48 hours and 14 days after vaccination.<br>EOM 3.4. Proportion of hospitalized infants requiring positive pressure ventilation in between 48 hours and 14 days after vaccination.<br>EOM 3.5. Proportion of discharged infants readmitted to the hospital within 14 days of vaccination. |
| **Estimated Time to Complete Enrollment:** | Approximately 28 months |

# 1 BACKGROUND

## *1.1 Background*

Premature infants (<37 weeks gestational age at birth) are at high risk for undervaccination. Despite concerns regarding the immaturity of the premature infant immune system, the majority of premature infants achieve antibody concentrations generally accepted to correlate with protection.[1] The Centers for Disease Control and Prevention Advisory Committee on Immunization Practices (ACIP) and the American Academy of Pediatrics have thus recommended that in most cases with the exception of hepatitis B vaccine, premature infants should receive all routinely recommended vaccines at the same chronological age as term infants.[2] Despite this recommendation, premature infants have been shown to be underimmunized at 6 months,[3] 12 months,[3,4] 24 months,[5] and 36 months[6] chronological age. Infants who are discharged from the neonatal intensive care unit (NICU) after lengths of stay ≥60 days are at particularly high risk, with only approximately 50% up-to-date at the time of discharge.[7] Vaccination delay at this earlier age thus contributes to undervaccination at later ages.

Premature infants are underimmunized for multiple reasons. In the general healthy infant population, vaccination rates are linked to a variety of factors, including socioeconomic status,[8] parental anxiety,[8] and young maternal age.[9] While these issues still may play a role for premature infants, especially considering the increased burden of prematurity among mothers of low socioeconomic status,[10] it is clear that additional factors have an impact on vaccination rates for premature infants hospitalized in the NICU. Some studies have shown lower birth weight to be a risk factor for undervaccination,[5,6] possibly indicating that more critical disease status may affect the decision to administer vaccines. On the contrary, diagnoses of congenital heart disease and bronchopulmonary dysplasia were associated with increased vaccination rates in another study of infants discharged from the NICU.[7] It is likely that factors affecting vaccination rates vary according to geographic region and individual NICU.

The perceived risk of reappearance or worsening of apnea after vaccination in premature infants likely contributes to undervaccination. Apnea is a common symptom noted in the NICU in the presence or absence of vaccination due to apnea of prematurity. Apnea of prematurity is a developmental disorder characterized by cessation of breathing and often accompanied by bradycardia, cyanosis, or pallor. Apneic events can be divided into 3 subtypes: central (failure of the infant to initiate a breath; obstructive (related to pharyngeal obstruction); and mixed (combination of central and obstructive). The pathogenesis of apnea of prematurity involves persistence of immature reflexes and immature responses to hypoxia and hypercapnia. The incidence of apnea is inversely proportional to the gestational age (GA) at birth, with essentially all infants ≤28 weeks' gestation diagnosed with apnea and 20% of those born at 34 weeks' gestation.[11] Most, but not all, of the U.S. Food and Drug Administration labels for the commonly administered infant vaccines mention premature infants (**Table 1**). While none of the vaccinations are specifically indicated in premature infants, several labels provide warnings that advise of the risk of apnea following vaccination in premature infants (**Table 1**). Awareness of this potential risk has originated from a number of retrospective and observational studies that have evaluated vaccination safety in premature infants (**Table 2**). The incidence of cardiorespiratory events (i.e. apnea and/or bradycardia) following vaccination ranged from 0-47% in these studies. Risk factors for cardiorespiratory events in some studies included lower gestational age and higher clinical disease severity (such as longer period of ventilation and presence of chronic lung disease). In several of these studies, infants served as their own controls, as they were observed pre-vaccination.

The relationship between vaccination and apnea has been studied extensively but the evidence is inconclusive for a causal association. While retrospective and prospective observational studies suggest a possible link between vaccination and apnea in premature infants, there are several inherent problems with these studies. Each study examines different

combinations of vaccinations, and they commonly have small sample sizes, use retrospective data, and vary in inclusion criteria and definition of adverse events. Observational studies that use immunized infants as their own controls also have an important flaw: clinicians are most likely to immunize an infant at a period of high stability. Thus, a small window of time prior to vaccination is likely to contain fewer cardiorespiratory events. After vaccination, infants may experience events associated with prematurity and disease status. Only one randomized controlled trial has been performed and showed no difference in prolonged apneic events between the immunized and control groups.[12] Infants in this study were enrolled at 56-60 days postnatal age. However, some infants in this study received DTaP alone, while others received DTaP-HBV-IPV. The variability in the evidence makes it inconclusive if the apnea described after vaccination in preterm infants is vaccine-related or a complication of underlying prematurity.

**Table 1. U.S. FDA labels for routine infant immunizations: indications and warnings for premature infants.**

| Immunization brand name | Component vaccines | Specific indication for premature infants | Warning in label for premature infants |
|---|---|---|---|
| Pediarix[13] | Diphtheria, tetanus, and acellular pertussis<br>Hepatitis B recombinant<br>Inactivated poliovirus | No | "Apnea following intramuscular vaccination has been observed in some infants born prematurely. Decisions about when to administer an intramuscular vaccine, including PEDIARIX, to infants born prematurely should be based on consideration of the individual infant's medical status, and the potential benefits and possible risks of vaccination." |
| Pentacel[14] | Diphtheria, tetanus, and acellular pertussis<br>Inactivated poliovirus<br>Haemophilus b conjugate | No | "Apnea following intramuscular vaccination has been observed in some infants born prematurely. The decision about when to administer an intramuscular vaccine, including Pentacel, to an infant born prematurely should be based on consideration of the individual infant's medical status and the potential benefits and possible risks of vaccination. The potential risk of apnea and the need for respiratory monitoring for 48 – 72 hours should be considered when administering the primary immunization series to very premature infants (born ≤ 28 weeks of gestation) and particularly for those with a previous history of respiratory immaturity. As the benefit of vaccination is high in this group of infants, vaccination should not be withheld or delayed." |
| Prevnar-13[15] | Pneumococcal 13-valent conjugate | No | "Apnea following intramuscular vaccination has been observed in some infants born prematurely. Decisions about when to administer an intramuscular vaccine, including Prevnar 13, to infants born prematurely should be based on consideration of the individual infant's medical status, and the potential benefits and possible risks of vaccination." |
| ActHIB[16] | Haemophilus b conjugate | No | None |
| Engenerix-B[17] | Hepatitis B recombinant | No | "The potential risk of apnea and the need for respiratory monitoring for 48 to 72 hours should be considered when administering the primary immunization series to very premature infants (born ≤28 weeks of gestation) who remain hospitalized at the time of vaccination and particularly for those with a previous history of respiratory immaturity. It is generally understood that the benefit of vaccination is high in very premature infants. The decision to vaccinate should be based |

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| | | | on careful consideration of the potential benefits and possible risks." |

**Table 2. Evidence of adverse cardiorespiratory events (including apnea) after immunization in premature infants.[1]**

| Immunization | Study design | N | Mean gestational age (weeks) | Cardiorespiratory (apnea and/or bradycardia) events | Follow-up | Factors associated with events |
|---|---|---|---|---|---|---|
| DTwP[18] | Retrospective | 97 | 28 | 20% | 24 hours | Younger gestational age<br>Longer ventilation<br>Chronic lung disease |
| DTwP ± Hib[19] | Prospective observational | 98 | 27 | 17% | 24 hours | Receipt of DTwP alone |
| Hib, HBV, DTaP, IPV[20] | Retrospective | 48 | 26 | 0% | 48 hours | N/A |
| DTwP+Hib[21] | Prospective observational | 97 | 27 | 12% | 72 hours | Lower weight<br>More severe apnea of prematurity<br>Chronic lung disease |
| DTwP, Hib, HBV[22] | Prospective observational | 79 | 28 | 30% | 24 hours | N/A |
| DTaP, IPV, Hib[23] | Retrospective observational | 78 | 28 | 47% | 48 hours | Severe clinical course<br>Persistence of cardiorespiratory symptoms at time of immunization |
| DTaP, IPV, Hib, HBV[24] | Retrospective | 53 | 28 | 13% | 72 hours | N/A |
| DTaP[12] | Randomized controlled | 191 | 27 | "Prolonged apnea"<br>16%: DTaP group<br>20%: control group | 48 hours | N/A |
| DTaP, IPV, Hib, HBV[25] | Prospective observational | 45 | 27 | 11% | 72 hours | Chronic diseases |
| Multiple, including HBV, DTaP, Hib, OPV[26] | Retrospective | 411 | 27 | 5% | 7 days | Previous sepsis<br>Longer prior duration of CPAP |
| Multiple, including HBV, DTaP, Hib, IPV, OPV, Strep pneumo, influenza[27] | Retrospective | 497 | Not provided | 13% | 48 hours | Younger postnatal age<br>Lower birth weight<br>More severe clinical illness |

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| DTaP, IPV, Hib or DTwP, IPV, HiB[28] | Retrospective | 124 | 27 | 45% | 72 hours | Lower weight |
| DTaP, Hib, meningococcal serogroup C[29] | Retrospective | 76 | 27 | 38% | 24 hours | N/A |

DTwP=diphtheria, tetanus, whole cellular pertussis; DTaP=Diphtheria, tetanus, acellular pertussis; HBV = hepatitis B virus; Hib = *Haemophilus influenzae* type B; IPV=inactivated poliovirus; OPV=oral poliovirus.

### *1.2 Summary & Rationale*

Premature infants are at high risk for apnea. Current published recommendations state that this group of infants should receive vaccinations according to chronological age, but frequently this does not occur. Clinicians often delay administration of vaccinations due to concern about the reappearance or worsening of apnea, but evidence to support a causal association is inconclusive. Further study is warranted to quantify the risk and clinical importance of apnea in premature infants after vaccination.

We therefore propose to conduct a randomized clinical trial to assess the risk of apnea in premature infants following routine vaccination. In addition, the study would provide more information about other adverse events following vaccination in the context of current US childhood vaccination practices. This study will provide evidence about the risk and clinical importance of apnea after vaccination in preterm infants and will broaden understanding of adverse events after vaccination in the context of current US vaccination practices. A better understanding of the safety of routine vaccination in preterm infants will assist with clinical decision-making and anticipatory guidance to parents which may improve timely vaccination in preterm infants. Information from this study will also help guide future research efforts to identify potential risk factors and prevention strategies for apnea after vaccination.

## 2 STUDY OBJECTIVES

**Primary Objective (PO):**

PO 1: To compare proportions of preterm infants with apnea in a 48-hour monitoring period after vaccination in the “vaccinated” group versus a 48-hour monitoring period after randomization in the “unvaccinated” group.

*The primary hypothesis is that the proportion of infants with apnea will be higher in the “vaccinated” group compared to the “unvaccinated” group.*

**Secondary Objectives (SO):**

SO 1: To compare the clinical importance of apneic events in a 48-hour monitoring period after vaccination in the “vaccinated” group versus a 48-hour monitoring period after randomization in the “unvaccinated” group.

SO 2: To compare proportions of preterm infants with severe cardiorespiratory events in a 48-hour monitoring period after vaccination in the “vaccinated” group versus a 48-hour monitoring period after randomization in the “unvaccinated” group.

**Exploratory Objectives (EO):**

EO 1: To compare proportions of preterm infants with temperature instability in a 48-hour monitoring period after vaccination in the “vaccinated” group versus a 48-hour monitoring period after randomization in the “unvaccinated” group.

EO 2: To compare proportions of preterm infants with other clinically important adverse events in a 48-hour monitoring period after vaccination in the “vaccinated” group versus a 48-hour monitoring period after randomization in the “unvaccinated” group.

EO 3: To compare clinically important adverse events occurring between 48 hours and 14 days after vaccination among infants in the vaccinated group who do, and do not have apnea in a 48-hour monitoring period after vaccination.

### 2.1 Study Outcome Measures as Related to Objectives

**Primary Outcome Measure (POM):**
POM 1.1: Proportion of infants with ≥ 1 apneic event in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.

**Secondary Outcome Measures (SOM):**
SOM 1.1: Average number of apneic episodes in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.

SOM 1.2: Average duration of apneic episodes in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.

SOM 1.3: Proportion of infants requiring any increase in respiratory support in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.

SOM 2.1: Proportion of infants with ≥1 severe cardiorespiratory event in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.

SOM 2.2: Proportion of infants requiring positive pressure ventilation in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.

## Exploratory Outcome Measures (EOM):
EOM 1.1: Proportion of infants with fever in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.

EOM 1.2: Proportion of infants with hypothermia in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.

EOM 2.1: Average number of oxygen desaturation events in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.

EOM 2.2: Average number of bradycardia events in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.

EOM 2.3: Proportion of infants requiring blood culture for and/or antibiotics (intravenously or intramuscularly) in the setting of blood culture for sepsis evaluation in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.

EOM 2.4: Proportion of infants with a serious adverse event in a 48-hour monitoring period after vaccination in the “vaccinated” group and a 48-hour monitoring period after randomization in the “unvaccinated” group.

EOM 3.1: Proportion of hospitalized infants with ≥ 1 episode of clinical apnea between 48 hours and 14 days after vaccination.

EOM 3.2: Proportion of hospitalized infants requiring any increase in respiratory support between 48 hours and 14 days after vaccination.

EOM 3.3. Proportion of hospitalized infants requiring blood culture and/or antibiotics (intravenously or intramuscularly) in the setting of blood culture for sepsis evaluation in between 48 hours and 14 days after vaccination.

EOM 3.4. Proportion of hospitalized infants requiring positive pressure ventilation in between 48 hours and 14 days after vaccination.

EOM 3.5. Proportion of discharged infants readmitted to the hospital within 14 days of vaccination.

# 3 STUDY DESIGN

This study is a prospective, randomized, open-label clinical trial to assess apnea in the 48 hours after randomization (“unvaccinated” group) versus 48 hours after vaccination (“vaccinated” group)with 13-valent Conjugate Pneumococcal Vaccine (PCV13), Diphtheria Toxoid, Tetanus Toxoid, and Acellular Pertussis Vaccine (DTaP), Hepatitis B vaccine (HBV), Inactivated Polio Vaccine (IPV), and *Haemophilus influenzae* Type B Vaccine (Hib) vaccines in premature infants <33 weeks and 0 days gestation at birth who have been hospitalized since birth and eligible to receive 2-month vaccines per the ACIP schedule.[30]

At least 300 infants will be enrolled over a period of 28 months at Duke University (Duke), University of North Carolina at Chapel Hill (UNC) and Cincinnati Children’s Hospital Medical Center (CCHMC). The first 12 infants (Duke: 4, UNC: 4, CCHMC: 4) will be enrolled in a pilot study to assess the feasibility of the protocol and study instruments. The remaining 288 will be enrolled after the pilot study; 86 infants at Duke, 86 infants at UNC and 116 infants at CCHMC.

On day 1, eligible infants will be randomized 1:1 to a “vaccinated” or “unvaccinated” group. Infants in the “vaccinated” group will be continued on cardiorespiratory and pulse oximetry monitoring and PCV13, DTaP, HBV, IPV, and Hib vaccines will be given within 6 hours of randomization. Vaccines will be administered per ACIP recommendations.[2] Infants in the “unvaccinated” group will be continued on cardiorespiratory and pulse oximetry monitoring and no vaccines will be administered. Continuous cardiorespiratory and pulse oximetry monitoring

are standard of care procedures for infants hospitalized in NICU. The study will collect data from the continuous cardiorespiratory and pulse oximetry monitors from randomization to 48 hours after randomization for infants in the unvaccinated group, and from randomization to 48 hours after vaccination for infants in the vaccinated group. Blinded analyst(s) at Duke University will evaluate data recorded by the cardiorespiratory and pulse oximeter monitors during this time period to determine the occurrence of apnea, bradycardia, and desaturation. In addition, information will be collected on increased respiratory support, sepsis evaluation and serious adverse events.

For infants in the "vaccinated" group, the study will also collect adverse events of clinical interest and serious adverse events occurring between the end of the 48-hour monitoring period and 14 days after vaccination. This information will be collected through parental report and review of medical records.

## 4 STUDY ENROLLMENT AND WITHDRAWAL

Subject Inclusion and Exclusion Criteria will be reviewed at Day 1 to assess eligibility for study participation.

### *4.1 Subject Inclusion Criteria*

Subjects who meet all of the following criteria will be eligible to participate in this study.

1. <33 and 0 days weeks gestational age at birth
2. ≥6 weeks and 0 days and ≤12 weeks and 0 days postnatal age at randomization
3. Remains hospitalized after birth (has never been discharged home)
4. Treating clinician deems infant eligible to receive 2-month vaccines
5. English- or Spanish-speaking parent(s)/legally authorized representative(s) (LAR(s))
6. Not planned for discharge within 60 hours of study entry
7. The parent/guardian must be willing to provide permission for their child to participate through the written informed consent process

### *4.2 Subject Exclusion Criteria*

Subjects who meet any of the following criteria will not be eligible to participate in this study:

1. Receipt of DTaP, IPV, PCV13, or Hib prior to enrollment. Previous administration of the first dose of HBV is permitted
2. Anticipated receipt of any vaccine other than DTaP, IPV, HBV, PCV13, or Hib during the first 60 hours after randomization
3. History of a severe allergic reaction (e.g. anaphylaxis) to a previous dose of any hepatitis B vaccine
4. History of a severe allergic reaction (e.g. anaphylaxis) to any component of the vaccines used in the study including neomycin, yeast and polymyxin B
5. History of latex allergy
6. Fever ≥38°C within 48 hours prior to randomization*
   **This may result in a temporary delay of randomization*
7. Active known respiratory infection within 48 hours prior to randomization*
   **This may result in a temporary delay of randomization*
8. Active infection being treated with systemic antimicrobials*
   **This may result in a temporary delay of randomization*
9. Requiring mechanical ventilation or support with nasal intermittent positive pressure ventilation (NIPPV)*
   **This may result in a temporary delay of randomization*
10. History of unstable progressive neurologic disorder of unknown cause
11. Known cause of apnea other than apnea of prematurity
12. Cyanotic heart disease (congenital or acquired)

13. Major invasive medical or surgical procedure (including circumcision) within 48 hours prior to randomization or anticipated to have major invasive medical or surgical procedure during the first 60 hours after *randomization**
    **This may result in a temporary delay of randomization*
14. Child or parent/LAR is an immediate relative of study staff or an employee who is supervised by study staff.
15. Any condition that would, in the opinion of the site investigator, place the participant at an unacceptable risk of injury or render the participant unable to meet the requirements of the protocol

### *4.3 Recruitment*

Participants will be recruited from Duke, UNC and CCHMC. Infants hospitalized in the NICU will be screened for eligibility daily by study staff. Parent(s)/LAR(s) of the infants will be approached for consent. Infants' parent(s)/LAR(s) may be approached for consent when the infant is eligible to receive 2-month vaccines, or for pre-consent prior to being eligible to receive 2-month vaccines. Initial discussions regarding the study may occur over the phone (see section 5.2), but written informed consent will be obtained prior to any study procedures. The clinical care team will be consulted prior to approaching parents (LARs) for consent.

### *4.4 Reasons for and Handling of Withdrawals*

The following may be reasons for study withdrawal:

- As deemed necessary by the principal investigator (PI).
- Parent(s)/LAR(s) withdrawal of permission for their infant to participate.
- Loss to follow-up.
- Termination of the study by the sponsor.

A parent/LAR may withdraw permission for their infant to participate at any time and for any reason, without penalty. Subjects who are withdrawn from the study prior to randomization will be replaced. Subjects who are withdrawn from the study after randomization will not be replaced. For subjects who were randomized, data collected prior to withdrawal from the study will be included in the study.

### *4.5 Termination of Study*

This study may be terminated for safety concerns of the principal investigators from any of the enrolling sites, the CDC, or participating Institutional Review Boards (IRBs).

## 5 STUDY SCHEDULE, PROCEDURES, & EVALUATIONS

### *5.1 Study day definitions*

| Table 3. Study Day definitions | | |
|---|---|---|
| | **Vaccinated group** | **Unvaccinated group** |
| Day 1 | R → V + 23:59 hours:minutes | R → R + 23:59 hours:minutes |
| Day 2 | V + 24 hours → V + 47:59 hours:minutes | R + 24 hours → R + 47:59 hours:minutes |
| Day 3 | V + 48 hours → V + 95:59 hours:minutes | |
| Day 4 | V + 96 hours → V + 119:59 hours:minutes | |
| Day 5 | V + 120 hours → V + 143:59 hours:minutes | |
| Day 6 | V + 144 hours → V + 167:59 hours:minutes | |
| Day 7 | V + 168 hours → V + 191:59 hours:minutes | |
| Day 8 | V + 192 hours → V + 215:59 hours:minutes | |
| Day 9 | V + 216 hours → V + 239:59 hours:minutes | |
| Day 10 | V + 240 hours → V + 263:59 hours:minutes | |
| Day 11 | V + 264 hours → V + 287:59 hours:minutes | |
| Day 12 | V + 288 hours → V + 311:59 hours:minutes | |
| Day 13 | V + 312 hours → V + 335:59 hours:minutes | |
| Day 14 | V + 336 hours → V + 359:59 hours:minutes | |

R = time of randomization. V = time of vaccination. If infants in the vaccinated group are not vaccinated, then “V” should be replaced by “R + 12 hours”. Note that for vaccinated group, “Day 1” will be longer than 24 hours.

### *5.2 Schedule of Events*

Infants meeting the proposed eligibility criteria will be recruited. Written informed consent for the infants to participate will be obtained from parent(s)/LAR(s) prior to conducting any study procedures. **Table 3** describes the proposed schedule of study visits.

| Table 3. Study Procedures. | | | | | |
|---|---|---|---|---|---|
| **Procedure** | **Day -60 to Day 1** | **Day 1** | | **Days 1-2** | **Days 3-14** |
| **Location of procedure** | **NICU** | **NICU** | | **NICU** | **NICU or phone call** |
| **Group (Vaccinated, Unvaccinated, or Both)** | Both | Vaccinated | Unvaccinated | Both | Vaccinated |
| **Informed consent & Medical Release of Information** | X | | | | |
| **Review Eligibility Criteria** | X | X | X | | |
| **Demographic information** | X | | | | |
| **Birth and delivery information** | X | | | | |
| **General medical history** | | X | X | | |
| **Targeted medical history** | | X | X | | |
| **Respiratory support at time of randomization/vaccination** | | X | X | | |
| **Concomitant medications** | | X | X | X | X |
| **Randomization** | | X | X | | |
| **Procedures** | | | | X | |
| **Cardiorespiratory monitoring data capture** | | | | X | |
| **Vaccination** | | X | | | |
| **Clinically important adverse events** | | X | X | X | X |
| **Serious adverse events** | | X | X | X | X |

***Study Day -60 to Day 1***

- Obtain parental permission by written informed consent and a release of medical record information
- Review and confirm study eligibility, or likely study eligibility for pre-consent
- Obtain demographic, birth, and delivery information

***Study Day 1***

- Review and confirm study eligibility
- Obtain medical history both general and targeted (including respiratory support at time of randomization/vaccination and previous history of Hepatitis B vaccination)
- Obtain concomitant medication use within 1 week prior to randomization
- Randomize study participant to vaccination (vaccinated group) or no vaccination (unvaccinated group) (Section 5.5.1)
- Administer study vaccines to vaccination group. In order to limit differences between the two groups due to lag in vaccination after randomization, vaccines will be administered within 6 hours of randomization; however, vaccination up to 12 hours post-randomization will be permitted. A time window for vaccination is provided in order to allow for flexibility in coordination of vaccination with care/assessment times, family visits, other procedures.
- Record clinically important adverse events (including apnea) and serious adverse events

***Study Days 1-2***

- Cardiorespiratory monitoring (standard of care), with collection of printed or digital output
- Pulse oximetry monitoring (standard of care)
- Record concomitant medications

- Record standard of care procedures (including, but not limited to, any surgical procedures, eye exams, central line placements)
- Record clinically important adverse events (including apnea) and serious adverse events
- Of note, the unvaccinated group completes the study 48 hours after randomization.

***Study Days 3-14 (Vaccinated group only)***
**This period of time (day 3-14) will begin at 48 hours after vaccination.*

- Record clinically important adverse events (including apnea) and serious adverse events in vaccinated group only
    - If infant is discharged from the hospital prior to day 14, then follow-up of events will occur via telephone call with the parent/LAR and/or chart review of outpatient clinic notes or inpatient clinic notes (if infant is rehospitalized and these are available).

### *5.3 Parent/LAR Permission Process (Informed Consent)*

The consent process will take place in the NICU or in a private conference room if preferred by the parent(s)/LAR(s). Initial discussions regarding the study may occur over the phone. Study staff will be available to answer all parent/LAR questions before and after permission is obtained. Parent(s)/LAR(s) will be given as much time as needed after being approached about their infant participating in the study and needing to decide whether or not to participate. We anticipate that the initial consent discussion, including presenting the information in the consent document and answering questions will take about 30 minutes. Parent(s)/LAR(s) will have the opportunity to take the consent form home and discuss the document with other family members or friends. During the consent process, it will be emphasized that participation is voluntary and that parents can withdraw permission for their infant to participate at any time. Permission will not be obtained from parent(s)/LAR(s) who do not read, who are blind, or who do not read/understand English or Spanish. Parent(s)/LAR(s) will be given a copy of the signed informed consent to take home with them. The original copy of the consent will be kept in the study records and a third copy will be included in the infant's medical record per local requirements.

### *5.4 Data Collection*

Study staff will collect participant data at times according to **Table 1**.

***Demographic information***

The participant's postnatal age (days), race/ethnicity, sex, and insurance payer status will be obtained from the electronic health record (EHR) at the time of randomization.

***Birth and delivery information***

The subject's birth and delivery information including gestational age, birth weight, multiple birth, reason/indication for premature delivery will be captured from the EHR.

***Medical history***

The participant's medical history including but not limited to the following (apnea of prematurity, respiratory support at 36+0 weeks postmenstrual age (if ≥36+0 weeks postmenstrual age at randomization), necrotizing enterocolitis, intraventricular hemorrhage and grade, periventricular leukomalacia, and seizures) be captured by the EHR. Current weight will also be recorded.

***Respiratory support at time of randomization/vaccination***

Respiratory support at time of randomization in the unvaccinated group and vaccination in the vaccinated group, including mode (noninvasive positive pressure ventilation, continuous positive airway pressure (CPAP), nasal cannula >1 liters per minute, nasal cannula <1 LPM, or none) and fraction of inspired oxygen ($FiO_2$), will be obtained from the EHR.

***Concomitant medications***
The participant's exposure to all prescribed medications in the 1 week prior to randomization will be obtained from the EHR. Start and stop dates will be collected. In addition, caffeine and acetaminophen administration will be collected during the study period with start/stop dates.

***Cardiorespiratory monitoring data capture***
All participants will have data captured from their continuous cardiorespiratory and pulse oximetry monitoring after randomization. Capture of continuous monitoring data will begin at randomization and end at 48 hours after vaccination (vaccinated group) or randomization (unvaccinated group). If infants in the vaccinated group are not vaccinated by 12 hours after randomization, capture of monitoring data will end at 60 hours after randomization. At the end of the 48-hour monitoring period, study staff at all enrolling sites will collect digital or printed output of data from cardiorespiratory monitors for all infants and send to the analysts at Duke University who will be blinded to the randomization group of the participants. To ensure the analysts are blinded to the treatment arms, the data obtained from the monitor for each infant will not include any personal or study identifiable information. Each output will be assigned a non-sequential coded number before they are sent to the analysts. The blinded analysis will interpret the output and record the date, time, and duration of each cardiorespiratory event (apnea, bradycardia, desaturation, and severe cardiorespiratory event) as defined below.

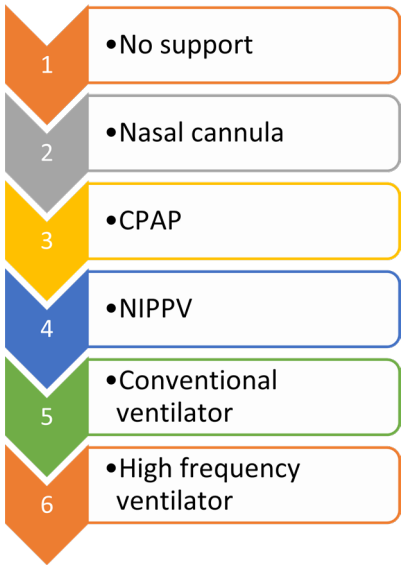
***Procedures***
Any procedures done as part of routine care (including, but not limited to, surgical procedures, eye exams, and central line placements) that occur beginning at randomization and ending at 48 hours after vaccination (vaccinated group) or randomization (unvaccinated group) will be recorded. Procedures occurring >60 hours after randomization in the vaccinated group will not be recorded. Data recorded will include the type of procedure, date/time of the procedure, length of the procedure, and a narrative describing any other important clinical information regarding the procedure.

***Clinically important adverse events: days 1-2***
Clinically important adverse events will be collected from randomization to 48 hours following randomization (unvaccinated group) or vaccination (vaccinated group). The following clinically important adverse events will be collected:

1. Apnea *(POM 1.1, SOM 1.1, SOM 1.2)*

   a. *Definition*: Apnea will be defined as a pause in respirations of >20 seconds, or a pause in respirations of >15 seconds with associated bradycardia (heart rate <80 beats per minute).
   b. *Method of collection*: Obtained from the cardiorespiratory monitor as described above.
2. Increase in mode of respiratory support *(SOM 1.3)*
   a. *Definition:* Increase in mode of respiratory support will be defined as progression from any lower level at baseline (at time of randomization in the unvaccinated group and vaccination in the vaccinated group) to any higher level (**Figure 2**). In addition, any increase in nasal cannula flow rate, or continuous positive airway pressure (CPAP) will be considered an increase in mode of respiratory support.
   b. *Method of collection*: Respiratory data will be collected from the EHR daily, and the highest level of support that is maintained for >1 hour will be recorded. In addition, start/stop date/time for NIPPV and mechanical ventilation will be recorded.
3. Severe cardiorespiratory event (*SOM 2.1)*
   a. *Definition:* Respiratory pause of >30 seconds in duration or bradycardia of <60 beats per minute for >10 seconds
   b. *Method of collection:* Obtained from the cardiorespiratory monitor as described above.
4. Positive pressure ventilation delivered via bag-valve mask (*SOM 2.2*)
   *a. Definition:* Infant with unexpected clinical decompensation requiring positive pressure ventilation via bag-valve mask.
   *b. Method of collection:* Obtained from the EHR as documented by a clinical provider (e.g., physician, nurse, respiratory therapist).
5. Fever *(EOM 1.1)*
   a. *Definition*: Elevated temperature ≥38°C.
   b. *Method of collection*: Highest daily temperature will be recorded from the EHR, in addition to how the temperature was obtained (skin, axillary, rectal, etc.) and infant's environment (open crib, radiant warmer or isolette).
6. Hypothermia *(EOM 1.2)*
   a. *Definition*: Decreased temperature <36°C.
   b. *Method of collection*: Lowest daily temperature will be recorded from the EHR, in addition to how the temperature was obtained (skin, axillary, rectal, etc.) and infant's environment (open crib, radiant warmer or isolette).
7. Oxygen desaturation *(EOM 2.1)*
   a. *Definition:* Oxygen desaturation will be defined as a $SpO_2$ <88% for >10 seconds.
   b. *Method of collection*: Obtained from the cardiorespiratory monitor as described above.
8. Bradycardia *(EOM 2.2)*
   a. *Definition:* Bradycardia will be defined as a heart rate <80 beats per minute lasting >10 seconds.

**Figure 2. Progression in respiratory support.**




CPAP=continuous positive airway pressure. NIPPV=non-invasive positive pressure ventilation

   b. *Method of collection*: Obtained from the cardiorespiratory monitorg as described above.
9. Blood culture for sepsis evaluation *(EOM 2.3)*
   a. *Definition:* A sepsis work-up will be defined each day as a blood draw to obtain blood culture.
   b. *Method of collection:* Data will be collected from the EHR (date/time of blood culture).
10. Antibiotics administered due to sepsis evaluation *(EOM 2.3)*
   a. *Definition:* New receipt of intravenous or intramuscular antibiotics will be recorded if they were started in conjunction with a blood culture during the 48-hour monitoring period after vaccination in the "vaccinated" group and the 48-hour monitoring period after randomization in the "unvaccinated" group
   b. *Method of collection:* Data will be collected from the EHR (date/time of start of the first new antibiotic).

***Clinically important adverse events: days 4-14 (vaccinated group only)***

1. Clinical apnea *(EOM 3.1)*
   *a. Definition*: Clinical apnea will be defined as an apneic event that is documented by the nurse as part of standard care.
   b. *Method of collection*: Events will be reviewed and collected from the EHR. Date, time, and duration of events will be noted when available from nursing documentation. Associated bradycardia and desaturation will be collected if available.
2. Increase in mode of respiratory support *(EOM 3.2)*
   a. *Definition*: Increase in mode of respiratory support will be defined as progression from any lower level at baseline (end of study period on day 3) to any higher level (**Figure 2**). In addition, any increase in nasal cannula flow rate, CPAP pressure, or NIPPV pressure will be considered an increase in mode of respiratory support.
   b. *Method of collection*: Respiratory data will be collected from the EHR daily, and the highest level of support that is maintained for >1 hour will be recorded.
3. Blood culture for sepsis evaluation *(EOM 3.3)*
   a. *Definition:* A sepsis work-up will be defined as a blood draw to obtain blood culture.
   b. *Method of collection:* Data will be collected from the EHR (date/time of blood culture).
4. Antibiotics administered due to sepsis evaluation *(EOM 3.3)*
   a. *Definition:* New receipt of intravenous or intramuscular antibiotics will be recorded if they were started in conjunction with a blood culture
   a. *Method of collection:* Data will be collected from the EHR (date/time of start of the first new antibiotic).
5. Positive pressure ventilation delivered via bag-valve mask (*EOM 3.4*)
   *a. Definition:* Infant with unexpected clinical decompensation requiring positive pressure ventilation via bag-valve mask.
   *b. Method of collection:* Obtained from the EHR as documented by a clinical provider (e.g., physician, nurse, respiratory therapist).
6. Hospital readmission *(EOM 3.5)*
   a. *Definition:* Among infants who are discharged from the hospital, hospital readmission (yes/no) will be recorded along with date of readmission and reason for readmission. Scheduled, elective readmissions (e.g. surgical procedures) will not be counted.
   b. *Method of collection:* Data will be collected from the EHR and/or follow-up phone call to parent/guardian

***Serious adverse events (SAEs)*** *(EOM 2.4)*
An SAE is defined as an adverse event that results in one of the following outcomes during the infant's participation in the study:

- Results in death
- Is life-threatening (defined as immediate risk of death at the time of the event)
- Requires inpatient hospitalization (i.e. re-admission) or prolongation of inpatient hospitalization
- Results in a persistent or significant disability/incapacity
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Study site investigators will assess relatedness to study procedures and vaccines for all SAEs (related, possibly related, or unrelated).

### 5.4 Reporting Adverse Events

SAEs will be reported to the CDC and all participating IRBs according to institutional requirements.

If indicated, adverse events (AEs) will be reported to the CDC's Vaccine Adverse Event Reporting System (VAERS). The National Childhood Vaccine Injury Act requires healthcare providers to report:

- Any adverse event listed by the vaccine manufacturer as a contraindication to further doses of the vaccine; or
- Any adverse event listed in the VAERS Table of Reportable Events Following Vaccination [PDF - 75KB] that occurs within the specified time period after vaccination.

In addition, CDC encourages reporting of any clinically significant adverse event that occurs in a patient following a vaccination, even if there is uncertainty regarding if a vaccine caused the event.

### *5.5 Follow-up on days 3-14 for infants in the vaccinated group*
**This period of time (day 3-14) will begin at 48 hours after vaccination.*
Infants in the vaccinated group will be followed for the above clinically important AEs, SAEs, and concomitant medications on days 3-14. If an infant in the vaccinated group is discharged or transferred prior to day 14 (with no EHR accessible), then these data will be obtained via follow-up phone call from the study staff to the parent/guardian.

### *5.6 Treatment Assignment Procedures*
This is an open-label, prospective, randomized study for infants who are eligible to receive 2-month vaccinations. To the extent feasible, all infants randomized to the vaccinated group will be given the same type/brand of vaccines at all sites. Infants in the unvaccinated group will not receive any vaccine during the study.

#### 5.6.1 Randomization
Participants will be randomized (1:1) to either vaccinated or unvaccinated groups using a permuted block randomization scheme stratified by gestational age (<28 weeks, ≥28 weeks) and study site (i.e. Duke University Medical Center, University of North Carolina at Chapel Hill

Hospital, and Cincinnati Children's Hospital Medical Center). Randomization will occur within 6 hours of anticipated vaccination of infants in the vaccinated group, but infants will be allowed to receive vaccines up to 12 hours post-randomization. The first 12 infants enrolled in the Pilot Phase I will be randomized in a separate permuted block by manual randomization (e.g., envelopes). The project statistician at Duke University will generate permuted block randomization schemes, which will be uploaded to REDCap for the full study. The randomization schedule will not be available to the study staff, so the next randomization allocation will not be known before randomization occurs. Following confirmation of study eligibility criteria, participant randomization will be through REDCap with treatment allocation recorded on the case report form. In the event that REDCap is unavailable, manual randomization will occur through the use of envelopes. The project statistician will prepare 20 envelopes per site that will use the same randomization strategy as the primary scheme embedded in REDCap. When manually randomizing, the team member will pull the next envelope in order. In order to capture the allocation per subject, a separate form in REDCap will be used by the personnel to add the assignment. A log will need to be kept at the site capturing these instances.

### 5.6.2 Blinding

This study will be open label and study staff, caregivers, and parent(s)/LAR(s) will not be blinded to treatment arm assignments. The output from the cardiorespiratory monitors will be read by analysts who are blinded to the treatment arm assignments.

### 5.5.3 Vaccine Supply, Storage, and Administration

*Vaccine Supply and Storage*

Pharmacy-supplied US licensed vaccines will be administered to the vaccination group as part of this study. The following vaccine brands will be used for this study: PCV13 (Prevnar® 13, Pfizer); DTaP-IPV-HBV (Pediarix®, GlaxoSmithKline); and Hib (ActHib®, Sanofi Pasteur). However, given the potential for unforeseen manufacturing issues/specific product shortages, the use of alternate U.S.-licensed vaccines may be required.

*Vaccine Administration*

Only infants randomized to the vaccinated group will be vaccinated during the protocol specified observation period. A single administration of PCV13 and DTaP-HBV-IPV and Hib comprises intramuscular delivery of 0.5 mL total volume of each vaccine. For each infant, all vaccines will be administered simultaneously by licensed nurses within the study sites. Injections given in the same thigh should be separated by 1 inch or more, if possible, so that any local reactions can be differentiated. The research staff will record vaccine brand, lot numbers, dose, date/time and site of vaccine administration. For continuity of care purposes, documentation of vaccine administration will be done in a manner that the infant's healthcare provider can access.

After administration, used study syringes will be disposed of according to standard operating procedure.

Although oral rotavirus vaccine is recommended by the ACIP for infants at 2-months of age, this vaccine will not be administered during the study to prevent potential nosocomial spread of this live vaccine virus.

## 6 STATISTICAL CONSIDERATIONS

### *6.5 Sample Size and Power Estimation*

**Power considerations related to the primary objective**

The study has approximately 84.3% power to reject the null hypothesis of no difference in the proportion of infants with apnea in the "vaccinated" group compared to the "unvaccinated" group based on a two-side alpha 0.05 chi-square test with N=135 per group. This assumes that the proportion of infants with apnea will be 15% in the "unvaccinated" group and 2-fold higher (30%) in the "vaccinated" group. This assumption is based on a review of the literature[14] and expert opinion. The study allows for a 10% drop out rate to give a total sample of 300 infants.

During the pilot study, at least 12 infants will be enrolled to assess feasibility. Following completion of the pilot study, a total of at least 288 infants will be enrolled.

### *6.6 Analysis Plan*

#### 6.6.1 Pilot Study

The pilot phase of the study will include 12 infants. There will not be any formal analysis of study data. However, the feasibility benchmarks listed in **Table 5** will be documented for the first 12 patients to assess protocol adherence. These subjects will contribute to the final sample for analyses in the study objectives.

**Table 5: Benchmarks for Pilot**

| Benchmark | Target |
|---|---|
| Among pre-screened and eligible, LARs approached for consent | 70% |
| Among approached for consent, consent obtained | 40% |
| Documentation of reason for non-consent (of those approached) | 80% |
| Among consent obtained, percent randomized | 80% |
| Administration of vaccines in the vaccination group within 6 hours of randomization | 60% |
| Administration of vaccines in the vaccination group within 12 hours of randomization | 80% |
| Entire 48-hour monitoring period assessed for apnea events | 80% |

#### 6.6.2 Full Study

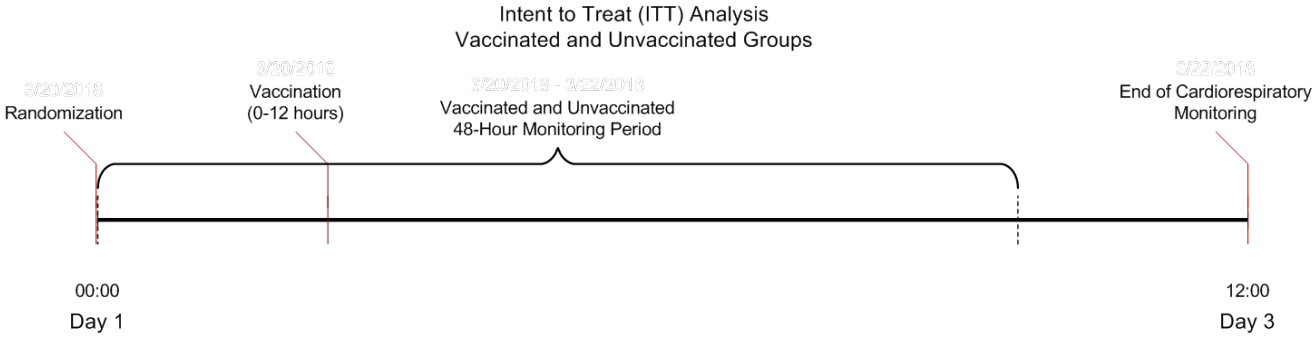
##### 6.6.2.1 Study Populations

We will define 3 study populations for the purpose of analyses.

1) Intent-to-treat (ITT) population
    - Definition: The ITT population includes any infant that was enrolled and randomized in the study.

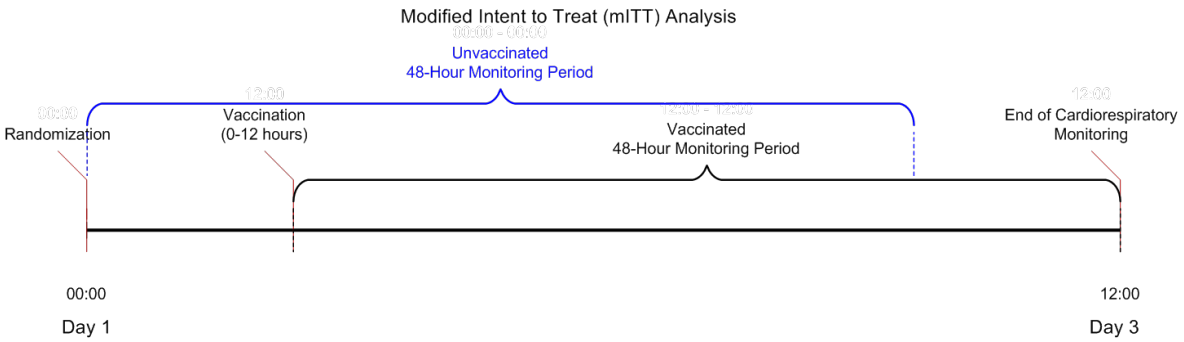
- Analysis: For the ITT analysis, study outcomes in the 48-hour monitoring after randomization will be evaluated in both vaccinated and unvaccinated group. Infants will be analyzed in their assigned treatment arms irrespective of receipt of vaccine.

Figure 3. Intent to Treat (ITT) Analysis




2) Modified intent-to-treat (mITT) population
   - Definition: The mITT population includes any infant that was enrolled and randomized in the study.
   - Analysis: For the mITT analysis, infants will be analyzed in their assigned treatment arms irrespective of receipt of vaccine. Study outcomes will be included in the analysis as follows:
     - Vaccinated group: study outcomes in the 48-hour monitoring after vaccination. If vaccination does not occur by 12 hours after randomization, then study outcomes will be assessed between 12 and 60 hours after randomization.
     - Unvaccinated group: study outcomes in the 48-hour monitoring period after randomization.

Figure 4. Modified Intent-to-Treat (mITT) Analysis




3) Per protocol (PP) population
   - Definition: The PP population includes any infant that was enrolled, randomized and did not have any major protocol violations as determined by the study investigators.

- Analysis: For the per protocol analysis, study outcomes will be included in the analysis as follows:
    - Vaccinated group: study outcomes in the 48-hour monitoring after vaccination. The analysis will exclude infants who do not receive vaccine by 12 hours.
    - Unvaccinated group: study outcomes in the 48-hour monitoring period after randomization. The analysis will exclude infants who are vaccinated during the study period.

The mITT population will be the primary analysis population for all planned analyses for the study objectives. For comparison, pre-specified secondary analyses will also be performed using the ITT and PP populations. The ITT population will only be analyzed for the primary and secondary objectives. The PP population will be analyzed for all planned objectives as supporting analyses.

#### 6.2.2.2 Interim Safety Data Review

Given that this study involves administering U.S.-licensed vaccines recommended by the ACIP for preterm infants and included as part of routine clinical care, there will not be a designated data safety monitoring board for this study. However, due to the fragility of the study population and interventional design, one interim safety data review will be performed with the goal of identifying unexpected safety concerns of clinical importance. The safety data review will provide the study the opportunity to identified unexpected safety concerns and make changes to the protocol if needed. The interim safety data review will be done by a safety monitoring panel with relevant expertise, comprised of experts who are not co-investigators on this study. The safety population for the interim safety review will include infants who were enrolled and randomized. Infants who participated in the pilot study will also be included. The safety monitoring panel will review clinical narratives of SAEs. If the CDC and study investigators determine additional analyses or reviews are needed, efforts will be made to conducted additional analysis or reviews that will not include analyzing the primary endpoint as a first step. This is to avoid introducing bias or increasing sample size needs for statistical power.

Enrollment will continue during the interim safety data review.

#### 6.2.2.3 Final study analysis

A final analysis of the data will occur after all infants have completed the study and will include data from infants enrolled in the pilot study.

All proportions specified below in the objectives will be compared by group (unvaccinated group or vaccinated group) using a Mantel-Haenszel statistic in a stratified analysis by site (i.e. Duke, UNC and CCHMC) to control for the randomization blocks at the two-sided alpha 0.05 level unless otherwise specified. The odds ratio and corresponding 95% confidence interval will also be calculated and presented. There will be no adjustment to the alpha level made for the secondary and exploratory objectives.

<u>Primary Objective 1 - To compare proportions of preterm infants with apnea in a 48-hour monitoring period after vaccination in the “vaccinated” group versus a 48-hour monitoring period after randomization in the “unvaccinated” group.</u>
The proportion of infants with ≥ 1 apneic event apnea (as defined in Section A.5.3) will be presented for each group and compared using a Mantel-Haenszel statistic.

Secondary Objective 1 - To compare the clinical importance of apneic events in a 48-hour monitoring period after vaccination in the “vaccinated” group versus a 48-hour monitoring period after randomization in the “unvaccinated” group.
For this objective we will present the proportions of infants in each group who required increase in respiratory support (as defined in Section A.5.3) and compare these proportions using a Mantel-Haenszel statistic. We will also report summary statistics regarding the number of distinct episodes and the duration of episodes, including mean, median, standard deviation, interquartile range, and range. We will compare the number of distinct episodes of apnea and the duration of episodes between groups using the Wilcoxon rank sum test.

Secondary Objective 2 - To compare proportions of preterm infants with severe cardiorespiratory events in a 48-hour monitoring period after vaccination in the “vaccinated” group versus a 48-hour monitoring period after randomization in the “unvaccinated” group.
For this objective we will present the proportion of infants in each group experiencing a severe cardiorespiratory event (as defined in Section A.5.3) or received positive pressure ventilation at least once during the 48 hours after randomization/vaccination and compare these proportions using a Mantel-Haenszel statistic.

Exploratory Objective 1 - To compare proportions of preterm infants with temperature instability in a 48-hour monitoring period after vaccination in the “vaccinated” group versus a 48-hour monitoring period after randomization in the “unvaccinated” group.
For this objective we will present the proportion of infants the proportion of infants in each group experiencing fever and the proportion in each group experiencing hypothermia (as defined in Section A.5.3) at least once during the 48 hours after randomization/vaccination and compare these proportions using a Mantel-Haenszel statistic.

Exploratory Objective 2 - To compare proportions of preterm infants with other clinically important adverse events in a 48-hour monitoring period after vaccination in the “vaccinated” group versus a 48-hour monitoring period after randomization in the “unvaccinated” group.

For this objective we will present the proportion of infants the proportion of infants in each group experiencing other clinically important adverse events (as defined in Section A.5.3) at least once during the 48 hours after randomization/vaccination and compare these proportions using a Mantel-Haenszel statistic. We will repeat these analyses for serious adverse events. We will report SAE narratives and relatedness descriptions (see section 5.3).

Exploratory Objective 3 - To compare clinically important adverse events occurring between 48 hours and 14 days after vaccination among infants in the vaccinated group who do, and do not have apnea in a 48-hour monitoring period after vaccination.
For this objective we will present the proportion of infants in the vaccinated group with and without apnea in a 48-hour monitoring period who experience each of these clinically important adverse events (as defined in section A.5.3) at least once between 48 hours and 14 days after vaccination. These subgroup comparisons will be conducted a Fisher’s Exact Test at the alpha 0.05 level.

### *6.7 Data Management*
The amount of data that will be collected for the proposed project will be substantial and will require a sophisticated, practical and flexible system. The novel Vanderbilt-designed resource developed specifically for online collection of research information, the Research Electronic Data Capture (REDCap) platform, will be used to design study forms, including the reaction forms, and short customized questionnaires to collect information from study subjects. This

system will be used by all the study sites (i.e. Duke, UNC, and CCHMC). All electronic linkages will fulfill regulations for protection of human subjects and requirements to minimize the risk of breach of confidentiality. After initial set-up, the work load required for electronic data collection will be substantially reduced (description of REDCap resources below).[31] Duke investigators have previously used the REDCap system for collection and analysis of large quantities of data. All study-related documents containing protected health information, e.g. enrollment logs, case report forms completed by study participants, will be maintained in secure research offices at Duke, UNC and CCHMC, which are accessible to research staff only.

### 6.7.1 Research Electronic Data Capture (REDCap)

Investigators within the NIH-funded Clinical and Translational Research Unit at Vanderbilt have developed REDCap (http://project-redcap.org/), to collect and manage data for diverse clinical and translational research studies. REDCap was designed around the concept of giving research teams an easy method to specify project needs and rapidly develop secure, web-based applications for collection, management and sharing of research data. REDCap accomplishes these key functions through use of a single study metadata table referenced by presentation-level operational modules. Based on this abstracted programming model, databases are developed in an efficient manner with little resource investment beyond the creation of a single data dictionary. The concept of metadata-driven application development is well established, and the critical factor for successful data collection lies in creating a simple workflow methodology allowing research teams to autonomously develop study-related metadata in an efficient manner. The product includes secure institutional data hosting and include full audit-trails in compliance with Health Insurance Portability and Accountability Act (HIPAA) security requirements. The REDCap Consortium is comprised of 2318 active institutions. The REDCap currently supports 68,000 projects with over 89,000 users spanning numerous research focus areas across the consortium. The current project will use this software application for the design of electronic forms to collect information from study participants, to link the baseline data, sample collection date, and laboratory results in an automated database family, to perform data cleaning and data quality assurance efficiently, and to design an analytical dataset for the analysis of the project data.

Data will be entered directly into the REDCap database by members of the study team at the study sites. Study investigators will be responsible for assuring that all paper records are securely stored according to the requirements of their IRBs. The study investigators will be responsible for assuring the accuracy of the data entered from the paper forms into REDCap, as appropriate. Only the assigned identifiers will be used in REDCap. Therefore, personal health identifiers will not appear in the REDCap database.

In order to perform data cleaning and data quality assurance efficiently, numerous built-in filters and checks for consistency of the data including range and limit checks, branching logic and pull down menus to limit choices for categorical variables to a pre-specified list will be implemented and performed automatically to minimize data entry error. The data will be randomly sampled and checked against source records on a regular basis. The data and related analytical datasets will also be stored at all the study sites (i.e. Duke, UNC, and CCHMC) with secured password-protected computers. Coded data without personal identifiers will be made available to the CDC and transferred using a secure transfer method.

## *6.8 Role of the CDC Investigators in the Project*

This study is funded by a CDC contract with Duke University (and subcontract with UNC) and CCHMC as Task Orders in the CISA Project Contract. The Duke University PI will oversee the study in partnership with the CCHMC's PI. CDC staff will collaborate with both sites to develop

the protocol, conduct the study, ensure the study is aligned with US Department of Health and Human Services public health priorities, and analyze the data and disseminate the results. CDC may receive access to coded data not containing any directly identifying information.

# 7 HUMAN SUBJECTS

## *7.5 Human Subjects Involvement, Characteristics, and Design*

Duke and CCHMC investigators, along with subcontractor investigators, will be responsible for submitting the protocol, informed consent and any written or verbally conveyed materials specific to this project to their institutional review boards. CDC staff will be responsible for submitting materials to the CDC for review and obtain reliance on Duke IRB.

To facilitate subject recruitment at the practices, we will request a waiver of consent and HIPAA authorization for ascertainment (identification, selection) and/or recruitment of potential subjects while recording identifiable private health information (PHI) prior to obtaining the subject's consent. This information will be obtained from review of the electronic scheduling and medical record systems in the clinics in order to determine eligibility for study enrollment. We will review information only the minimum amount of information necessary to determine potential eligibility, e.g. to consent will be used to recruit and screen only. Use of PHI in this manner involves no more than minimal risk to subjects and no information will leave the study sites.

Continuing reviews will be submitted to the IRBs on an annual basis. Protocol deviations or concerns about study integrity will be reported promptly to the overseeing IRB in accordance with institutional requirements.

## *7.6 Sources of Material*

Medical history, vaccination history, and concomitant medication history will be obtained from the EHR. Demographic information will be obtained from the EHR or parent/LAR. Adverse events will be recorded using the output from cardiorespiratory monitors (for cardiorespiratory events only), EHR, and/or phone call to the parent/LAR (days 4-14).

## *7.7 Potential Risks and Benefits*

PCV13, DTaP, IPV, HBV, and Hib are US- licensed vaccines approved for use in infants ≥6 weeks of age. These vaccines are recommended by the ACIP for use in preterm infants.[30] Participants will be provided with the CDC Vaccine Information Statements (VIS) for infant 2-month vaccinations (https://www.cdc.gov/vaccines/hcp/vis/vis-statements/multi.pdf).

Risks of the DTaP-IPV-HBV combination include minor problems such as soreness, redness, swelling, or pain where the shot was given, fever, drowsiness, irritability/fussiness, vomiting, and loss of appetite. Other potential adverse reactions include a small increased risk of apnea in premature infants.

Most common risks of PCV13 include fever, injection site erythema, induration, or swelling. Uncommon symptoms include apnea.

Most common risks of Hib include injection site erythema, induration, or swelling. Uncommon symptoms include apnea.

Problems that could happen after any injected vaccine:

- Any medication can cause a severe allergic reaction, including anaphylaxis. Such reactions from a vaccine are very rare, estimated at about 1 in a million doses, and

would happen within a few minutes to a few hours after the vaccination. As with any medicine, there is a very remote chance of a vaccine causing a serious injury or death.

There is a small potential risk that infants in the no vaccination group could develop infection due to one of the organisms covered by the vaccine during the 48-hour monitoring period that vaccination is withheld. After their participation in the study, the study team will communicate with the clinical team to facilitate vaccination of these infants in a timely manner.

There is the potential risk of loss of confidentiality about information obtained as part of this study.

### *7.8 Adequacy of Protection Against Risks*

#### 7.8.1 Protections against Risk

Participants' parent(s)/LAR(s) will be counseled on possible side effects following vaccination and followed closely during the 14 days post-vaccination for clinical status changes. Subjects with a prior history of any severe reaction following HBV administration will be excluded from study enrollment.

Infants in the unvaccinated group will complete the study 48 hours after randomization, after which clinicians may administer the recommended vaccines in a timely fashion, limiting the risk.

Every effort possible will be made to keep information about participants confidential. Computerized participant information will be kept in password-protected files on secured servers. Any publications resulting from this work will not contain any identifiable participant information.

#### 7.8.2 ClinicalTrials.gov Requirements

The project will be registered on ClinicalTrials.gov.

### *7.9 Human Subjects*

In obtaining and documenting informed consent, the Investigator and study team will comply with the applicable regulatory requirements, Good Clinical Practices, and ethical principles. The parent or LAR must sign and date the written informed consent form prior to initiation of any study procedure.

#### 7.9.1 Vulnerable Subjects Research

*Vulnerable subjects*

Children are a vulnerable research population and require additional protections when they are potential research subjects. This is a minimal risk study, involving the administration of routine infant vaccinations in a manner that is consistent with ACIP recommendations. Because this study is no more than minimal risk, the permission of only one parent/LAR will be obtained. Because the participants will be infants they will not be capable of providing assent.

## REFERENCES

1. Bonhoeffer J, Siegrist CA, Heath PT. Immunisation of premature infants. Arch Dis Child 2006;91:929-35.
2. Kroger AT, Duchin J, Vázquez M. General best practice guidelines for immunization. Best practices guidance of the Advisory Committee on Immunization Practices (ACIP). 2017.
3. Vohr BR, Oh W. Age of diphtheria, tetanus, and pertussis immunization of special care nursery graduates. Pediatrics 1986;77:569-71.
4. Meleth S, Dahlgren LS, Sankaran R, Sankaran K. Vaccination status of infants discharged from a neonatal intensive care unit. CMAJ 1995;153:415-9.
5. Davis RL, Rubanowice D, Shinefield HR, et al. Immunization levels among premature and low-birth-weight infants and risk factors for delayed up-to-date immunization status. Centers for Disease Control and Prevention Vaccine Safety Datalink Group. JAMA 1999;282:547-53.
6. Langkamp DL, Hoshaw-Woodard S, Boye ME, Lemeshow S. Delays in receipt of immunizations in low-birth-weight children: a nationally representative sample. Arch Pediatr Adolesc Med 2001;155:167-72.
7. Navar-Boggan AM, Halsey NA, Escobar GJ, Golden WC, Klein NP. Underimmunization at discharge from the neonatal intensive care unit. J Perinatol 2012;32:363-7.
8. McNutt LA, Desemone C, DeNicola E, et al. Affluence as a predictor of vaccine refusal and underimmunization in California private kindergartens. Vaccine 2016;34:1733-8.
9. Feemster KA, Spain CV, Eberhart M, Pati S, Watson B. Identifying infants at increased risk for late initiation of immunizations: maternal and provider characteristics. Public Health Rep 2009;124:42-53.
10. MacDorman MF. Race and ethnic disparities in fetal mortality, preterm birth, and infant mortality in the United States: an overview. Semin Perinatol 2011;35:200-8.
11. Henderson-Smart DJ. The effect of gestational age on the incidence and duration of recurrent apnoea in newborn babies. Aust Paediatr J 1981;17:273-6.
12. Carbone T, McEntire B, Kissin D, et al. Absence of an increase in cardiorespiratory events after diphtheria-tetanus-acellular pertussis immunization in preterm infants: a randomized, multicenter study. Pediatrics 2008;121:e1085-90.
13. "PEDIARIX- diphtheria and tetanus toxoids and acellular pertus s is ads orbed, hepatitis b (recombinant) and inactivated poliovirus vaccine combined injection, suspension" drug label. Revised 10/2016, GlaxoSmithKline Biologicals SA.
14. "PENTACEL- diphtheria and tetanus toxoids and acellular pertussis adsorbed, inactivated poliovirus and haemophilus b conjugate (tetanus toxoid conjugate) vaccine" drug label. Revised 10/2013, Sanofi Pasteur Inc.
15. "PREVNAR 13- pneumococcal 13-valent conjugate vaccine injection, suspension" drug label. Revised 04/2017, Wyeth Pharmaceutical Divis ion of Wyeth Holdings LLC, a s ubsidiary of Pfizer Inc.
16. "ACTHIB- haemophilus influenzae type b strain 1482 capsular polysaccharide tetanus toxoid conjugate antigen" drug label. Revised 04/2016, Sanofi Pasteur Inc.
17. "ENGERIX-B - hepatitis b vaccine (recombinant) injection, suspension" drug label. Revised 03/2010, Dispensing Solutions Inc.
18. Botham SJ, Isaacs D. Incidence of apnoea and bradycardia in preterm infants following triple antigen immunization. J Paediatr Child Health 1994;30:533-5.
19. Botham SJ, Isaacs D, Henderson-Smart DJ. Incidence of apnoea and bradycardia in preterm infants following DTPw and Hib immunization: a prospective study. J Paediatr Child Health 1997;33:418-21.

20. Ellison VJ, Davis PG, Doyle LW. Adverse reactions to immunization with newer vaccines in the very preterm infant. J Paediatr Child Health 2005;41:441-3.
21. Sanchez PJ, Laptook AR, Fisher L, Sumner J, Risser RC, Perlman JM. Apnea after immunization of preterm infants. J Pediatr 1997;130:746-51.
22. Pourcyrous M, Korones SB, Crouse D, Bada HS. Interleukin-6, C-reactive protein, and abnormal cardiorespiratory responses to immunization in premature infants. Pediatrics 1998;101:E3.
23. Pfister RE, Aeschbach V, Niksic-Stuber V, Martin BC, Siegrist CA. Safety of DTaP-based combined immunization in very-low-birth-weight premature infants: frequent but mostly benign cardiorespiratory events. J Pediatr 2004;145:58-66.
24. Schulzke S, Heininger U, Lucking-Famira M, Fahnenstich H. Apnoea and bradycardia in preterm infants following immunisation with pentavalent or hexavalent vaccines. Eur J Pediatr 2005;164:432-5.
25. Faldella G, Galletti S, Corvaglia L, Ancora G, Alessandroni R. Safety of DTaP-IPV-HIb-HBV hexavalent vaccine in very premature infants. Vaccine 2007;25:1036-42.
26. Hacking DF, Davis PG, Wong E, Wheeler K, McVernon J. Frequency of respiratory deterioration after immunisation in preterm infants. J Paediatr Child Health 2010;46:742-8.
27. Klein NP, Massolo ML, Greene J, Dekker CL, Black S, Escobar GJ. Risk factors for developing apnea after immunization in the neonatal intensive care unit. Pediatrics 2008;121:463-9.
28. Lee J, Robinson JL, Spady DW. Frequency of apnea, bradycardia, and desaturations following first diphtheria-tetanus-pertussis-inactivated polio-Haemophilus influenzae type B immunization in hospitalized preterm infants. BMC Pediatr 2006;6:20.
29. Slack MH, Schapira C, Thwaites RJ, Andrews N, Schapira D. Acellular pertussis and meningococcal C vaccines: cardio-respiratory events in preterm infants. Eur J Pediatr 2003;162:436-7.
30. 2018 Recommended Immunizations for Children 7-18 Years Old. 2018. 2018, at https://www.cdc.gov/vaccines/schedules/downloads/teen/parent-version-schedule-7-18yrs.pdf.)
31. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform 2009;42:377-81.