TRIAL OF LATE SURFACTANT FOR PREVENTION OF BRONCHOPULMONARY DYSPLASIA (BPD)

A Study in Ventilated Preterm Infants Receiving Inhaled Nitric Oxide

"THE TOLSURF STUDY"

PROTOCOL V.9

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NHLBI Study # U01-HL- 094338
FDA IND #79,367

Clintrial # NCT 01022580

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NHLBI Study # U01-HL-094355

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10.17.12

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GLOSSARY

BPD	bronchopulmonary dysplasia	NNT	number needed to treat
CCC	clinical coordinating center	NO	nitric oxide
CI	confidence interval	OI	oxygenation index [(MAPxFIO$_2$)/PaO$_2$]
CRF	case report form	OR	odds ratio
DCC	data coordinating center	PDA	patent ductus arteriosus
DPPC	Dipalmitoylphosphatidylcholine	PL	phospholipid
ELGAN	Extremely low gestational age newborn	PMA	post menstrual age
FIO2	fraction of inspired oxygen	PVL	periventricular leukomalacia
iNO	inhaled nitric oxide	RDS	respiratory distress syndrome
IVH	intraventricular hemorhage	ROP	retinopathy of prematurity
MAP	mean airway pressure	RSS	respiratory severity score (MAPxFIO$_2$)
mRNA	messenger ribonucleic acid	SP	surfactant protein
NCPAP	nasal continuous positive airway pressure	STads	adsorption surface tension
NEC	necrotizing enterocolitis	STmax	maximum surface tension
NICU	neonatal intensive care unit	STmin	Minimum surface tension

OVERVIEW

Infants born prematurely are at risk for both respiratory distress syndrome (RDS) and bronchopulmonary dysplasia (BPD), defined as a continuing requirement for ventilatory support and/or supplemental oxygen at 36 wk postmenstrual age (PMA). BPD affects more than 70% of infants of < 28 wk gestation who require ventilatory support after 7 d of age. Severe forms of BPD are associated with long-term pulmonary disability, neurodevelopmental abnormalities and death. It is estimated that there are up to 15,000 new cases of BPD annually in the United States.

Most very premature newborn infants are deficient in pulmonary surfactant at birth, and current clinical care includes surfactant replacement therapy to reduce the incidence and severity of RDS. Despite surfactant treatment at birth, premature infants often need mechanical ventilatory support and/or supplemental oxygen during the first wk of life and many have a continuing requirement for respiratory support after the first wk, often requiring reintubation or increased ventilatory support. We found that most of these infants experienced respiratory deteriorations that were associated with dysfunctional surfactant and low content of surfactant proteins (SP) B and C [1]. Although inhaled nitric oxide (iNO) started between 7 and 14 d of age significantly improves outcome in this group of infants [2,3], these infants still have episodes of surfactant dysfunction.

We propose that episodes of surfactant dysfunction in chronically ventilated infants contribute to development of BPD by increasing lung inflammation and injury secondary to greater exposure to oxygen and volutrauma, and by restricting distribution of iNO secondary to atelectasis. Based on these observations, we propose to conduct a multicenter, randomized, controlled trial of surfactant treatment for 524 infants $\leq$28 wk gestation receiving iNO for lung disease requiring mechanical ventilation beyond 7 d of age. *We hypothesize that administration of surfactant in addition to iNO in ventilated premature infants between 7 and 14 d of age will improve lung function and respiratory status, increase benefit from iNO, increase survival without BPD, and will be safe and well tolerated.* Specific Aim 1 will assess the effect of late doses of surfactant in infants receiving inhaled iNO on survival without BPD in ventilated extremely low gestational age newborn (ELGANS) infants.

Aim 2 assess effects of late surfactant treatment on surfactant status and lung inflammatory biomarkers and establishes a DNA repository for genomic studies of the pathogenesis of BPD.

1.0 SCIENTIFIC BACKGROUND

1.1 Pathophysiology of Bronchopulmonary Dysplasia (BPD). Infants born prematurely often have respiratory failure because of structurally immature lungs, deficiency of pulmonary surfactant, primitive respiratory drive, disturbed intrauterine environment, and susceptibility to infection. The need for assisted ventilation and supplemental oxygen in these infants contributes to a form of chronic lung injury initially described by Northway et al. [4] as BPD. In recent years, with increased survival of ELBW

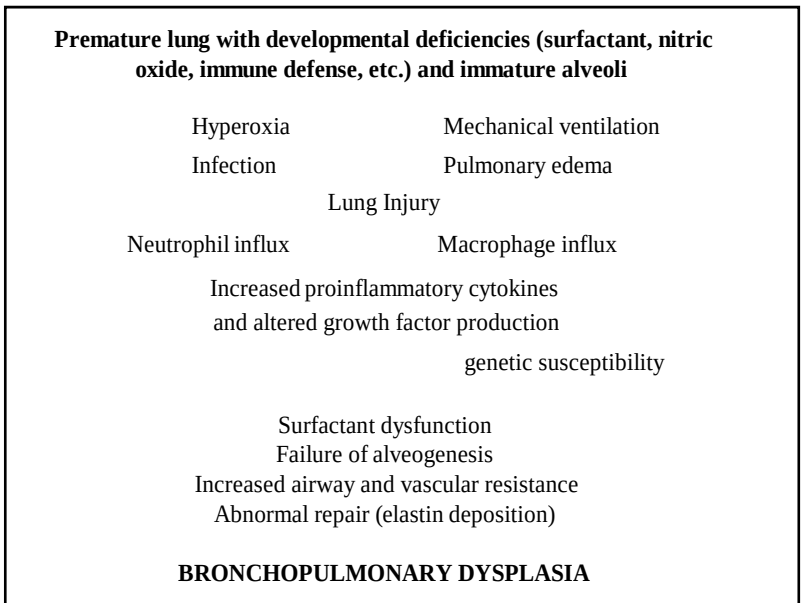


Figure. Simplified schema of the pathogenesis of BPD and proposed role of surfactant dysfunction. Lung injury in the immature lung secondary to hyperoxia, mechanical ventilation and infection initiates an inflammatory response and altered growth and inflammatory factor milieu. One response to these changes is decreased SP-B/C content, which results in inactive surfactant. Loss of SP-B and surfactant function increases lung susceptibility to injury from both oxygen and infection, promoting and prolonging the inflammatory and growth factor response. This continuing insult contributes to the long-term changes in lung structure that characterize BPD.

infants, another form of BPD has been identified. This "new BPD" is characterized by impaired alveolar development with excess tone and reactivity of pulmonary arterial and airway smooth muscle [5-8]. As described in the report validating the NIH consensus definition of BPD, severe BPD is defined as a requirement for ventilatory support and/or supplemental oxygen >30% in an infant at 36 wk PMA [9]. The etiology of BPD is clearly multifactorial and involves derangements in multiple aspects of lung function (e.g., surfactant production), repair from injury (e.g. elastin deposition) and growth and development (e.g. alveologenesis). Various factors contribute to this process, including a susceptible host with immature lung structure, and developmental deficiencies of factors crucial to lung development and function such as surfactant, nitric oxide, innate immune defense, and antioxidant capability (Figure). It is quite unlikely that a single therapy targeted at one component of the pathogenesis (e.g., iNO to improve lung development) will be sufficient to completely prevent BPD. Rather, a combination of treatments directed at different aspects of the pathogenesis may be expected to provide additive benefit.

1.2 Epidemiology of BPD. There are approximately 29,000 infants of 500-999 g birth weight (≤28 wk GA) born annually in the U.S. For the high risk sub-group of these infants who continue to require mechanical ventilation at 7 d of age, BPD occurs in ~70%. Pulmonary compromise of infants with BPD often continues for two or more years, making the prevalence of the disorder as high as 30,000 cases in the United States alone. The acute and chronic sequelae of BPD often result in multiple emergency room visits, re-hospitalizations and long-term pulmonary disability (including asthma and susceptibility to infection), and increased risk of late mortality and neurodevelopmental delay.

1.3 Pulmonary surfactant. Pulmonary surfactant is a mixture of lipids and proteins that provides the surface-active alveolar film required for normal lung function and survival. The predominant surfactant phospholipid is disaturated phosphatidylcholine (dipalmitoylphosphatidylcholine, or DPPC), a highly

compressible lipid that provides a surface film at end-expiration, reducing surface tension to nearly zero to prevent collapse of alveoli and terminal airways. Two of the surfactant-associated proteins, SP-B and SP-C, are intimately associated with surfactant lipids and are crucial for surfactant film formation and function in the lung. SP-B is also required for proper packaging of surfactant in lamellar bodies within type II respiratory epithelial cells [10]. Most ELGAN infants have a developmental deficiency of surfactant at birth. Production of active surfactant, with normal levels of SP-B and SP-C, develops after birth but is delayed as long as three wk in some infants [11]. Numerous clinical trials have established the safety and efficacy of surfactant treatment of premature infants immediately after delivery [12, 13], with decreased mortality and incidence of air leak syndromes.

Most infants who remain intubated beyond the first wk of life experience one or more episodes of dysfunctional surfactant, defined as an abnormal minimum surface tension value (>5 mN/m) *in vitro* for surfactant isolated from tracheal aspirate samples. Moreover, episodes of dysfunctional surfactant are highly associated (p=0.005) with respiratory deteriorations and increased requirement for ventilatory support [1]. This observation provides the rationale for later doses of surfactant to prevent episodes of respiratory decompensation.

A variety of commercial surfactant formulations are safe and effective when administered to premature infants at birth. For this clinical trial of late surfactant treatment we have selected Infasurf® (ONY inc), a natural surfactant extracted from bovine lung lavage fluid, which has consistent amounts of SP-B (0.9% phospholipid) and SP-C (1.5% phospholipid). Animal studies indicate an important role for surfactant in prevention of lung injury and resistance to infection. Alterations in surfactant function and/or SP concentrations have been reported in patients with Acute Respiratory Distress Syndrome (ARDS) [14], pneumonia [15], viral bronchiolitis [16], adult chronic lung disorders [17] and *Pneumocystis* infections [18].

To date, there are limited data evaluating surfactant therapy for premature infants who require continuing ventilatory support beyond one wk of life. Pandit et al. [19] reported a study of 10 premature infants, median gestational age 25 wk, at 9-30 d of life who were given a single dose of a commercially available bovine lipid extract surfactant. Median FiO_2 decreased significantly by 24 h and remained significantly less than at study entry for 72 h. A report from Bissinger et al. [20] also demonstrated a transient improvement in oxygenation of premature infants >7 d of life with diffuse lung disease or respiratory decompensation, after treatment with 2 doses of surfactant. Katz and Klein [21] reported a retrospective cohort study of 25 premature infants, median gestational age of 24.7 wk, receiving booster surfactant treatment on median d 12 of life for worsening oxygenation. The surfactant treatment was well tolerated, and 70% of those treated had a short-term improvement in Respiratory Severity Score (RSS, mean airway pressure x FiO_2) after surfactant therapy.

1.4 Pilot Trials. We have recently conducted two late Infasurf® surfactant treatment pilot trials in premature infants who were not receiving iNO. In the first pilot trial, "Pilot Trial of Surfactant Booster Prophylaxis For Ventilated Preterm Neonates <1000 g Birth Weight, the 2-dose Protocol," premature infants requiring intubation and mechanical ventilation at 7-10 d of life were enrolled and were given a standard dose of Infasurf® surfactant, which was repeated in 1 wk if the infant remained intubated. Tracheal aspirates were collected before, and at various times after, each surfactant dose, and were analyzed for surfactant function and composition. Clinical outcome data, including daily respiratory severity scores, were also collected. Forty-four infants, median birth weight 713 gm (range 430-995), gestational age 25.4 wks (range 23-28 wks), were enrolled in this pilot trial. A separate group of historical controls were identified from the SCOR Pathophysiology of BPD research program and the NO CLD inhaled nitric oxide trial databases, and yielded 41 premature infants requiring intubation and mechanical ventilation beyond the 1st wk of life. Mean birth weight (740±223 gm) and gestational age at birth (25.6±1.5 wk) were very similar to those entered into the pilot trial.

Infants in the historical control group had a significant, progressive, daily increase in Respiratory Severity Scores throughout the entire 2nd wk of life, as compared to baseline (d 8) values. Infants in the surfactant pilot trial had an initial improvement in respiratory severity scores after the first surfactant dose as compared to baseline scores, but similar to historical controls, had significantly higher respiratory severity scores than baseline by 5 d after initial surfactant treatment. Of note, 65% of tracheal aspirate samples obtained before enrollment into the surfactant pilot trial (n=17) had abnormal surface tension, confirming the earlier finding that surfactant dysfunction is common in

infants requiring mechanical ventilation after 7 d of age. The incidence of abnormal minimum surface tension (>5 mN/m) the day after treatment was 38%. However by study d 3 and 7, 58% and 60% of tracheal aspirate samples obtained again had abnormal surface tension, suggesting a loss in benefit at the third study day after initial treatment.

Based on these Respiratory Severity Score and tracheal aspirate data, a second pilot surfactant replacement trial, "Pilot Trial of Surfactant Booster Prophylaxis For Ventilated Preterm Neonates Less <1250 g Birth Weight, the 3-Dose Protocol," was designed. This second pilot study enrolled 43 premature infants with birth characteristics similar to infants of the first pilot (median weight 738 (range 440-1050 gm), median gestational age 25.2 wk (range 22.3–28 wk). The infants in this second pilot trial were dosed with a standard dose of Infasurf® surfactant at study entry, at d 3 and again at 1 wk if the infant remained intubated. Tracheal aspirates and clinical data were collected as previously outlined. Unlike the 2-dose surfactant trial, Respiratory Severity Scores in the 3-dose pilot trial were not significantly higher at 7 d after the initial surfactant dosing, suggesting the more frequent dosing was more beneficial. For the combined pilot trials, respiratory severity scores following the final doses of surfactant at 1 wk were significantly reduced compared to baseline (RSS 5.5 vs. 4.4, p=0.003).

A total of 184 doses of Infasurf® were given in the two pilot trials (74 doses in the 2-dose protocol, 110 doses in the 3-dose protocol). Transient adverse reactions related to the medication instillation occurred in only 10% of the 184 doses and were limited primarily to the known complications of surfactant administration: 13 brief bradycardia-desaturation episodes, and 5 episodes of plugged endotracheal tube. Thus, the surfactant administration was generally well tolerated. Furthermore, there was a significant reduction in respiratory severity score at 60 and 120 min after each surfactant dose (4.56 pre vs. 4.16 at 60 min, and 4.12 at 120 min, p<0.0001) for the combined trials. Specifically, there was no exacerbation of standard neonatal morbidities, such as intraventricular hemorrhage, pulmonary hemorrhage or patent ductus arteriosus around the surfactant dosing in either trial.

However, in spite of these initial improvements in RSS, survival without BPD at 36 wk was still only 25% in these infants (19% in the "2-dose" and 30% in the "3-dose" protocol), suggesting that in these very ill preterm infants requiring ventilation at 7-10 d of age, late treatment with surfactant alone at the dosing schedule used would not result in a significant improvement in survival without BPD.

In order to further evaluate the severity of illness in the infants in the pilot studies, we compared them to the infants in the placebo arm of the NO CLD trial who were intubated and enrolled between 7 and 14 d of age. That group of infants had a 25% survival without BPD confirming that these infants are indeed among the preterm infants most at risk for BPD, and consistent with the concept that treating just one aspect of their multifactorial disease is unlikely to result in clinically important improvement in survival without BPD.

The TOLSURF Pilot study: We conducted a randomized, blinded, pilot study at a subset of 9 of the TOLSURF sites to determine if combined iNO and surfactant therapy would decrease or eliminate episodes of dysfunctional surfactant in treated infants. As of 10/9/09, 85 infants were enrolled and the steering committee decided to close enrollment so that all data and sample collection could be completed before the main study starts. There were no safety issues.

1.5 Inhaled Nitric Oxide for the prevention of BPD

1.5.a. Animal Studies. A number of studies in a variety of animal models have addressed effects and mechanisms of iNO on lung development and injury related to BPD [22-28]. These data indicate that NO is required for normal lung development, that NO production is deficient after premature birth, and that replacement iNO therapy, over a period of weeks, is beneficial in the injured lung.

1.5.b. Clinical Trials. iNO is established as an effective treatment for persistent pulmonary hypertension occurring in term newborn infants [29-31]. The success of iNO treatment of the term infant led to studies of iNO treatment in the preterm infant that have focused on either 1) treatment of critically ill infants at the time of birth with the hypothesis that treatment might result in improved survival [32-34], or 2) treatment of preterm infants with established BPD [35, 36] or at high risk of BPD [2, 37, 38].

1.5.c. Prevention of BPD with the use of inhaled Nitric Oxide (iNO): the NO CLD Trial. We recently published the results of our multicenter randomized clinical trial of iNO to prevent BPD in high risk preterm infants (2,3) The trial was conducted between May, 2000 and April, 2005 at 21 NICUs in 14

cities across the United States and involved infants of birth weights between 500 and 1250 g who continued to require ventilatory support between 7 and 21 d of age. The mean±SD birth weight of the infants enrolled was 766±161 g in the treated group and 759±155 g in the placebo group. There was no difference between the treatment groups in gestational age, racial background, number who received antenatal glucocorticoids, surfactant at birth, or vitamin A. The primary outcome in that trial was survival without BPD at 36 wk PMA. For the infants enrolled between 7 and 14 d (as will occur in the proposed surfactant trial), the rate of survival without BPD at 36 wk PMA was 49% in the treated group and 27% in the placebo group (p=0.005). The relative benefit of treatment was 1.91(1.31-2.78) with NNT =4. In that trial (as is proposed in TOLSURF) all infants from a given pregnancy, although assigned a randomization number, received the same therapy. This required biostatistical analysis techniques (GEE and Multiple Outputation) that take into account clustering [40].

Inhaled nitric oxide treatment in this group also shortened duration of both hospitalization (by mean of 9 d), ventilatory support (by mean of 7 d), and fewer infants were discharged on supplemental oxygen therapy. A cost effectiveness analysis done by John Zupancic [41] found that there was an average saving of ~$5,000 for infants treated with iNO between 7 and 14 d. We saw no clinical or laboratory evidence of short-term adverse effects. In evaluation of the one-year pulmonary outcome of this group of infants; there was a significant decrease in the number of children who received inhaled steroids, supplemental oxygen or bronchodilators [42]. We found no change in the incidence of standard neonatal co-morbidities, namely infection, PDA, NEC, extension of IVH/PVL, or ROP, and no elevation of methemoglobin levels in any of the iNO-treated infants. The long-term neurodevelopmental outcome data from the NO CLD trial indicate that there is no deleterious outcome related to iNO treatment [43, 44] We believe that the results of the NO CLD Trial, along with the findings in other trials and the biological plausibility demonstrated in animal studies, indicate that iNO is an important treatment modality for reducing the incidence and severity of BPD and has become accepted therapy for prevention of BPD in many NICUs.

1.5d.NIH Consensus Conference on iNO in the Preterm Infant. A Consensus Conference was held in Bethesda on Oct 27-29, 2010. The panel drew the following conclusion:

" Taken as a whole, the available evidence does not support use of iNO in early routine, early rescue, or later rescue regimens in the care of premature infants <34 weeks gestation who require respiratory support".

However, within the text of the statement, the panel expanded upon the evidence relevant to this conclusion:

"Infants treated with early routine and early rescue iNO (see Introduction for definitions of these groups) had no significant reduction in death, BPD, or the composite outcome of death or BPD. However, infants treated in the later rescue group, predominantly represented by one, large multicenter trial (the NO CLD trial) in which the treatment protocol was unique not only in the timing of initiation, but also in dosing and duration, revealed an overall reduction in the composite outcome of death or BPD and a post hoc finding of greater efficacy when treatment was initiated during the second postnatal week, as compared with the third postnatal week."

The findings of the NO CLD study are currently being tested in a second multicenter RCT being conducted by IKARIA, using the same protocol and enrolling infants between 5 and 14 days of age.

Although no safety issues have been raised concerning the use of iNO by this protocol, there is concern that some insurance companies will no longer pay for iNO. Although many of the sites participating in TOLSURF have reaffirmed that they believe the evidence in favor of administration of iNO by this protocol is an "accepted practice", appropriate for this patient population, they are evaluating the charges for iNO that might be passed on to the families. INO will be provided at no charge by IKARIA to infants enrolled in the trial.

1.6 Interaction Between Surfactant And Nitric Oxide. We plan to study the addition of late surfactant administration to infants at high risk for BPD who are receiving iNO therapy. To our knowledge there has been only one published study in animals addressing the potential interaction of the 2 treatments. A study in piglets examined responses to iNO and surfactant with acute lung injury secondary to *E. coli* sepsis [45]. Both treatments improved the respiratory status at 24 h compared to control, and combined therapy provided the best outcome. These findings support the safety of combined iNO and surfactant treatment and suggest additive benefit in this experimental model.

A number of studies have examined effects of iNO on endogenous surfactant. In many cell culture and animal studies, which used relatively high doses of NO, exposure reduced surfactant recovery or inhibited surfactant function [46-52]. More relevant, chronic exposure of infant baboons to a clinically relevant dose of iNO had beneficial effects on surfactant. iNO-treated animals had improved surfactant phospholipid/protein ratio and efficiency of SP-B/-C to promote low surface tension was increased [39].

Because of reported negative effects of iNO on surfactant in some studies, as well as the vulnerability of the surfactant system in preterm infants, we prospectively examined surfactant function and composition as part of the NO CLD Trial. We collected tracheal aspirates from a subpopulation of study infants and determined effects of iNO on recovery of surfactant as well as surface tension properties. Aspirate samples were collected at study entry (just prior to receiving study gas) and at 24-48 h, 4 d, and then weekly after initiation of study gas until extubation.

Surfactant function data were obtained at study entry for 83 infants (41 iNO-treated infants and 42 placebo infants). The mean gestational age (25.5 wk), birth weight (725-755 g) and racial distribution were similar for the two groups. The mean age at enrollment was 15 d for both groups and the severity of lung disease at enrollment, as assessed by the RSS, was similar between groups. The infants in this study were representative of the entire population of infants in the NO CLD Trial with regard to primary outcome and the demographic factors [53].

Values for minimum surface tension were obtained by pulsating bubble surfactometer; this measurement reflects the ability of surfactant to form a stable surface film with a normal value for minimum surface tension of <5 mN/m. After initiation of treatment, minimum surface tension increased in placebo infants and decreased in iNO-treated infants, with the largest difference occurring on d 4 (p=0.02 by Student's t test). In addition, surface tension values were significantly different over time between groups during study gas exposure for up to 26 d using mixed effect analysis (p=0.039). We cannot establish from this study whether the improved function occurs only at the higher NO dose (20 ppm) or is a transient response unrelated to dose [53].

As described earlier [1], we have found that premature infants with lung disease experience episodes of surfactant dysfunction throughout the time that they are intubated. We thus examined for an effect of iNO therapy on episodes of dysfunctional surfactant, defined as a value for minimum surface tension of >5 mN/m, measured while the infant remained intubated after initiation of study gas. Data for this analysis were available for 38 infants in each group for a mean duration of 3.9 and 3.1 wk (p=0.03) after trial entry for control and treated infants, respectively. The mean percent of samples taken over time while intubated that had dysfunctional surfactant was 53.4% for control infants and 48.1% for treated infants (NS). Thus, despite an initial lowering of minimum surface tension after beginning iNO, treated infants continued to experience episodes of dysfunctional surfactant during the time they remained intubated. This finding supports our proposal that infants on iNO might benefit from later doses of surfactant to prevent or ameliorate the respiratory deteriorations accompanying surfactant dysfunction.

We also evaluated the relationship between surface tension and the primary clinical outcome in the trial. Survival without BPD in the iNO-treated group was 60% for infants who had a decrease in minimum surface tension between d 3-7 after study entry compared to 25% for those infants without improved surfactant function. In the placebo group, 25% of infants who had a decrease in minimum surface tension had a favorable outcome compared to 53% of the infants without improved surfactant function. None of these differences were statistically significant, although the study was not sufficiently powered to address this question.

2.0 HYPOTHESIS AND SPECIFIC AIMS

2.1 Hypothesis and Rationale for Trial

In this trial, we hypothesize *that late doses of surfactant, in addition to iNO, administered to Extremely Low Gestational Age Newborn (ELGAN) infants ≤28 w gestation who continue to require mechanical ventilation between 7 and 14 d of age will increase survival without BPD. We further hypothesize that there will be no adverse effects of surfactant treatment on short- or long-term outcomes.*

The proposal for a trial of late surfactant therapy combined with iNO to reduce episodes of dysfunctional surfactant and incidence of BPD as well as later pulmonary morbidity is based on the following observations and arguments:

- Although iNO treatment reduces the occurrence and severity of BPD, approximately 44% of treated infants who were intubated at enrollment in the NO CLD Trial still had BPD. This response is consistent with BPD as a multi-factorial disease and with effects of iNO that are limited to specific factor(s) involved in the pathogenesis. Based on data from animal models of BPD, we speculate that iNO treatment of infants promotes alveolarization and reduces smooth muscle cell hyperplasia in airways. However, there is no evidence that iNO as used in the NO CLD Trial, affects pulmonary inflammation or oxidative stress, or promotes long-term improvement in surfactant function. Thus, it seems clear that further significant clinical improvement in lung disease of premature infants will require multi-factorial therapy.
- At this time iNO therapy, as used in the NO CLD trial, is the only intervention that reduces the incidence and severity of lung disease without apparent short- or long-term adverse effects in the group of preterm infants still intubated between 7 and 14 days. Although iNO therapy was accepted practice in the units participating in the pilot study and in most of the units participating in the large trial, there is concern about the potential cost for families in light of the NIH Consensus Conference statement, and therefore the gas will be provided at no charge to infants participating in the study. Since the incidence of BPD and other measures of pulmonary morbidity are substantial in this population despite iNO, it remains appropriate to investigate new therapies in conjunction with iNO.
- Both control and iNO-treated infants have episodes of dysfunctional surfactant. These events are associated with clinical deteriorations in respiratory status that require increased oxygen and/or ventilatory support. Based on animal findings, we propose that these episodes increase exposure of infants to hyperoxia, barotrauma and associated mediators of inflammation and oxidative stress that cause lung injury. We propose that late treatment with surfactant will reduce the amount of lung injury and improve infant outcome.
- Late doses of surfactant are associated with transient improvement in the severity of lung disease. We propose that repeated late surfactant treatments will translate into improved pulmonary outcome at 36 wk PMA and beyond. Based on data for the duration of intubation in infants receiving iNO, we believe that infants will benefit from prophylactic surfactant therapy between 1 and 4 wk of age.
- Although late surfactant treatment in our pilot trials stabilized the RSS for 3-5 d, there was no apparent improvement in outcome at 36 wk PMA compared to the matched placebo infants in the NO CLD trial. We believe that 2 factors contributed to this finding: 1) The duration of effective replacement surfactant in the pilot protocols, which were designed for safety, was ≤2 wk; thus, many infants remained intubated and likely experienced additional episodes of dysfunctional surfactant and further respiratory deteriorations, and 2) none of the infants received iNO therapy as administered in the NO CLD trial. The proposed trial will use more doses of surfactant in iNO-treated infants. Based on data from the current TOLSURF pilot, it appears that dosing should occur every 2 to 3 d.
- We propose that iNO and surfactant will have interactive effects to improve the respiratory status and outcome of premature infants. In infants with existing (or impending) surfactant dysfunction, late surfactant treatments will improve pulmonary compliance and reduce atelectasis. In addition to directly reducing pulmonary inflammation and oxidative stress, surfactant treatment will improve the distribution of iNO to the lung periphery, thereby enhancing benefits related to alveolar development. Improved alveolization secondary to NO therapy, in the presence of active surfactant, will improve oxygenation and decrease the need for respiratory support. We propose that these combined, interactive effects of iNO and late surfactant will be reflected in reduced incidence and severity of episodes of surfactant dysfunction without any short term adverse effects.

2.2 Specific Aims

Aim 1. Assess the effect of late doses of surfactant in ventilated ELGANS receiving inhaled nitric oxide on survival without BPD.

We will conduct a multi-center placebo-controlled trial of late surfactant treatment in addition to iNO in infants at high risk of BPD. We will enroll 524 infants ≤28 wk GA who are intubated requiring mechanical ventilator support between 7 and 14 d of age. Infants will be treated with either surfactant (Infasurf®) or sham (no intervention). Study drug or placebo treatment will be repeated at intervals of 2 +/- 1 d to a maximum of 5 doses through the 3rd wk of life if ventilator support is still required. Tracheal aspirate (TA) samples will be collected prior to initiation of therapy and before subsequent doses while the infants are still intubated for isolation and analysis of surfactant. The trial will have sufficient statistical power to detect an increase in survival without BPD from 44% to 57%. Secondary outcomes include pulmonary outcome at 40 wk PMA as well as pulmonary and neurodevelopmental outcome at 12 and 22 to 26 months of corrected age.

We will collect clinical data to investigate effects of surfactant treatment on both short-term respiratory support needed, as assessed by the Respiratory Severity Score, and pulmonary status at 36 wk (survival without BPD). We also will monitor for evidence of toxicity as indicated by any of the known complications of surfactant administration as well as by the occurrence and severity of other common morbidities of preterm infants, including intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL), patent ductus arteriosus (PDA), necrotizing enterocolitis (NEC), sepsis and retinopathy of prematurity (ROP). We expect that combined iNO and surfactant therapy will be safe.

Aim 2. Assess effects of late surfactant treatment on surfactant status and lung inflammatory biomarkers, and establish a DNA repository for genomic studies of the pathogenesis of BPD.

There is currently very little information regarding the pharmacodynamics and biochemical effects of late surfactant treatment in iNO-treated infants. In contrast to surfactant treatment at birth, which involves lungs deficient in endogenous surfactant, later treatment occurs in the presence of endogenous surfactant with varying degrees of activity. Efficacy of late surfactant therapy will be influenced by the functional status of the endogenous surfactant pool, relative pool sizes of replacement and endogenous surfactant, efficiency of replacement surfactant in reaching terminal airways and alveoli and extent of mixing with endogenous surfactant, content of SP-B and SP-C in endogenous surfactant, and clearance rate of lipid and protein components of administered surfactant. These biochemical issues relate to the appropriate dose and treatment interval for late surfactant. To address these topics, we will assess composition (SP-B and total protein content relative to phospholipid) of surfactant isolated from TA samples of study infants. We previously established that SP-B content is inversely related to surfactant function *in vitro* [1]. *We hypothesize that SP-B content will increase after Infasurf® treatment, and that infants with improved surfactant function will have lower RSS and better outcome.* These studies will continue our preliminary laboratory observations in the pilot trials, expanded to include control infants who do not receive surfactant. In addition, we will determine the profile of selected inflammatory cytokines in TA of infants with and without late surfactant treatment to investigate safety of the treatment as well as potential anti-inflammatory responses. *We hypothesize that the combination of iNO and repeated surfactant treatments, starting between 7 and 14 d of age, will reduce pulmonary inflammation as reflected in concentrations of biomarkers in TA fluid.* With a large and detailed clinical database plus the laboratory data, the TOLSURF Study also provides a unique opportunity to investigate genetic contributions to the development of BPD as well as responsiveness to surfactant therapy. As part of the trial, a DNA repository will be developed for analysis of specific gene polymorphisms in separately funded initiatives.

3.0 RESEARCH DESIGN

We will perform a randomized, blinded, multicenter, placebo-controlled trial of late surfactant treatment administered to ventilated preterm infants ≤ 28weeks gestation beginning between 7 and 14

days of age. Randomization will be stratified by site and gestational age at birth. (< 26 weeks or 26 to 28 weeks GA).

3.1 Sites Involved. Subjects will initially be recruited from eligible inpatients within the Neonatal Intensive Care Units listed in **Appendix A section 23.0**

Study sample size is 524 patients. There is no enrollment restriction based on gender, ethnicity, or race. Enrollment is expected to take about 38 to 40 months plus follow-up through 22 - 26 months corrected age.

3.2 Site Activities. See Table for timing of procedures and data and sample collection. All sites will:

- Screen infants ≤28 w/0 d GA for eligibility and enrollment
- Collect clinical data on pulmonary course and outcome
- Assess the incidence of common co-morbidities of preterm infants including infection, intracranial hemorrhage, necrotizing enterocolitis and retinopathy of prematurity.
- Collect samples of tracheal aspirates at specified times for analysis of surfactant function and inflammatory markers. As well as biomarkers of long term pulmonary disease.
- Evaluate long-term pulmonary and neurodevelopment outcome through 22 -26 months of age.

TABLE 3.1 STUDY PROCEDURE CHART

Study Day/Corrected Age	Pre-Study	**0**	**2[b]**	**4[b]**	**6[b]**	**8[b]**				**1wk post iNO**	**36 wks**	**40 wks**	**3, 6 & 9 & 18 mos**	**12 mos**	**22 - 26 mos**
Informed consent	**X**														
Randomization		**X**													
Tracheal Aspirate[a]		**X**	**X**	**X**											
Study Surfactant or sham procedure[a]		**X**	**X**	**X**	**X**	**X**									
iNO		**20**		**10**			**5**	**2**	**0**						
Oxygen reduction challenge											**X**	**X**			
Respiratory status questionnaire												**X (DC)**	**X**	**X**	
Neurodevelop-mental evaluation														**X**	**X**

[a] If remains intubated and mechanically ventilated. Obtain prior to study dose.
[b] ± 24 h
. Tracheal aspirate samples will be collected before Infasurf/Sham dosing procedures #1,#2 and #3.

4.0 ELIGIBILITY

4.1. Inclusion Criteria
- ≤ 28 0/7 wk gestational age
- Day of life 7-14
- Intubated and mechanically ventilated
- Plan to treat with iNO

4.2. Exclusion Criteria
- Serious congenital malformations or chromosomal abnormality (see MOP)
- Life expectancy <7 d from enrollment
- Clinically Unstable (see Table 4.3 below and MOP)
- Less than 48 h from last clinical dose of early surfactant
- Unlikely to be able to collect primary endpoint data at 36 weeks

TABLE 4.3 DEFINITION OF CLINICALLY UNSTABLE INFANT

1. Active Pneumothorax with chest tube
2. Active Pulmonary Hemorrhage
3. Uncontrolled hypotension requiring more than 20 mc/kg/min dopamine or 2 pressor agents for > 24 hours
4. Acute NEC –less than 24 hours from diagnosis or surgery
5. Untreated culture positive sepsis (<6h)
6. RSS >14
7. Clinical team feels infant would not tolerate dosing procedure

NOTE: Once these issues resolve the infant may be considered either for enrollment in TOLSURF with initial dosing or for re-dosing if dosing was delayed

5.0 RECRUITMENT AND RANDOMIZATION

5.1 Recruitment. To facilitate timely enrollment in the trial, infants ≤ 28 w GA will be screened at age 1-4d by the study physicians and nurses for eligibility and exclusion criteria and a brochure explaining the study in lay language will be provided to the family. Records of potential candidates for the study will then be reviewed by one of the study investigators and the child's condition discussed with the attending physician. If appropriate, the infant's parents will be approached by one of the investigators to obtain informed consent preferably within the first 3 days with the expectation that if the infant meets criteria between 7 and 14 d of age, he/she will be randomized and enrolled at that time. It is expected that there will be a reasonably high acceptance rate by parents for participation in this study because a) these infants, if still requiring ventilation between 7 and 14 d, have only a 44% chance of survival without BPD at 36 wk PMA with usual therapy (including iNO) alone; b) late treatment with surfactant (Infasurf®) represents extra doses of a substance (surfactant) that their infant received at birth as part of standard clinical care. Infants who are still intubated or reintubated between d 7 -14 will be enrolled in the trial, begun on iNO, and randomized to receive surfactant study drug or sham placebo treatment.

5.2 Accrual - Meeting Recruitment Targets**.** We have chosen sites for this trial that can be expected to enroll a minimum of 8 to 10 infants/year in the study (many expect to enroll more than 15/year).
We plan to recruit at 19 sites (5of which have two hospitals) Based on our experience to date with the TOLSURF Pilot study, we anticipate that the participating sites will meet their enrollment targets and that subject accrual will follow the projected timeline. We plan 6 months (from 9/15/09) for finalizing preparations to begin enrollment in the study. We expect that the 7 sites (9 hospitals) involved in the pilot will be able to begin enrolling by March, 2010 (averaging 5 infants/month). We expect 3

additional sites(4 hospitals) to begin enrolling by mid June (increasing the monthly enrollment for the 10 initial sites to 8/mo and an estimated 39 infants in the first 6 mos - 9/15/10). We expect that another 2-3 sites will be ready to enroll by 9/15/10 and the final 3 ready by 12/15/10 to reach the goal of enrolling 11/month by the end of the second 6 mo and 16/mo in year 2 and 3. This projection would lead to 111 infants in year one (by 3/15/11), additional 192 in year 2 (303 total) and 192 in year 3 (495 total) expecting to complete enrollment in 38-39 mo. However, we recognize that some sites might fail to meet enrollment targets for a variety of reasons. Accordingly, we will assess site enrollment every 6 mo after initiation of the study. Should enrollment at an individual site fall below 4 in 6 mo, the PI and Clinical Steering Committee will give warning and evaluate whether it is appropriate to drop the site from the trial. If no patients are entered during a 6 mo period we will recommend to the DSMB that the site be dropped and a new site recruited. If a single site appears to be enrolling more than 20% of the total infants enrolled in the trial we will temporarily limit enrollment at that site.

5.3 Randomization. Randomization tables will be prepared by the DCC and sent to the Pharmacist at the sites. This study has been designed to allow for randomization 8 AM to 5 PM during week days. The site will phone the Project Director and confirm that the infant meets the eligibility criteria and determine if the infant is part of a multiple birth. If eligibility is confirmed, a study ID number will be assigned to the patient and instructions given to the site to enroll the infant. Tracheal Aspirate samples will be obtained. The physician will write the order to RRT to begin iNO and to the pharmacy to assign the next treatment to the infant. The Project Director will complete an entry on her log giving the date, time, patient ID, confirmation number, etc. These can later be confirmed against the clinical center records. Only the pharmacist will be unblinded to treatment assignment. Patients will be stratified by gestational age (<26 and 26-28w) and by site and randomized to one of two groups: a) iNO with sham instillation and b) iNO and Infasurf®. To ensure balance within clinical centers and with respect to the key prognostic variable, gestational age, the randomization will be implemented using randomly permuted blocks of length 4 and 8, randomly varying, to ensure approximate balance at every time point and to make successive assignments harder to guess. The sequence of assignments for each site and stratum will be prepared in advance by the DCC. Since we have found that parents of multiple births who participate in a clinical study have a strong preference that each of their children receives the same treatment, in this trial, the first infant will be randomized according to the permuted block design. Subsequent infants from a multiple birth will be assigned to the same treatment group as the first infant. This is equivalent to randomizing the mother and will be accounted for in the analysis as described below.

6.0 STUDY TREATMENT

6.1 Inhaled Nitric Oxide. All of the infants in this study will receive iNO. The gas will be administered in the dosing schedule used in the NO CLD trial starting at 20 ppm for 4 +/- 1d, weaning to 10 ppm, 5 ppm and 2 ppm at weekly intervals for a total minimum treatment of 25 d. Gas will be continued by NCPAP or nasal cannula if the infant is extubated. INO will be provided at no charge by IKARIA, Inc.

6.2 Late Surfactant Treatment. Infasurf (ONY Inc., Amherst, NY) is an FDA-approved, commercially available extract of bovine natural surfactant, which contains phospholipids, neutral lipids and a consistent concentration of SP-B and SP-C (0.9% SP-B to phosphatidylcholine ratio). The efficacy of Infasurf® at birth has been demonstrated in multiple trials and this formulation will be used for the study surfactant at all study sites. The lot number for each dose of Infasurf® will be recorded. Infasurf® will be provided at no charge by ONY, Inc.

6.3 Instillation Procedure. Infants must be considered clinically stable at the time of enrollment (see Table 4.3 and MOP for definition). Either Infasurf® surfactant or a sham treatment is administered as soon as possible after the initiation of iNO, using standard procedures and precautions as for newborn treatment. In order to maintain blinding, screens are set around the bedside, shielding the infant from personnel in the room. Monitors are on and active, but silenced. Endotracheal tube (ETT) patency and appropriate position should be assured prior to instilling study drug. Study drug is usually

administered by one or more respiratory therapists who are trained in the instillation procedure. Other members of the health-care team step away from the bedside during study drug instillation. An individual skilled in intubation must be present in the nursery at the time of study drug instillation in case of dislodging or plugging of the endotracheal tube requiring re-intubation. There will be no manipulation of the ETT of infants in the sham treatment group. Study drug is administered per the institution's regular surfactant administration policy and care is taken to maintain lung inflation during instillation. Ventilator adjustment guidelines will be provided to address the response to any significant changes in oxygenation, ventilation, heart rate or chest wall movement during or after the procedure. The dosing respiratory therapists will attempt to wean the ventilatory support back to baseline settings over a 20 min period, after which the audible alarms are reset and the screens are removed. Vital signs, saturations, severity score and ventilator settings will be recorded prior to and at 60 and 120 minutes after each study procedure. Subsequent ventilatory management is at the discretion of the clinical medical team, with a common ventilator strategy to maintain adequate lung inflation and minimize overdistention and oxygen exposure. See the MOP for a detailed description of the procedure.

6.4 Blinding of Surfactant/Placebo Administration. The study drug, prepared by the pharmacist, is 3 ml/kg Infasurf® surfactant or an equivalent volume of air as placebo; the air is not administered. The drug is drawn up in two half doses (1.5 ml/kg) and sent to the nursery in syringes covered in an opaque envelope to maintain blinding. All parents, study and clinical staff, except the "dosing" respiratory therapist and the research pharmacist are blinded to treatment assignment. Clinical respiratory therapy staff, but not the "dosing" therapist, may make suggestions about ventilator manipulations in study infants immediately after study drug delivery. Every attempt will be made to limit unblinding of study subject assignment. Staff involved in the dosing procedure will collect information about how the infant tolerates dosing (bradycardia,desaturation or reintubation) and fax to the DCC separate from the rest of the CRF. They will not be involved in any other aspect of data collection, determination of the endpoint at 36 or 40 weeks – or administering the O2/flow challenge test. We have previous experience with randomization in the pharmacy [54] and unblinded administration of treatment by a respiratory therapist (NO CLD Trial). In neither trial was there an instance of unblinding of the PI, and several of the trial centers also have experience with blinding of surfactant treatment in the manner proposed.

6.5 Retreatment with Surfactant/Placebo. If the infant remains intubated 1-3 days after the first study drug treatment, a second dose of the same assigned study treatment is administered using the same procedure. Up to 3 additional doses of study drug (total 5 doses) may be given at 1-3d intervals if the infant remains intubated. Retreatment intervals after the first dose for this trial are based upon respiratory severity score data and tracheal sample analysis from the first three surfactant pilot trials. This treatment schedule will provide active surfactant for infants into the third or fourth wk of life. We have found that the highest incidence of respiratory deterioration associated with surfactant dysfunction occurs during the postnatal interval from 7 to 28 d [1]. Should the infant be considered clinically unstable by the physicians at the time when a dose (see Table 4.3 and as defined in the MOP) should be given, but later stabilizes during the study treatment period, the infant will continue with study drug dosing as per the dosing schedule outlined above, and is still eligible for a total of 5 study doses during the 3 weeks after enrollment.

6.6 Patients Extubated within 24 Days of Study Initiation. Patients who are extubated between Study Day 1 and Study Day 25 will continue on iNO, but will not have subsequent tracheal aspirates obtained, and will not receive subsequent surfactant/placebo doses. However, if an infant is re-intubated, the patient will have subsequent tracheal aspirates obtained, and will receive surfactant/placebo, according to the study schedule. For infants who are extubated, and then require re-intubation, a tracheal aspirate sample will be obtained within 24 h of reintubation, and tracheal aspirate sampling will then continue until 3 TA samples a have been obtained, as outlined above, while the infant is receiving study drug.

7.0 STUDY TREATMENT MODIFICATIONS

7.1 Indications for Interruption of Study Drug Administration If infant is deemed clinically unstable (Table 4.3) by the attending physician (including RSS > 14 for > 8 hours or as otherwise defined in the MOP) dosing will be delayed until RSS is < 14 and infant considered stable by the clinical team.

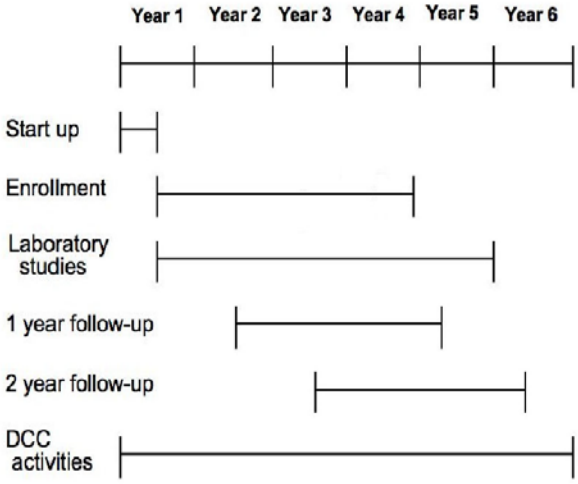
7.2 Stopping Study Drug Administration for Individual Patient. If the infant experiences severe respiratory decompensation immediately after the study drug administration procedure with a sustained (for 24 h or more) change in RSS above baseline of > 8 no further doses will be given but data collection will continue.

7.3 Open Label Use of Late Surfactant. If the attending physician deems that the infant would benefit from late doses of surfactant the following procedure is to be followed:

- The decision must be approved by the site PI and reported to the CCC within 72 h
- Surfactant will not be furnished free for this use
- DO NOT UNBLIND – continue the protocol as appropriate
- Data and sample collection and AE reporting on infants treated Open Label should continue as per study protocol.
- A Protocol violation form must be filed

8.0 OUTCOME MEASURES

8.1 Primary Outcome. The primary outcome is survival without BPD at 36 wk PMA. This definition is currently used as the primary outcome for other trials related to premature infant lung disease. BPD is diagnosed, as in our NO CLD Trial, by a requirement at 36 wk PMA for ventilation and/or supplemental oxygen determined by a room air challenge test for those infants still receiving oxygen. Infants at 36 wk PMA who require mechanical ventilation and any level of supplemental oxygen, or oxygen >30% without assisted ventilation, are diagnosed with BPD and considered "severe" BPD by the NIH Consensus definition [9]. Infants at 36 wk PMA on ≤30% effective oxygen are evaluated for oxygen requirement by a room air challenge administered by a member of the investigative team (Complete description in the Manual of Operations). For infants in room air, we will also record Oxygen saturation and, if available, PCO2. On the basis of the NO CLD trial results in infants enrolled between 7 and 14 d, we estimate that incidence of BPD-free survival at 36 wk will be 44% in the infants receiving only iNO (placebo group). We feel that a 13% absolute improvement in outcome would be clinically important and lead to change in practice and therefore estimate 57% disease-free survival in the Infasurf® group. We calculate that a sample of 524 will provide 80% power in 2-sided tests to detect the proposed increase in 36-wk BPD-free survival from 44% to 57%. Based on actual enrollment by the sites that participated in the NO CLD and/or the late surfactant pilot trials, and expected enrollment at the new sites we expect to complete enrollment in 38-39 m, (see section 5.2 on Accrual)Year one began September 15, 2009.



8.2 Secondary Outcomes. There are 4 secondary outcomes related to the severity of BPD and other effects of treatment on respiratory status: a) discharge home or off respiratory support at 40 wk PMA (term), b) measures of BPD severity, c) pulmonary outcome after discharge and, d) pulmonary and neurodevelopmental outcome through 2 y of age.

8.2.a. Discharge home or off respiratory support. At 40 wk PMA we will record the number of infants who have been discharged to home or are off respiratory support in the hospital. From the NO CLD trial, we know that in the group of infants enrolled between 7 and 14 d and ventilated at enrollment (the population proposed for this study) 44% of control infants compared with 63% of those treated with iNO were discharged or off support by 40 wk (P=0.01) We also found that status at 40wk is predictive of treatment with bronchodilators at 1 year [55]. At 40 wk, if appropriate, we will again perform an oxygen challenge test to determine if the infant still requires supplemental oxygen.

8.2.b. Severity of BPD. Due to the selection criteria for infants in this trial, virtually all of the infants who will be defined as having BPD at 36 wk will have the "severe" form as defined by the NIH consensus [9]. Duration of ventilatory support, supplemental oxygen and hospitalization will be evaluated as further measures of clinically important disease severity as well as indicators of health services resource use.

8.2.c. Pulmonary outcome at 12, 18 and 22- 26 mo of age.

We will evaluate post discharge pulmonary morbidity through use of a respiratory questionnaire administered at discharge as well as questionnaires administered every 3-4 months either at clinic visits – or by phone- addressing medication use, ER visits and respiratory hospitalizations. We have received permission from Dr. Tim Stevens and the Steering Committee of the NICHD Neonatal Network to modify the tool that they have used for these evaluations in the NICHD SUPPORT (The Surfactant Positive Airway Pressure and Pulse Oximetry Trial).

8.2.d. Neurodevelopmental outcome at 22 - 26 mo of age. BPD has a profound negative impact on neurodevelopmental outcomes. We anticipate that decreasing BPD will improve neurodevelopmental outcome. On the other hand, late treatment is a new application for surfactant and could have unforeseen effects on neurodevelopment. Thus, there is a strong rationale for a comprehensive outcome assessment program. All of the centers involved in this trial have active neonatal developmental follow-up programs. As in the NO CLD trial, all the sites will have a "Gold Standard" Bayley III examiner for assessment at age 22 -26 mo. The MOP provides a description of the assessments to be done as well as the "Gold Standard" certification process. The early detection of impaired survivors allows implementation of intervention services, which may reduce the burden of disability in the survivors. We fully acknowledge the limitations of these early evaluations for the prediction of future intellectual and school performance. However, if late surfactant is efficacious in improving survival without BPD, but survivors demonstrated more pronounced early neurodevelopmental abnormalities, it would be important to have this follow-up information before this therapy became widely used. We do not expect late surfactant to have a deleterious effect on outcome.

9.0 DATA COLLECTION AND EVALUATION

9.1 Clinical Data Collection. The clinical data includes details about respiratory support, development of BPD, selected medications, and significant co-morbidities. See the CRFs. The respiratory data includes the type of respiratory support, FiO2, and ventilator settings, which will be used to calculate daily respiratory severity scores (RSS = MAP X FiO2). Other respiratory outcomes include the length of ventilatory support, duration of oxygen supplementation, and hospitalization. BPD will be diagnosed at 36 wk PMA using the oxygen/flow challenge test similar as employed in the NO CLD trial. Respiratory status at 40 wk PMA will be assessed using the same oxygen challenge test when appropriate. Data on medication administration, including use of steroids, vitamin A, caffeine, antibiotics and indomethacin will be collected. The incidence of co-morbidities related to prematurity and other adverse events potentially related to tracheal aspirate sampling or administration of surfactant will be recorded. Infants will be followed through 22- 26 mo of age for pulmonary and neurodevelopmental outcomes. Standardized pulmonary questionnaires will be administered (usually by phone) every 3-4 mo until 1 y of age and again at 22 - 26 m of age in order to collect information

about respiratory status, ER visits and hospitalizations and pulmonary medications. See list of information to be collected in MOP.

9.2 Other Clinical Management. Other therapies for treatment of BPD, in particular, postnatal glucocorticoid treatment, will be administered according to clinical management guidelines agreed upon by all investigators, as was the case with the NO CLD Trial. see Appendix B for guidelines) As part of the education and site initiation visit, the PI will discuss clinical protocol guidelines with the attending neonatologists at that site. The protocols include, but are not restricted to, the use of surfactant at birth, treatment with caffeine, Vitamin A use, ventilator management and respiratory gas parameters, oxygen saturation targets, the use of NCPAP and high flow nasal cannula, the use of corticosteroids both for BPD and hypotension, as well as rescue treatment (open label) with surfactant for severe lung disease. The investigator steering committee will review these guidelines in light of current practice on at least an annual basis, and the sites will be monitored for compliance by staff during site monitoring visits.

9.3 Data Quality Assurance. We will monitor the accuracy of data entry by the sites both internally and externally. For internal monitoring, completed CRFs are reviewed prior to entry into the database, clarifying issues as necessary with site coordinators and acting as liaison between the sites and the DCC as needed. These steps are already in place and functioning in the TOLSURF Pilot Study. External monitoring will consist of regular monitoring visits to every site while actively enrolling. Initial monitoring visits will take place after the first 6-8 patients have completed data collection and then after every 10 to 15 infants are subsequently enrolled and CRFs completed. All medical charts will be monitored and compared to the CRFs for meeting entry criteria, adherence to protocol, primary and secondary outcomes, and adverse event reporting. In addition, a 25% random sample of all data points in the CRFs will be compared with the medical record. Any outstanding data queries will be resolved with the research coordinator at the time of the visit. After each study site visit a report will be prepared and copies sent to the Study File, the DCC, the study PI (R. Ballard), the site PI, the site coordinator and the Project Director (N. Newton).

As part of the overall QA effort, we will examine various measures of study implementation across sites. In particular, recruitment, retention, data completeness, and measurement precision will be tabulated and compared across sites and will be included in our web-based reports. QA efforts and site visits will be focused on any sites that show evidence of problems.

10.0 SAMPLES AND LABORATORY STUDIES

10.1 Tracheal Aspirate Samples.

Each tracheal aspirate sample is centrifuged soon after collection to provide a cell pellet and a supernatant fraction. After shipment to UCSF, the supernatant is centrifuged to isolate a large aggregate surfactant fraction that is assayed as previously described [1] for both phospholipid and surfactant protein concentration under the direction of Dr. Philip Ballard. The supernatant fraction after surfactant isolation is assayed for selected cytokines using a multiplex assay. The TA cell pellet is used for isolation of DNA to develop a repository for genetic studies of gene variants. All tracheal aspirate samples are labeled by study ID number and date/time, without other identifiers. . Patient name, initials, birth date and other potential identifiers are known only to investigators at each study site and this information is not available to Dr. P. Ballard and other laboratory personnel. The study samples are stored in a locked freezer and access to the Ballard laboratory requires a coded identification card that is available only to approved laboratory personnel. Analyses of data from study samples as related to clinical parameters is performed by personnel in the DCC. Should any portion of samples remain, we request that it be stored, labeled only by study number, in the laboratory of Dr. P. Ballard for further testing of substances identified as related to airway inflammation and the development of BPD (this is addressed specifically in the consent form and reflected in the CRF).

11.0 ADVERSE EVENT AND PROTOCOL VIOLATION REPORTING

11.1 Adverse Event Reporting. Adverse events will be recorded surrounding the study drug administration, up until 7 d after dosing is completed, at 36 wk PMA and through to discharge. and a summary for each infant will be reported by the sites to the CCC and DCC at 36 wk PMA and discharge. See Table 11.1

11.1.a.Serious Adverse Events (SAE). The most important SAE is death occurring up to 7 d. after dosing completed. This requires expedited reporting (see flow chart). In addition, severe, cardiopulmonary decompensation requiring CPR with cardiac meds/compressions within 4 h of dosing or increase in RSS of > 5 over baseline and sustained > 24 h within 4 h of a dosing procedure, severe pulmonary hemorrhage, pneumothorax requiring a chest tube, or significant PIE within 24 h of a dosing procedure, will be reported to the IRBs, CCC, DCC, NHLBI, the DSMB and the FDA by the investigators. Unexpected events thought to be related to the study drug will also be considered SAEs. See flow chart for expected timing of reporting. A neonatologist independent of this study will serve as the data safety monitoring officer, and will monitor all serious adverse events concurrently. Each death will be reported in a detailed letter to the FDA as well as to appropriate IRBs and the DSMB.

11.1.b. Adverse Events. (see table 11.1)will primarily be i) related to study drug dosing, ii) the incidence of the known co-morbidities of prematurity in these critically ill infants including sepsis, IVH, PVL, NEC, PDA, and ROP, iii) other relevant pulmonary or cardiovascular complications including pulmonary hypertension or airway abnormalities, iv) complications related to tracheal aspirate sampling, v) requirement for CPR involving cardiac medications and chest compression, vi) unexpected events, and will be reported to the DSMB at regular intervals as part of safety monitoring.

TABLE 11.1 PROPOSED SAE AND AE REPORTING
(See Table 11.2 in MOP and Flow Chart for timing of reporting)

SAEs (these are only within study dosing time plus 7 d)

1. Death (only SAE requiring expedited reporting)
2. Beginning within 4 h of dosing:
    a. Severe cardiopulmonary decompensation requiring CPR with cardiac medication & chest compressions
    b. Increase in RSS >5 sustained for >24 hours
3. Within 24 hrs of dosing:
    a. Severe pulmonary hemorrhage
    b. Severe PIE
    c. Pneumothorax requiring chest tube
4. Unexpected and related to study drug administration

AE's

1. Death >7 d after dosing protocol (also fill out death form)
2. Problems with obtaining tracheal aspirate samples
3. Within 4 h of dosing procedure (separate confidential form completed by dosing team).
    a. Prolonged (>60 seconds)bradycardia/desaturation
    b. Endotracheal tube problems requiring reintubation
4. CPR requiring cardiac meds and compressions
5. Hypotension requiring vasopressor support w/ dopamine > 20 mcg/kg/min or 2 pressor agents for > 24 h
6. Co-Morbidities occurring after enrollment in study (details are described in CRF and MOP)
    a. Neurologic (IVH, PVL, hydrocephalus)
    b. GI (NEC, perforation, surgery)
    c. Pulmonary (PIE, pulmonary hemorrhage, pneumothorax, tracheomalacia, stenosis)
    d. Cardiovascular (PDA -with or without surgery, pulmonary hypertension)
    e. Sepsis (bacterial, fungal, viral)
7. Unexpected adverse events (medication errors, catheter complications etc)

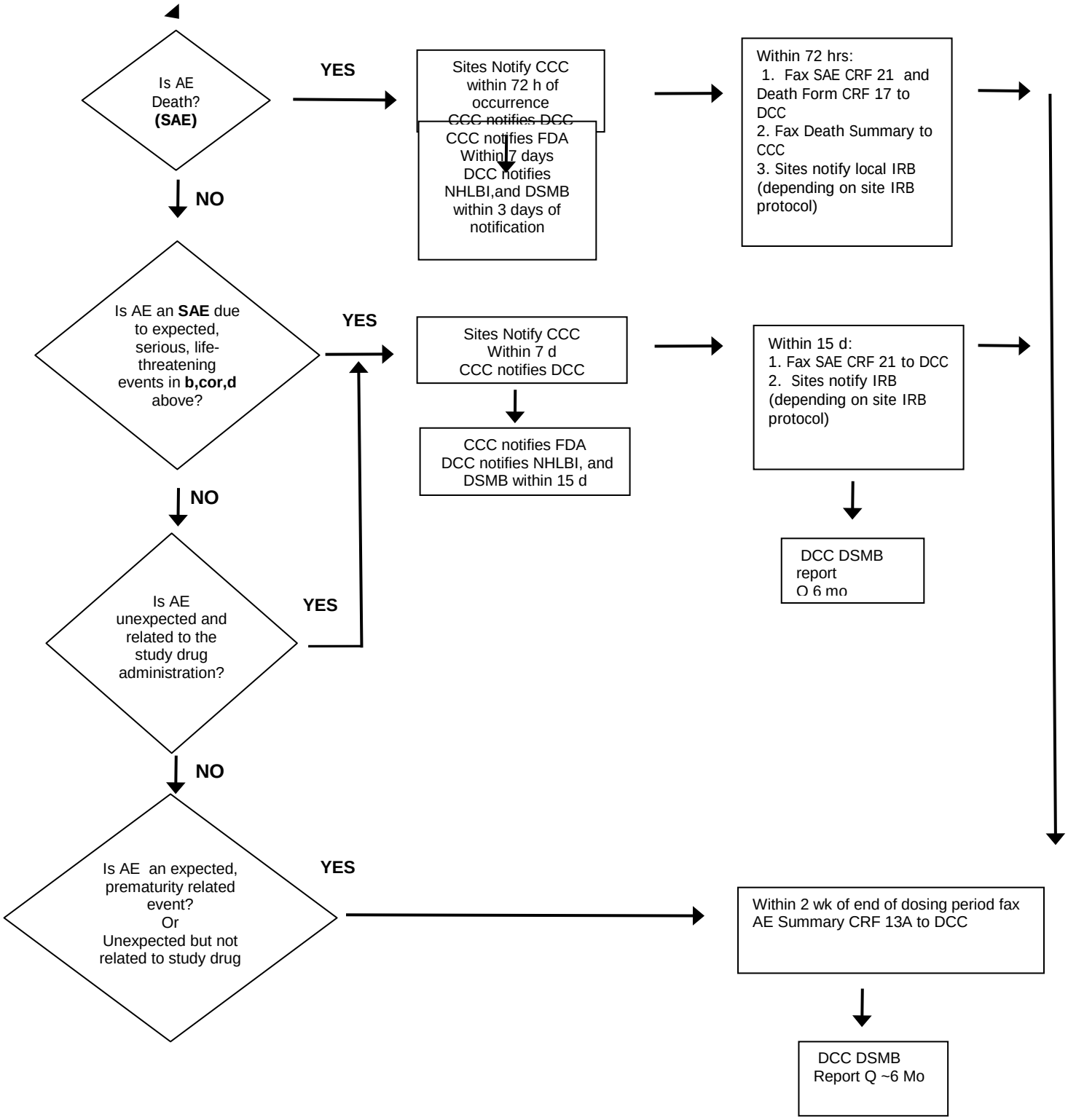
NOT TO BE CONSIDERED AEs – common problems encountered in the clinical care of these infants such as feeding intolerance, or electrolyte imbalance will not be considered AEs (see MOP)

TABLE 11.3 SERIOUS ADVERSE EVENT/ADVERSE EVENT FLOWCHART

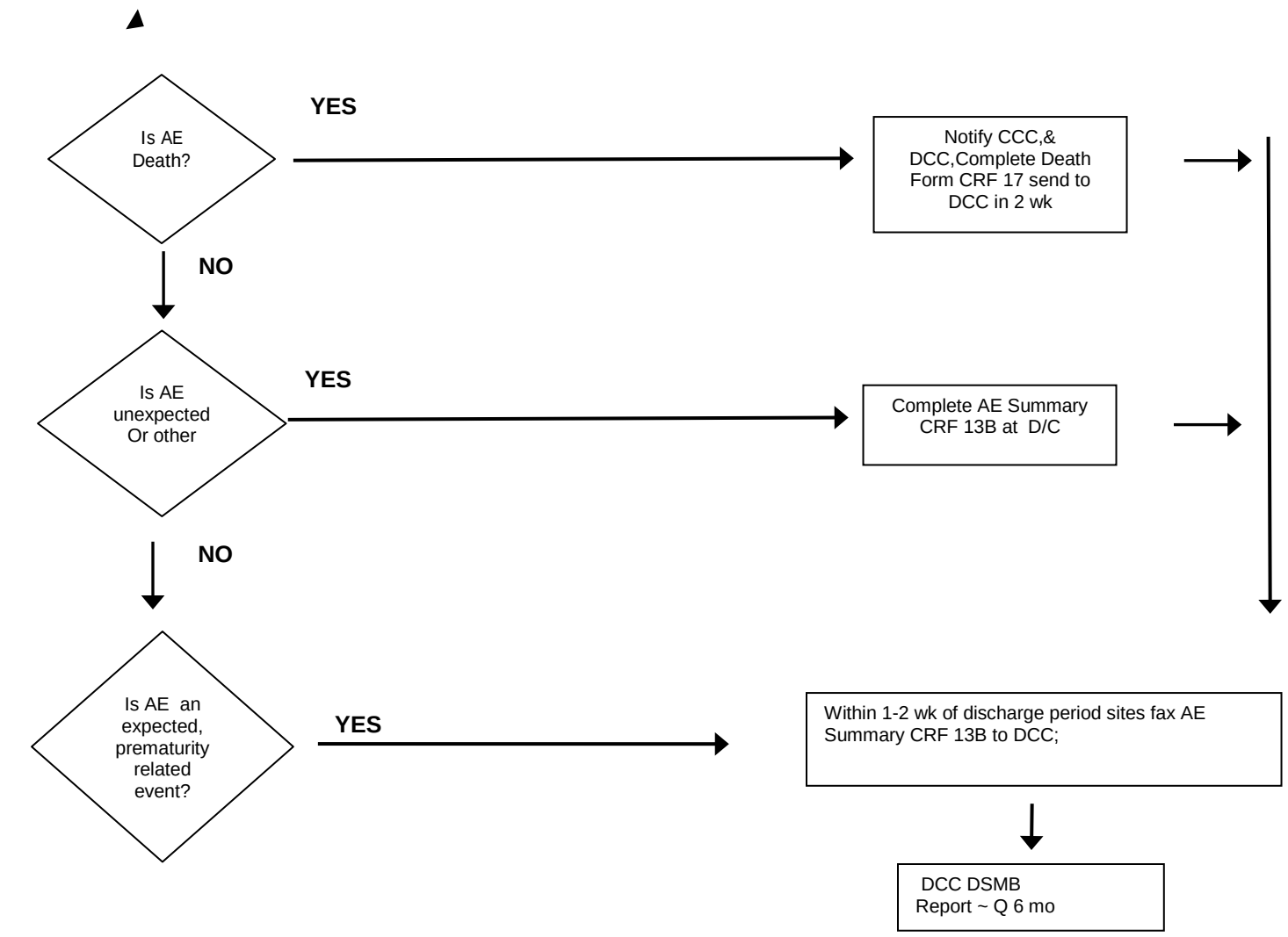
SAE's include the following events:

a) death if it occurs between enrollment and 1 week (7 days) after dosing completed:
b) severe respiratory decompensation requiring CPR with chest compressions and cardiac meds within 4 hours of dosing
c) Increase in RSS >5 from baseline within 4 hours of dosing sustained for >24 hours
d) severe pulmonary hemorrhage, severe PIE, or pneumothorax within 24 hours of dosing
e) unexpected and related to study drug administration

EVENT OCCURRED BETWEEN ENROLLMENT AND 7 DAYS AFTER DOSING COMPLETED:



EVENT OCCURRED > 7 DAYS AFTER DOSING COMPLETED:



11.2 Protocol Violation/Deviation Reporting. Violations of the study drug administration protocol require completion of a Protocol Violation Form and notification to the CCC and DCC within 3 working d.

11.2.a Major Protocol Violations Include**:**

- Enrollment in light of exclusion criteria
- Consent obtained not in accordance with IRB guidelines
- Unblinding of study personnel
- Study drug administration or dosing error
- Inhaled NO not given
- Open label surfactant administered

11.2.b Protocol Deviations include:

Failure to perform oxygen/flow reduction test as indicated

- iNO not given according to protocol
- deviation from clinical guidelines

12.0 STATISTICAL CONSIDERATIONS

12.1 Sample Size and Power.

12.1.a. Assumptions for calculation of sample size. Our calculations are based on a comparison of the proportion of infants who achieve the primary endpoint (BPD-free survival at 36 wk) in the two treatment groups. The detailed analysis methods are described in more detail below. In summary, we use a significance level of 0.0488, reflecting a single interim analysis. In analyses of 36-wk BPD-free survival among infants 7-14 d old at study entry in the NO CLD trial, the inflation of the variance of the treatment effect estimate in GEE analysis, relative to an analysis assuming independence, is approximately 1.05. This value is used in the sample size calculation.

12.1.b. Placebo/ iNO-only event rate. The primary endpoint for the study is BPD-free survival at 36 wk. This study will be limited to infants between 7 and 14 d, who comprised about half the NO CLD sample, and benefited more than older infants. Specifically, the BPD-free survival rate in this group was 49.1% in the iNO group [2, 3], and 44% in the subgroup of these infants who were intubated at enrollment (infants could be enrolled in NO CLD who were on NCPAP). On this basis we have assumed that the 36-wk BPD-free survival rate will be 44% in the iNO-only group in the proposed study. The impact of alternative assumptions are shown in section 12.1.e below.

12.1.c. Treatment effect size. In designing the NO CLD trial [2, 3], we calculated the sample size to ensure power to detect a 12.5% absolute increase in 36-wk BPD-free survival. The 12.5% absolute improvement was felt to be clinically important at that time and we believe is still a relevant magnitude of effect. In designing the current trial, we have increased the absolute improvement slightly to 12.9% in order to accommodate our approved samples size of 524. Given a 44% disease-free survival proportion in the iNO-only (surfactant placebo) group, this would correspond to a 36-wk BPD-survival rate of 56.9% in the NO+surfactant group. For comparison purposes, the treatment effect observed in the NO CLD study in the 7 to 14 d subgroup was much larger: disease-free survival to 36 wk was 25% in the placebo group compared to 44% in the NO-treated group (an absolute difference of 19%). The impact of alternative treatment effect assumptions are shown in section 12.1.e below.

12.1.d. Sample size results. Using these parameter estimates and standard methods for binary outcomes, we calculate that a sample of 524 will provide 80% power in 2-sided tests to detect the proposed increase in 36-week BPD-free survival from 44% to 56.9%.

12.1.e Power and sample size under alternative incidence and effect size assumptions**.** The two tables below show the power and sample size under alternative assumptions about the incidence rate in the placebo arm and the (absolute) increase in the surfactant arm.

Table: Power (n=524) for alternative incidence and effect sizes

	Increase in outcome in surfactant arm					
Incidence in placebo	**6%**	**9%**	**12%**	**13%***	**14%**	**15%**
40%	24.0	48.9	74.2	80.1	86.5	90.9
44%	23.7	48.4	73.9	80	86.4	90.8
48%	23.6	48.5	74.2	80.3	86.8	91.1

Sample size (power=.8) for alternative incidence and effect sizes

	Increase in outcome in surfactant arm					
Incidence in placebo	**6%**	**9%**	**12%**	**13%***	**14%**	**15%**
40%	2320	1050	599	521	443	387
44%	2357	1061	603	524	445	388
48%	2364	1060	599	520	441	384

*Actual percentage 12.9%, rounded to 13%

12.2.General Statistical analysis considerations. An intention-to-treat (ITT) approach [56] will be used, with all randomized infants to be included in the analysis. Tests of the primary hypothesis of the effect

on treatment on BPD-free survival at 36 wk will use a two-sided significance level of 0.0488, to reflect a single interim efficacy analysis using O'Brien-Fleming boundaries. All secondary hypotheses will be tested at the nominal 5% 2-sided significance level, with no formal adjustment for multiple comparisons. Our inference will be focused on the primary outcome. In the analysis of secondary outcomes and in other secondary analyses, we recognize that this might increase type-I error rates, due to multiple testing. To alleviate that concern, we will clearly distinguish primary from secondary endpoints as well as exploratory analyses, look for consistency among multiple endpoints, and be cautious in our interpretation of marginally significant findings, particularly unexpected results not motivated by *a priori* hypotheses**.**

12.2.a. Clustering of outcomes within multiple births. Outcomes in this study will potentially be clustered within multiple-birth sibships. To address this, we will use generalized estimating equations (GEE) models with robust standard [57] for continuous and binary outcomes, treating gestational age stratum as a fixed effect. GEE models are implemented in Proc Genmod in SAS.

12.2.b. Missing data. The extent of missing data will be reported in all statistical analyses. For the primary outcome, the overall rate is expected to be low, since most measurements are to be obtained while the infants are hospitalized, none are invasive, and almost all are part of regular clinical care. Therefore, no specific missing data imputation methods are considered for the baseline data and short-term outcomes. If there are cases with missing data for the primary outcome, they will be considered to have BPD. Covariate adjustment will be used in the analysis of longer-term endpoints to account for between-group differences potentially arising from mortality and loss to follow-up (see below). Additional sensitivity analyses will be undertaken using multiple imputation of the missing outcomes among surviving infants [58].

12,2,c. Analyses restricted to surviving infants remaining in follow-up. Some secondary outcomes, in particular pulmonary status at 1 yr and neurodevelopmental impairment at 2 yr corrected age, will be analyzed only among surviving infants who remain in follow up. To deal with potential confounding of treatment effects by differences among the infants available for re-assessment, we will use regression models to adjust for all important baseline predictors of both mortality and the outcome of direct interest. Beginning with prognostic covariates at least weakly associated with death or loss to follow-up, we will use backward deletion with a liberal retention criterion to select covariates for these models.

12.3 Statistical Analysis Plan: Primary Outcome. The primary analyses will use a GEE logistic model to assess the effects of treatment on the proportion of infants alive and free of BPD at 36 wk. This model will include randomization stratum as a covariate. We expect that BPD-free survival status at 36 weeks will be observed for all infants.

In this analysis, BPD-free survival, is treated as a dichotomous (yes/no) variable. It cannot be treated as a conventional survival outcome, because at randomization, all infants are alive but meet the criteria for possible BPD. Over the 36-wk follow-up, two events might occur: resolution of lung disease or death. The complication is that at 36 wk, treatment failure is defined by censoring of time to resolution of BPD, or occurrence of death, while treatment success is defined by resolution of BPD signs and symptoms, plus censoring of time to death. This is in contrast to usual clinical event endpoint trials (eg., cancer trials) assessing recurrence-free survival, for example, where failure is defined by tumor recurrence *or* death, and success is defined by censoring of *both* failure time outcomes. Because follow-up at 36 wk is expected to be complete, we resolved this difficulty by proposing use of a dichotomous outcome with GEE logistic model for the binary outcome defined as being alive and free of BPD at 36 wk.

12.4 Statistical Analysis Plan: Secondary Analyses Of Primary Outcome

12.4.a. Checking balance by treatment on baseline covariates. We expect randomization to result in well-balanced baseline characteristics, particularly for gestational age and clinic, factors on which the randomization will be stratified. We will assess balance between treatment groups, in terms of age at randomization, gestational age, gender, race, IVH, severity score, surfactant status, previous extubation, and sepsis. On the basis of this assessment, we will perform secondary analyses of treatment effects adjusting for any baseline prognostic characteristics sufficiently imbalanced to confound the treatment effect estimates.

12.4.b. Subgroup analyses. We will perform pre-specified subgroup analyses to examine differences in the effect of treatment across important subgroups as defined by gestational age (<26 wk, ≥26-28 wk), gender, race (white, black, Hispanic, other), severity score at enrollment (split at the median), surfactant status (abnormal vs normal, defined below), and previous extubation. We will adhere to guidelines for subgroup analyses recently published to minimize type 1 errors [59]. These guidelines include, for example, relying primarily on test of interaction for assessment of difference between subgroups and being clear in the reporting of results to clarify that negative results (i.e. no difference in treatment between subgroups) should be interpreted cautiously in light of limited power.

12.5 Statistical Analysis Plan: Secondary Endpoints and Specific Aim 2

12.5 a. 40 wk outcome- discharged home or off respiratory support. This secondary endpoint will be defined as positive if the infant is at home or still in the hospital but off respiratory support at 40 wk. A negative outcome will be defined as death before 40 wk or still in hospital on respiratory support. This outcome will be analyzed using a GEE logistic model controlling for randomization stratum. Discharge or off respiratory support will also be evaluated at 44 wk.

12.5. b. Severity of BPD. The Fine-Gray regression model [60] will be used to assess treatment effects on severity of BPD among affected infants, with severity measured by duration of ventilation, oxygen support, and hospitalization. In analyzing these survival time outcomes, the Fine-Gray model accounts for mortality as a competing risk, as well as censoring of durations for infants still on ventilation or oxygen support, or hospitalized at the end of follow-up. The model is implemented in the *cmprisk* function in R [61].

12.5.c. Pulmonary status at 12 and 20 months of age. Following analyses used in the NO CLD Trial [42], pulmonary status at one year will be assessed in terms of use of bronchodilators, inhaled steroids, systemic steroids, diuretics, and supplemental oxygen. The effect of treatment on these binary endpoints will be assessed using GEE logistic models, adjusting for baseline prognostic covariates. Sensitivity analyses using multiple imputation of these endpoints for surviving children will also be conducted. A parallel analysis will be conducted using the two year follow-up data.

12.5.d. Neurodevelopmental impairment (NDI) at 22 - 26 mo of age. GEE logistic models adjusted for baseline prognostic covariates will be used to assess treatment effects on neurodevelopmental impairment between 22 and 26 months, as measured by the Bayley III assessment. Sensitivity analyses using multiple imputation of these endpoints for surviving children will also be conducted.

12.5.e. Changes in surfactant levels (Specific Aim 2). To assess the effects of treatment on changes from baseline in surfactant levels for Aim 2, we will use linear models adjusting for the baseline level and estimated using GEE with robust standard errors and normalization of the outcomes as appropriate. In addition, we will use GEE logistic models to assess treatment effects on abnormal surfactant composition during the course of the study, controlling for baseline surfactant values. In this analysis, missing follow-up surfactant status will be imputed as abnormal for infants who have not survived, and as normal for those who have been extubated. If the data significantly departs from normality, we will perform the analysis using the log-transformed values. Results from the NO-CLD and pilot studies of late Infasurf treatment indicate that ~50% of infants will have surfactant with low surfactant protein B at study entry.

Results for these endpoints will be clearly presented as secondary, and thus should not affect the interpretation of the p-value for the primary endpoint of the trial.

12.6 Exploratory Analyses. In exploratory analyses we will examine baseline predictors of study outcomes, both overall and within treatment group. For example, we will examine gestational age, gender, and race as predictors of duration of ventilatory support. We will also consider treatment effects of steroid use early in the original hospitalization, and assess mediation of the treatment effect by this co-treatment. Finally, we will assess treatment effects on additional measures at 2 yr, including growth parameters, neurologic status, vision status, pulmonary status (pulse oximetry in room air, continued oxygen utilization, and use of systemic or aerosolized medications).

For Specific Aim 2, as an exploratory analysis, levels of 12 cytokines will initially be assessed on a random sample of 100 infants. We will compare the change from baseline to d 3 and d 6 for treated versus placebo groups For any of these cytokines that show a significant trend, defined as a

difference between treatment groups significant at a p< 0.1, the remaining 400-424 samples will be analyzed. We consider this a reasonable tradeoff between type-I and type-II error in the initial screening. We do not plan to adjust the significance levels for results using all samples, but will cautiously interpret them as hypothesis generating and requiring confirmation.

Because a large number of exploratory analyses will be performed, with the potential to inflate the type-I error rate, the results will be interpreted cautiously, and used to help interpret results for the primary and secondary outcomes or as hypothesis-generating.

13.0 DATA COLLECTION AND MANAGEMENT

13.1 Data Collection. All data will be collected on machine-readable case report forms (CRFs) that are developed specifically for the study. These forms will include screening, enrollment and follow-up data for infants.

The infant's parent or legal guardian must read, understand, and sign an IRB approved informed consent form. Additionally, it should be recorded in the infant's medical record that the infant is involved in this study. The Investigator must retain the signed consent form in a secured location.

Investigators must provide all information required by the protocol on the CRFs provided for the study (see Manual of Operations).

13.2 Data Management. The UCSF Data Coordinating Center (DCC) data system combines decentralized data submission, centralized and remote data editing, and a centralized database structure designed to collect, transfer, and store data for large-scale multi-center clinical studies. Clinical sites submit data to the DCC on machine-readable, validated case report forms (CRFs) via secure fax. After the data have been electronically received by the DCC, they are assessed for quality via automated and manual processes and then written to the study database. Queries (data discrepancies) are generated (hourly during business hours) to identify potential errors in the study data and are immediately accessible via a secure study web site so that the CCC project director (PD) can consult with the onsite study coordinator to resolve them in a timely manner. Original source documents are kept by the site in a secure location for their records.

The study coordinator at each site (or designate) will review all CRFs for completeness, accuracy and consistency prior to faxing into the data system. Should any questions arise during this review, all queries will be referred back to the site investigator for resolution prior to faxing the forms to the DCC.

After forms are submitted to the DCC, they are subject to image verification by a DCC trained data editor and then exported to a SQL database where metadata tables authenticate the data as valid and pre-programmed logic checks look for discrepant or missing data. Data queries are posted to a secure website and addressed by the CCC PD in consultation with the onsite study coordinator. Query resolution that requires a change in how the data form is completed are recorded on the paper form, dated and initialed at the clinical site. The CCC PD is responsible for entering data changes using the data management tools on the secure, password-protected study website. An audit table generated during the editing process contains a complete record of the changes and automatically generates an audit trail.

Components of the web site dedicated to data management operations include the following features:

- data query and edit tables
- missing and rejected forms listing
- data inventory
- audit trail

13.3 Data Audit. After all patients' data have been submitted and data queries addressed, the database will be considered ready to lock. At this time, a random 10% sample of patients will be audited (all data points) for accuracy between the source paper CRF at the clinical site and the study database.

13.4 Data Security and Confidentiality. The DCC employs procedural and technical controls to ensure the security, integrity and confidentiality of subject data that are in compliance with established

standards for Information Technology Security. Multiple levels of data security are in place designed to prevent unauthorized access and limit authorized access to the computer systems and prevent data corruption and loss.

At the procedural level, all DCC personnel sign a confidentiality agreement and undergo security awareness training for HIPAA and sensitive data handling. Remote users also receive training prior to gaining access to the data system. Access to study data within the system is granted on as as-needed basis and further restricted by defined user roles. User authentication is by unique username and password. User accounts are configured to lock out a user after three failed log-in attempts and to log-off after 30 minutes of inactivity. Study website and database access requires a network domain account with appropriate account-specific permissions on the database. All requests for new accounts and access to the database must be documented by a System Access Request Form and signed by the DCC PD. Subject files containing the original CRFs are maintained at the clinical sites. Website communications are encrypted at the 128-bit level using an SSL certificate issued by Verisign (Verisign, Inc, Mountain View, CA).

Computer programs are fully documented tested and subject to change controls in concurrence with FDA-mandated requirements for pharmaceutical studies. Audit trails are automatically generated for both study data and for meta-data (i.e., forms, query logic, data dictionary, and log-on table) housed in SQL databases, which show who made a change, date and time a change was made, description of the data or meta-data item affected, the old and new value, and the reason for the change.

The DCC network is privately maintained, hardware fire-walled and none of the workstations or database servers can be directly addressed from outside the Local Area Network. All study data are stored on SQL servers at the DCC at 185 Berry St., San Francisco. All servers are housed in a new (2005) state-of the-art secure server room. Access to the server room is via a limited access suite occupied by the Information Technology (IT) staff. Both the suite and server room doors are fitted with an Access Control System. Only critical IT staff members are allowed to enter the room. All others who enter the server room (e.g. air conditioning repairman) must be accompanied by a member of the IT staff and their visit is logged. Servers are protected from viruses by Network Associates Netshield 4.x, Groupshield, and VirusScan Enterprise 7.x (McAfee, Santa Clara, CA). This software automatically checks for virus signature file updates from Network Associates' FTP and HTTP sites once an hour. Anti-virus software is monitored and IT personnel are notified in the event that the software stops functioning on a particular server.

Each server is backed-up nightly to disk and mirrored to a "failover" site at the co-location facility at 650 Townsend St., San Francisco. These two sites have copies of the study database and all associated systems required to carry on the study in the event of a disaster in one of the locations. In addition, back up copies of the entire enterprise (databases, user workstations, file servers, etc) are archived in Sacramento, California by Recall, Inc. This will protect the study data in case of a natural disaster affecting the San Francisco Bay Area.

The disclosure of individual health information will comply with local, state, and federal laws and regulations (including the Privacy Rule under the Health Insurance Portability and Accountability Act [HIPAA] of 1996) relating to the privacy, security and confidentiality of health information collected for research purposes. Subject confidentiality will be protected thorough a multi-tiered approach.

13.4.a. Consent. Only subjects with a signed IRB-approved consent form at the clinical center will have their data included in a DCC dataset.

13.4.b Subject identifiers. Subject data submitted to the DCC are identified by a study ID number (screening ID), random acrostic secondary ID, and randomization number. Only the clinical center will have the key that maps the ID number, infant initials and randomization number to the subject's name and contact information at their site. At the clinical sites, all subject data are maintained in locked file cabinet secure locations with limited access by researchers and staff. Laboratory samples will be identified by a study ID number, secondary ID and randomization number. Tracheal aspirate samples will be stored in the laboratory of Philip Ballard MD at UCSF. A list of each sites' subjects will be kept separately in password protected or locked files within that site investigator's office.

13.4.c. Data submission. Data are sent from the clinical sites to the DCC by secure fax to the DCC network.

14.0 DOCUMENTATION AND RECORD RETENTION

14.1 Documentation. Each site must provide the CCC project director or designate with the following documents prior to study initiation. A copy of these documents must be maintained in the investigator's study files.

- IRB approved informed consent form
- All IRB approvals and correspondence (including approved revisions, protocol, advertisements, etc.)
- Copies of all correspondence pertaining to the study (excluding any budgetary matters)
- Copies of all serious adverse events submitted to the IRB
- Copy of all safety reports.

14.2 Record Retention. The clinical site is responsible for maintaining all records (i.e., case report forms, original data, screening logs, signed informed consent forms, correspondence, etc.) until notified, in writing, by CCC, that these records are no longer needed. The Investigator must notify CCC project director if the site or records are relocated, if the investigator leaves the institution, etc. and a new address for the records must be provided.

15.0 INTERIM SUMMARIES AND ANALYSES FOR THE DSMB

15.1 Interim Summaries. The DSMB will be provided with interim summaries of recruitment and demographics, and periodically with summaries of safety. Initially, these reports will be created semi-annually and the frequency will increase or decrease at the discretion of the DSMB. They will summarize data accrual and quality, show whether recruitment is proceeding as scheduled, and whether all sites are recruiting approximately as expected. The reports will include demographics and some baseline characteristics, such as distribution among the two gestational age groups, and birth weight. Safety summaries will consist of adverse events tallies including response to study drug dosing. The safety summaries will be by treatment, labeled as A and B. We will provide a summary individual report for each death, and for any unexpected events. We will summarize study drug procedure interruptions or errors, prematurity-related adverse events while on treatment + 7 days, and unexpected events while on treatment. We will also summarize all other adverse events on those patients followed to outcome and discharge. Percentages and differences by treatment will be provided with confidence intervals. No formal statistical inference will be performed. The summaries will be overall, by gestational age group, sex, race, severity and relationship to treatment as assessed by the site investigator.

The CCC PI will be seeing the overall number of adverse events (not by treatment) in the open session reports and in on-going monitoring activities between DSMB meetings. If it is noticed that an adverse event is occurring at a higher frequency than expected, and this was not apparent at a previous summary for the DSMB, the DCC will forward a summary of the event between reports to the chair of the DSMB, who will determine whether to convene the DSMB and what further information is needed.

15.2 Interim Analysis. Two formal efficacy interim analyses will be performed. The first will be performed when 25% of the data are complete to 36 weeks (131 infants) and the second when approximately 57% (301 enrolled before August 1,2012) of the data have been collected.. The data for the second interim analysis is expected to be available for analysis approximate 26 months after active enrollment start. The interim analysis will address efficacy outcomes of the study and will report p-values. Formal stopping rules for the efficacy outcomes have been established as a critical value of 4.33 and 2.96 at the first and second interim analyses respectively. In order to maintain the overall significance level at 0.05, a small adjustment will be made in the final significance value from 1.96 to 1.9686 to account for the interim analyses. In addition, a futility analysis will be carried out by the DCC and presented to the DSMB with each interim analyses. Additional details of the interim analysis plan and stopping rules are provided in the Data and Safety Monitoring Board Charter.

16.0 PROTOCOL AMENDMENTS

Any changes to the protocol require a written protocol amendment that must be approved by Steering Committee and the DSMB prior to implementation. Amendments that affect patient eligibility, study protocol, or consent changes require additional approval by the IRB at each site. These amendments, should they be required, will become a part of the protocol and maintained by the Investigator as part of the study documentation.

Amendments affecting only administrative aspects of the study do not require formal IRB approval, however the IRB at each of the sites must be informed of such administrative changes.

16.1 Other Changes In Study Conduct. Changes in the study conduct are not permitted. Any unforeseen changes must be recorded in the clinical study report.

17.0 STUDY ORGANIZATION AND ADMINISTRATION

The PI (RAB) will chair an executive committee consisting of Drs. P. Ballard, J. Merrill, R. Keller, D. Black, the NHLBI Project Scientist Carol Blaisdell and the Project Director, (N Newton) that will meet at least monthly to assure smooth operation of all aspects of the study. The PI will also chair a meeting initially monthly and at least quarterly (conference call) of the clinical steering committee, which includes members of the executive committee, appropriate staff from NHLBI as described in the UO1 terms and agreement document, as well as Drs. Truog, , Ryan, Durand, and Eichenwald and the Research Coordinator(J Asselin) to guide clinical aspects of the trial. The study CCC PI will be available on a daily basis for communication with the Associate Directors (JM and RK), the Project Director and the Research Coordinator as was the case in the NO CLD trial. This CCC core group will meet (teleconference as necessary) along with the director of the laboratory component (P Ballard) at least weekly to discuss trial operations and logistical issues as well as to develop recommendations for the steering and/or executive committees.

18.0 POTENTIAL RISKS, BENEFITS AND ALTERNATIVES

18.1 Potential Risks. The main potential risks for this study are the expected risks of surfactant administration. The most common adverse reactions associated with Infasurf® dosing are cyanosis, airway obstruction, bradycardia, reflux of the surfactant in the endotracheal tube, requirement for manual ventilation and reintubation. (see MOP for specific definitions) These events are usually transient and not associated with serious complications. We will minimize this risk by having respiratory therapists trained in surfactant administration present during study drug instillation, standardized instillation and ventilatory management protocols across all study sites, as well as physicians present within the nursery during drug administration, should the infant need to be reintubated. We feel that the risks of surfactant administration to neonates beyond the first week of life are equivalent to those of neonates given routine surfactant in the first 72 h of life, as evidenced by our prior prophylactic pilot trials' safety data. In addition, the studies evaluating surfactant replacement in neonates with early chronic lung disease [19-21] reported improvement in oxygenation, without untoward effects.

All the infants in this study will be receiving iNO therapy modeled after the regimen used in the NO CLD trial. If, for any reason, the clinical team feels that the infant does not tolerate iNO, the gas will be discontinued. The infant will remain in the study and all other study procedures will continue.

18.2 Potential Benefits. Those in the surfactant treatment arm may experience improvement in oxygenation and ventilation allowing a reduction in airway pressures and inspired oxygen concentration after surfactant therapy. There may be no direct benefit from surfactant treatment. The risk/benefit ratio is favorable.

18.3. Alternatives. The alternative is to continue current medical therapy as directed by the infant's medical team. Treatment with iNO according to the NO CLD protocol is accepted practice in many of the sites participating in this study, thus infants may receive iNO outside the study at their attending physician's discretion. Surfactant therapy is not standard of care for infants beyond the first week of life. Surfactant therapy within this study is limited to up to 5 study doses as outlined.

19.0. FINANCIAL CONSIDERATIONS

This is an investigator-initiated study. Infasurf® surfactant will be supplied free of charge by ONY Inc., Amherst, NY. INO will be provided free at no charge by IKARIA Inc. There will be no other financial compensation to subjects for participation.

20.0. DRUG HANDLING AND STORAGE

The Research pharmacist at UCSF will receive the Infasurf and coordinate dispensing the drug to the sites as has been done in the pilot study. Surfactant study drug storage will then be coordinated by the investigational pharmacy services at each participating hospital, and stored as per manufacturer's guidelines.

21.0. FEDERAL OVERSIGHT

This study has been submitted to the FDA for registration and received IND # 79,367 for the combined use of iNO and Infasurf. Dr. Roberta Ballard, UCSF, San Francisco CA is the holder of the IND. The trial has been registered with clinicaltrials.gov and has been assigned Identifier Number NCT 01022580

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23.0 APPENDIX A – CLINICAL SITES: (10.01.12)

1. UCSF Medical Center, San Francisco, CA
2. Children's Hospital and Research Center, Oakland, CA*
3. Alta Bates Summit Medical Center, Berkeley, CA
4. Children's Mercy Hospital, Kansas City, MO*
5. Women and Children's Hospital of Buffalo, Buffalo, NY
6. Children's Memorial Hospital, Chicago,IL
7. Northwestern Memorial Hospital, Chicago, IL
8. Baylor Childrens Hospital, Houston Texas(DROPPED)
9. Stonybrook University Hospital,Stonybrook,NY
10. University of Washington, Seattle,WA
11. UT Houston, Houston TX*
12. Wolfson Childrens, Jacksonville,FL
13. Shands Hospital, Jacksonville, FL
14. Wake Forest Univ – Brenner Hospital, Winston Salem, NC
15. Wake Forest Univ – Forsyth Hospital, Winston Salem, NC
16. CHC Minnesota, St Paul, MN*
17. CHC Minnesota, Minneapolis, MN
18. Medical University of South Carolina, Charleston, SC
19. UT Memphis, Memphis TN
20. University of Minnesota, Minneapolis, MN
21. All Children's Hospital, St Petersburg, FL
22. University of Calgary, Alberta CA = DROPPED
23. Florida Childrens' Hospital(FHC), Orlando FL – NEW SITE
24. Arkansas Children's Hospital , Little Rock, AR new site as of 10/1/12
25. University of Arkansas, Little Rock, AR –NEW site as of 10/1/12

*= 2 hospitals with same PIs involved in one city

24.0 APPENDIX B CLINICAL MANAGEMENT GUIDELINES (DRAFT from Exec committee 1.06.10)

24.1 Guidelines for ventilation & oxygenation

Oxygen saturation limits (until 32 weeks' PMA)

1. Target saturations 85-94%
2. Set saturation monitor limits (depending on individual center saturation targets)
    a. Lower: 80-85%
    b. Upper: 92-95%

Mechanical ventilation guidelines—conventional ventilation

1. Appropriate PEEP to maintain lung inflation. Usually 5-7 cmH_2O.
2. Tidal volume 3-7 mL/kg (usually 4-6)
3. PCO_2 target 45-70 mmHg
4. pH target ≥ 7.15 (most want >7.20)

Suggested criteria for reintubation (until 32 weeks' PMA)
Note: some patients, particularly if unstable or with increased work of breathing may be re-intubated earlier

1. Inability to maintain target saturation despite NCPAP > 8, nasal ventilation or high flow nasal cannula > 3 liters per minute with FIO2 > 0.6
2. PCO_2 consistently > 70 mmHg
3. pH consistently < 7.15
4. Recurrent or severe (requiring bag mask ventilation) apnea despite maximal caffeine therapy and NCPAP

24.2 Guidelines for caffeine citrate use

Caffeine citrate therapy should be considered for infants with gestational age less than 31 weeks, who are less than 10 days of age and require mechanical ventilation or CPAP, for the following indications: apnea prophylaxis, apnea treatment, facilitation of extubation.

Recommended regimens

1. Caffeine citrate IV/PO load 20 mg/kg/dose x1
2. Caffeine citrate maintenance IV/PO 5-10 mg/kg/dose once daily until 32-34 weeks post menstrual age even if infant remains intubated.

Considerations

1. If central apnea occurs / persists on caffeine therapy and other causes of apnea have been ruled out, then caffeine dose may be adjusted by increasing the maintenance dose by 1-3 mg/kg/dose (if HR < 180) to a maximum maintenance dose of 10 mg/kg/dose. In addition, one may also consider an additional caffeine bolus of 10mg/kg. When adjusting caffeine dose, give the bolus immediately regardless of timing of the last maintenance dose. Base timing of subsequent doses from the time the bolus is administered.

1. Schmidt B et al. Caffeine therapy for apnea of prematurity. NEJM 2006; 354:2112-2121.

2. Davis PG, Schmidt B, Roberts RS, Doyle LW, Asztalos E, Haslam R, Sinha S, Tin W; for the Caffeine for Apnea of Prematurity Trial Group. Caffeine for apnea of prematurity trial: benefits may vary in subgroups. J Pediatr. 2009 Nov 17. [Epub ahead of print]

24.3 Guidelines for glucocorticoids for lung disease

Potential candidates for initiation of glucocorticoids—***try to avoid administration!***

1. Infants at least 2 weeks of age
2. Other therapies optimized
3. RSS ≥ 7 (RSS = MAP x FiO_2)
4. No contraindications to glucocorticoid therapy
    a. Indomethacin exposure within 48h
    b. Systemic hypertension
    c. Active infection with < 24 hours of appropriate antibiotics

Regimen guidelines (**short course**)

1. Hydrocortisone (total dose 5 mg/kg)
   Day 1 – 3 mg/kg/d divided q 12h
   Day 2 – 1.5 mg/kg/d divided q 12h
   Day 3 – 0.5 mg/kg/d divided q 12h

2. Dexamethasone (total dose 0.45 mg/kg = HC 6.75-9 mg/kg (15 – 20x))
   Day 1 – 0.2mg/kg/d divided q12h
   Day 2 – 0.15 mg/kg/d divided q12h
   Day 3 – 0.1 mg/kg/d divided q12h

Regimen guidelines (**long course**)

Note: Discontinue or rapid taper (½ dose x 24h, ¼ dose x 24h then off) if no response after 48h. Response defined as ability to wean ventilator and oxygen.

1. Hydrocortisone (total dose 15 mg)
   Day 1-3 – 3 mg/kg/d divided q 12h
   Day 4-6 – 1.5 mg/kg/d divided q 12h
   Day 7-9 – 0.5 mg/kg/d divided q 12h

2. Dexamethasone (total dose 0.89 mg/kg = HC 13.35-17.8 mg/kg (15-20x))
   Day 1-3 – 0.15 mg/kg divided q 12h
   Day 4-6 – 0.1 mg/kg/d divided q 12h
   Day 7-8 – 0.05 mg/kg/d divided q 12h
   Day 9-10 – 0.02 mg/kg/d divided q 12h

Considerations

1. If infant requires surgery while on steroid course, consider stress dose hydrocortisone 2-4 mg/kg/d divided q 6-12h x 24h
2. If infant develops hypotension or other signs of adrenal insufficiency after completion of steroid course, go back on most recent dose x 24h, then taper to ½ dose x 24h and then ¼ dose x 24h
3. May repeat short course at 7-10d if infant meets criteria

24.4 **Guidelines for glucocorticoids for hypotension**

Potential candidates for initiation of glucocorticoids for hypotension

1. Inadequate response to vasopressor therapy (dopamine ≥ 20 mcg/kg/min ± dobutamine or epinephrine) with *either*
    a. persistent hypotension despite fluid resuscitation, or
    b. inability to wean medications for > 48h
2. No contraindications to glucocorticoid therapy
    a. Indomethacin exposure within 48h
    b. Active infection with < 24 hours of appropriate antibiotics

Regimen guidelines

1. Hydrocortisone (total dose 5 mg/kg). Some infants may respond to initial 1-2 doses, making further dosing unnecessary.
   Day 1 – 1-2 mg/kg/d divided q 8-12h
   Day 2 – 1 mg/kg/d divided q 8-12h
   Day 3 – 0.5 mg/kg/d divided q 12h

Considerations

1. If infant requires surgery while on steroid course, consider stress dose hydrocortisone 2-4 mg/kg/d divided q 6-12h x 24h
2. If infant develops hypotension or other signs of adrenal insufficiency after completion of steroid course, go back on most recent dose x 24h, then taper to ½ dose x 24h and then ¼ dose x 24h, then discontinue.

24.5 **Guidelines for Vitamin A therapy (if being used)**

Candidates for initiation of Vitamin A

1. All infants < 1000 g birth weight
2. Infants 1000-1250 g birth weight if ventilated > 24h

Dosing regimen

5000 Units IM every M, W, F x 4 weeks
May be discontinued prior to 4 weeks of treatment if infant reaches full enteral feeds (150 mL/kg of premature formula or fortified breast milk or 120 mL/kg of premature formula with 1 mL/d Poly-Vi-Sol)

Vitamin A use should be consistent among <u>all</u> infants at any site.