Steroids and Surfactant in Extremely Low Gestation Age Infants (**SASSIE**)

Pilot Dose Escalation Trial

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1. Objectives

The overall goal of this protocol is to determine the optimal dose and frequency of treatment with budesonide suspended in surfactant and administered intratracheally to effectively and safely treat intubated extremely low gestational age newborn (ELGAN, <28 weeks of gestational age) infants at risk for bronchopulmonary dysplasia (BPD).[1;2]

We hypothesize that an appropriate dose of budesonide delivered directly to the lung suspended in the surfactant calfactant (Infasurf) will be effective and safe in improving the infant's respiratory status, as demonstrated by an improvement in respiratory assessments at up to 28 days of age, and decreased inflammatory response (a critical contributor to BPD in ventilated preterm infants).

This is a dose escalation study of budesonide suspended in calfactant as the vehicle into the infant's lungs. Measures of drug efficacy will include standardized clinical assessments to 28 days of age and cytokine levels in tracheal aspirate. Dose-dependent safety will be measured by monitoring adverse events potentially attributable to study treatment and budesonide systemic pharmacokinetics.

Specific Aim 1/Primary Objective:
To determine the clinical and anti-inflammatory efficacy of escalating doses of budesonide suspended in calfactant and administered into the lungs of ELGANs by monitoring the infant's clinical outcome up to 28 days of age, including the Respiratory Severity Score (RSS) [3] and measuring tracheal aspirate cytokine levels.[1;2]

Dose escalation will be defined as effective at the dose level in which 5 of 8 infants achieve:

- At least 1 one of the following:
    1) Extubation within 72 hours of first dose or after $\leq$ 3 doses-without re-intubation before 28 days of age
    2) RSS on nasal continuous positive airway pressure (NCPAP) < 1.5 or on nasal cannula Fi02 <25% at $\leq$ 2L/min at 28 days of age, persisting for at least 72 hours.
    3) Cumulative supplemental oxygen < 4.2 from time of enrollment to 28 days of age[4] (see calculation below)
    4) No respiratory support at 28 days, including no supplemental oxygen by nasal cannula
- AND a $\geq$ 50% suppression of the tracheal aspirate interleukin-8 (IL-8) or CCL2 (chemokine ligand 2) at 24-72 hours (or prior to extubation if occurs at <24 hours after dosing) after the initial dose in 5 of 8 of the infants (this may be a different combination of 5 patients than those who met the above criteria).

Surfactant alone has been shown to transiently decrease RSS by 10%,[5] so a sustained decrease in RSS would indicate budesonide effects. Data from human fetal lung explant studies from Dr. Phil Ballard's laboratory[6] (see section 2d below) demonstrates that budesonide reversibly suppresses a variety of inflammatory markers from tracheal aspirates and that IL-8 and CCL2 are useful biomarkers for in vivo glucocorticoid anti-inflammatory effects. In fetal human lung explants, 30nM Budesonide decreased IL8 and CCL2 by ≥75% within the 24 hours of dosing. Data from a matched historical control group from the Trial of Late SURFactant (TOLSURF)[3] will be used to evaluate the clinical response to

administration of calfactant alone over the first 28 days as well as changes in cytokines with treatment in banked tracheal aspirate samples. Secondary analysis[4] of data from the TOLSURF study demonstrated that a cumulative supplemental oxygen (average daily FiO_2-0.21 x number of days being monitored) of > 4.2 from day of life 5 to 28 had a 70% specificity for the development of BPD and/or death. As long as there are no safety concerns, dose escalation will be increased by one more dosing level above this defined effective dosing level to assess for any potential further benefits in terms of respiratory status at 28 days of age and/or cytokine suppression.

Secondary Specific Aims/Secondary Objectives:

1. Safety Objectives:

Safety assessments will include the subject's vital signs, clinical laboratory testing, morbidities associated with prematurity, morbidities associated with administration of budesonide in calfactant, and Adverse Events (AE)s. Clinical parameters/ AEs of interest are those potentially consistent with elevated glucocorticoid levels and will be specifically evaluated. A Data and Safety Monitoring Board (DSMB) will be established to review safety data. The incidence of morbidities will be compared with the incidence of these morbidities reported in the TOLSURF study[3] due to the similarities in these populations.

2. Serial Budesonide Levels:

We will obtain dried blood spot samples (DBS; about 0.2 mL per sample)[7] at 15 minutes, and 1 and 4 hours after the first dose of budesonide in calfactant for budesonide levels. This is a total of 0.6 mL per patient or 1.2 mL/kg in a 500 gram newborn. Trough concentrations (Cmin)will be drawn before potential subsequent daily doses for a potential total of 7 total DBS or a total of 1.4 mL (2.8 mL/kg for a 500 gram baby over a five day period which does not represent an excessive blood loss). Analysis of the DBS and pharmacokinetic modeling are being performed within the Department of Pharmacology/ Toxicology at the University of Utah School of Medicine. Based on preliminary data in lambs,[8] we could expect a Cmin of 1.9 ng/mL at 9 hours post-dosing. Non-compartmental and population pharmacokinetic analyses will be performed to establish the extent of budesonide exposure. Commonly reported pharmacokinetic parameters will be calculated including: clearance, volume of distribution, area under the curve (AUC), half-life, peak concentration (Cmax), and trough concentration (Cmin).

3. Clinical Respiratory Outcomes:

Clinical respiratory status (RSS and oxygen requirement) at 28 days of age will be compared between dosing groups and also compared to the matched historical control patients from the TOLSURF study.[3] The individual patient's decrease in RSS will also be correlated with their budesonide exposure measures by the area under the concentration time curve (AUC).

4. Exploratory Measurements (Pulmonary Function Tests, Other Anti-Inflammatory Biomarkers, and Airway Microbiome Analysis)

Pulmonary function tests will be completed on subjects born at Oregon Health and Science University (OHSU) prior to the first dose of study medication, 24 hours after the first dose, prior to extubation and then at discharge at 34-36 weeks of post menstrual age. Passive respiratory compliance (Crs) and resistance (Rrs) will be measured with the single breath occlusion technique and functional residual capacity (FRC) with the nitrogen washout technique as previously described. [9-12] International criteria for testing will be followed to ensure testing quality.[9-12]

Once the dose of budesonide is identified that effectively suppresses IL8 and CCL2 , a limited set of tracheal aspirates (from infants treated with that dose) will be sent to Dr. Ambalavanan's laboratory at the University of Alabama for multiplex analysis of up to 32 cytokines of interest in the development of BPD[13] as well as airway microbiome analysis to assess potential modulation of the mucosal microbiome by glucocorticoids.[14] A limited set of tracheal aspirates will also be sent to Dr. Ambalavanan's laboratory after treatment of the first several patients for pilot analysis of the 32 cytokines of interest.

2. Background

a. Epidemiology and pathogenesis

Extremely low gestational age newborn (ELGAN, born at < 28weeks gestation) infants are at risk for BPD, a chronic lung disease that is associated with mortality, ongoing pulmonary morbidity, including asthma, and neurodevelopmental abnormalities.[15] BPD occurs in >70% of ELGANs who require ventilatory support after 3 days of postnatal life.[16;17]

BPD is the most common morbidity of ELGANs and despite significant focused research its incidence has remained largely unchanged for the last 20 years.[15] BPD develops in 10,000-15,000 infants in the United States per year and impacts the infant's pulmonary and overall health as well as the financial and emotional well-being of the family.[18] BPD is associated with increased respiratory diseases into childhood, recurrent hospitalizations, poorer neurodevelopmental outcomes, and importantly *lifelong alterations in lung function.*[19]

The pathogenesis of BPD is multi-factorial including intrauterine and extrauterine inflammation as well as hypoxia, hyperoxia, ventilator trauma and/or infection after birth in susceptible infants with immature lungs.[20] Inflammation is a common pathway of many factors contributing to the genesis and evolution of BPD. [21] *Despite much research, as outlined below, there are few therapies that have been shown to decrease BPD*. Because increased synthesis of proinflammatory cytokines is an early event associated with BPD, treatment to reduce inflammation and these cytokines has a good chance of reducing BPD.[22]

b. Current therapeutic approaches to attempt to prevent/decrease BPD

Randomized controlled trials (RCTs) to decrease BPD have included studies of pharmacologic treatments, respiratory care practices, fluid and nutritional therapies, and evidence-based standardized bundled practices. Three medications have been shown to prevent / decrease BPD: Caffeine, vitamin A and corticosteroids given during the first week of life.[23] The numbers needed to treat are approximately 10 (95% CI 7-16) for caffeine and 12 (95% CI 6-94) for vitamin A.[23] Prophylactic use of systemic corticosteroids during the first week of life is no longer recommended because of potential harmful effects that include intestinal perforation and increased incidence of cerebral palsy.[24] Both antenatal steroids and postnatal surfactant therapy decrease respiratory distress syndrome and death, but have not been shown to decrease BPD in survivors.[20] Inhaled nitric oxide (iNO) did not decrease BPD in an individual patient meta-analysis of 3,298 premature infants.[25] However, iNO did significantly reduce BPD in African Americans in a second individual participant data meta-analysis. Several pharmacologic trials proved ineffective in preventing BPD including: administration of superoxide dismutase, glutathione, cimetidine, and macrolide antibiotics.[15]

Many RCTs of modified respiratory support in very low birth weight (VLBW) infants have been performed with limited success. Several large trials[26-28] randomized VLBW infants to intubation

versus continuous positive airway pressure (CPAP) in the delivery room, but a significant decrease in BPD was not been clearly demonstrated. Some trials suggest that permissive hypercapnia may decrease BPD, but a RCT of early permissive hypercapnia in 220 patients was negative.[29] Trials comparing different modes of ventilation have also been largely negative, including a meta-analysis of independent individual patient data comparing high frequency oscillatory versus conventional ventilation.[30] Nutritional studies for BPD prevention, including randomization to aggressive versus conventional parenteral nutrition, early lipid administration, and restricted versus liberal water administration have been negative. Studies using implementation science methodologies of best practices have reported decreased BPD in single centers. However, a study with seven intervention centers using a cluster, randomized, controlled design showed no difference in BPD.[31]

Despite strong evidence implicating inflammation in BPD causation, strategies directed at modulating inflammation for the prevention of BPD have been limited or linked with significant adverse effects. Antenatal steroid use may modulate the severity of BPD, yet direct evidence for preventing BPD is lacking.[20] The potential role for other anti-inflammatory agents, such as azithromycin,[32] remains unproven, and the use of selective anti-cytokine therapies remains unstudied in this population. Preclinical studies have implicated the regulation of nuclear factor kappa B (NFKB) as a key determinant of the inflammatory response; however, its role supporting normal lung development may limit NFKB as a potential target in the preterm infant.[22] Mesechymal stem cell (MSC) therapy has been shown to prevent experimental BPD in rodents, and has potent anti-inflammatory properties in addition to its growth-promoting effects in the lung. Whether MSC or conditioned media from MSC may provide a novel and effective anti-inflammatory strategy for the prevention of BPD is unknown.[22]

The use of postnatal systemic corticosteroids (primarily dexamethasone) has a long but controversial history of use in treating ventilator dependent infants at high risk for developing BPD.[33-35] Postnatal use of dexamethasone became routine after the first reports of its benefits in the mid 1980's and practice evolved with infants being treated prophylactically with longer and higher doses starting as early as day one of life.[36] This practice stopped abruptly after a meta-analysis of controlled trials demonstrated an increased risk of cerebral palsy after systemic corticosteroids.[24] Unfortunately no distinction was made between targeted use of steroids in patients at risk for BPD and indiscriminant or prophylactic use. No studies analyzed the pharmacokinetics of dexamethasone in a systematic manner to determine what exposure was related to AE's. The subsequent statement from the American Academy of Pediatrics strongly recommended against systemic postnatal steroids outside of randomized clinical trials and this statement in itself contributed to the early stopping of an ongoing RCT of postnatal dexamethasone whose primary outcome was neurodevelopmental outcomes.[37] Several studies have now shown that coincident with the overall decreased use of postnatal steroids, the incidence of BPD is increasing.[38]Also, a meta-regression analysis demonstrated that if an infant's risk of BPD is >55%, the use of postnatal systemic steroids will decrease the risk of poor neurodevelopmental outcome. [39] The revised AAP statement is now more tolerant of targeted use of systemic corticosteroids deferring to the clinician to use clinical judgement with regard to potential benefits and risks in ventilator dependent infants at risk for BPD. [40]

c. Pilot studies/potential role of budesonide in surfactant

Given the limited number of therapies shown to effectively decrease BPD and the importance of inflammation in its pathogenesis, it is critical to find a method to administer glucocorticoids that will target their anti-inflammatory effects to the lung and minimize systemic effects. Budesonide is a glucocorticoid with strong local anti-inflammatory effects and limited transfer to the systemic circulation when given intratracheally with surfactant as the vehicle. Two published randomized trials [1;2] of about 380 patients have reported that intratracheal installation of budesonide combined with surfactant as the vehicle for distribution into the lungs significantly improved respiratory status and decreased the incidence of BPD or death in preterm infants without adverse side effects. Both studies reported no differences in the incidence of neuromotor dysfunction, mental or psychomotor developmental indexes, or physical growth parameters at follow-up at 2-3 years of age.[1;2;41] *The most recent study was accompanied by an editorial by highly respected neonatologists discussing the great promise of this therapy to prevent BPD.*[42]

The premise of using surfactant as a vehicle to deliver budesonide is based on surfactant's ability to generate a convection flow that can be used to deliver medications to the lung periphery.[43] Recent in vitro studies in Dr. Ballard's (co-investigator on present study) lab have shown supplementation of calfactant with budesonide did not alter either surface tension properties[6] (Table 1) and improved adsorption to the air-liquid interface.[44] (Figure 1) This is similar to findings from Yeh et al[2] who demonstrated that Survanta (100 mg or 4 mL/kg) mixed with budesonide (0.25 mg or 1 mL/kg) did not affect the biophysical and chemical properties of the surfactant.

Table 1. *In vitro* surface tension properties of calfactant supplemented with budesonide

Time (h)	STmin[a] (mN/m)	Time to STmin[a] (sec)
0	0.3 ± 0.7	10.5 ± 1.6
24	1.6 ± 0.3*	12.7 ± 3.3
48	1.2 ± 0.5*	12.6 ± 2.4

All values are within the normal range for unsupplemented Infasurf.;[a]STmin = minimum surface tension ; Values represent means ± SD (n=6 for time 0 and n=12 for 24 and 48 h exposure at room temperature).*p<0.01 vs time 0

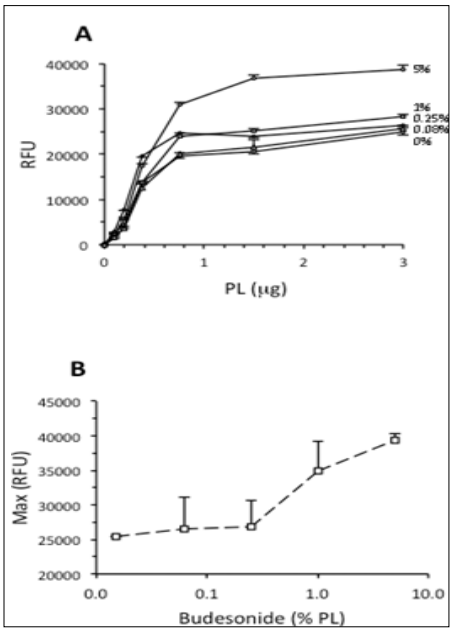

Figure 1 . Budesonide addition to calfactant:effect on adsorption. *A.Representative PL dose-response experiment for calfactant supplemented with budesonide 0-5% showing higher Max but similar ½Max with 5% budesonide (mean±SEM for triplicates). B. Budesonide dose-response curve for Max film formation at 1.5 µg PL. Data are mean± SEM for 4 experiments. The multimodal curve suggests a threshold beneficial effect between 1 and 5% budesonide.*[6]

d. Anti-Inflammatory Effects of Budesonide in Human Fetal Lung Explants Dr. Ballard's laboratory has completed a study of budesonide responses in fetal lung explants.[6] Explants of second trimester human fetal lung were cultured with or without dexamethasone, budesonide, and in the presence and absence of calfactant. Tissue and media were collected for LC/MS/MS, microscopy, assay of IL-8 and CCL2 cytokines by Western blotting and ELISA, and RNA analysis. Budesonide at 30 nM suppressed

medium levels of both cytokines by up to 80% at 12 hours and suppression was reversible on removal of steroid
(Figure 2). Results for cytokine suppression were similar for budesonide suspended in calfactant. In dose-response experiments, half-maximal suppression occurred at 0.04±0.02 and 0.05±0.02 nM for IL-8 and CCL2, respectively, consistent with the reported high potency of budesonide in other systems. Using RNAseq analysis, he identified 230 genes, including IL-8 and CCL2, that were decreased by >50% with a False Discovery Rate of <0.05 (data not shown due to space limitation), indicating that budesonide down-regulates a network of inflammatory mediators in lung tissue acting at the level of transcription. In studies using LC/MS/MS of explant tissue and culture medium,levels of the major budesonide metabolite (16α-hydroxyprednisolone) was <5% of the budesonide level. Thus, most metabolism of budesonide in vivo occurs in non-pulmonary tissues, primarily the liver.

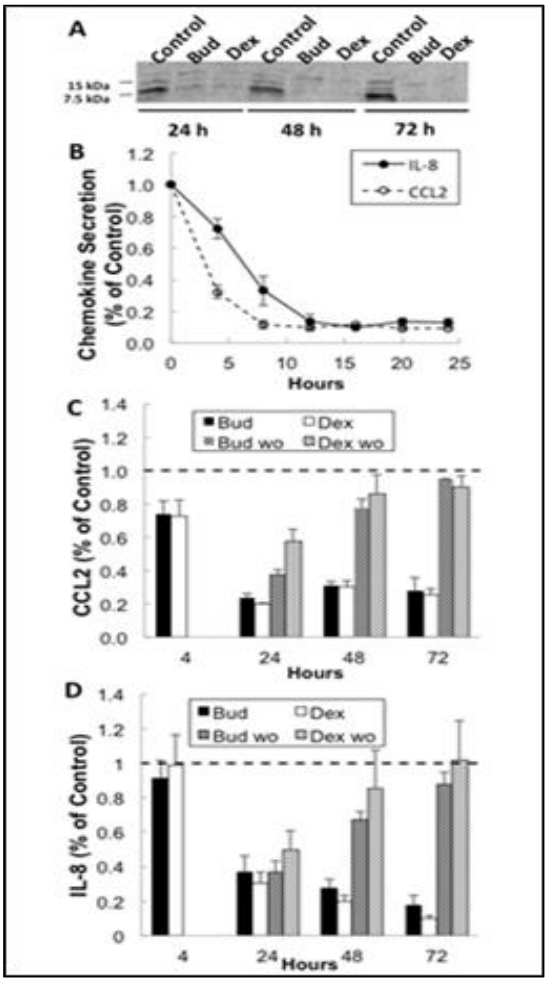

Figure 2: *Chemokine release from human fetal lung explants cultured with budesonide or dexamethasone is maximally decreased by 24 hours and reversed by 72 hours. (A) Representative CCL2 Western blot on explant culture media ± 30 nM budesonide or 30 nM dexamethasone collected every 24 hours (CCL2 N.D. in tissue, IL-8 N.D. by Western in media or tissue). (B) IL-8 and CCL2 levels in explant culture media as measured by ELISA reduced by up to 90% of control levels within 12 – 16 hours and 8 – 12 hours respectively (n=4, 30 nM budesonide). (C,D) Washout experiments exposed human fetal lung explants to 3 nM budesonide, 3 nM dexamethasone, or control media for 4 hours, followed by a saline rinse and replacement with steroid- and serum-free control media changed every 24 hours out to 96 hours. Levels of (C) CCL2 and (D) IL-8 were measured by ELISA in media collected every 24 hours. Explants cultured in budesonide or dexamethasone but not washed out were included for comparison. For CCL2, the differences between washout levels compared to control levels were statistically significant at all time points while IL-8 levels were significantly different at 48 and 72 hours. Dashed line indicates control levels. (wo = washout).*[6]

In preparation for this planned dose escalation trial, investigators from our group recently conducted a pharmacokinetic investigation of a combination budesonide and surfactant mixture that was instilled into the lungs of 7 preterm lambs.[8] All of the animals received 0.25 mg/kg budesonide with surfactant as the vehicle, which is the same dose used in the above trials by Yeh et al.[1;2] Plasma and tissue samples were obtained and assayed to quantify budesonide, 16α-hydroxyprednisolone, and budesonide palmitate concentrations using LC/MS/MS. Peak plasma concentrations were found to be inversely correlated with the oxygenation index (correlation coefficient of -0.75). Plasma budesonide concentrations were extremely low (~10% of expected) for two lambs that had high oxygenation indices and were excluded from further analyses. For the remaining 5 preterm lambs, a non-compartmental analysis demonstrated a mean AUC_{inf} of 148.8 (SD ± 28.2) ng*hr/mL, a half-life of 4.8 ± 1.8 hrs, and a peak concentration of 46.2 ± 17.7 ng/mL. Using population pharmacokinetic methods, a one-compartment model with an exponential residual error model and first-order absorption adequately described the data.[8] The apparent clearance and volume of distribution were estimated at 6.3 L/hr and 29.1 L, respectively.

Budesonide and budesonide palmitate were unable to be detected in the brain. *This finding suggests that intratracheal instillation results in local pulmonary deposition, with no evidence of budesonide accumulation in the central nervous system.* Overall, these results revealed that peak plasma budesonide concentrations are inversely correlated with the oxygenation index and that lung-specific delivery avoids budesonide accumulation in the brain.[8]

In the initial study by Yeh et al.,[1] 10 of the preterm infants treated intratracheally with 0.25 mg/kg budesonide mixed with surfactant had venous blood samples collected at 30 min, 1, 2, 4, and 8 hrs post-dosing to permit a pharmacokinetic analysis. The mean budesonide concentration observed at 30 mins was 20.9 (SD ± 14.4) ng/mL. The mean budesonide concentration measured at 8 hrs was 9.9 ± 8.2 ng/mL. Over the first 8 hrs following dosing, the mean AUC was calculated to be 115.7 ng*hr/mL. Of note, there was one neonate that had budesonide concentrations measured that were an order of magnitude lower than those observed among his/her peers, which may reflect insufficient lung recruitment. Yeh et al.[2] also demonstrated significant decreases in IL-8 in tracheal aspirates at 12 hour, 3-5 days, and 7-8 days after treatment.

A dose escalation study is needed to provide critical data about the most efficacious and safest dose for reducing BPD in premature infants, hence the design of a multicenter RCT to evaluate this potential therapy for BPD.

3. Study Design

This is a phase I/II open-label study to determine the lowest, safe, effective dose of budesonide given with calfactant as the vehicle; we will perform an unblinded dose escalation study. We will administer four dosing levels of budesonide suspended in calfactant beginning with 0.025 mg/kg of budesonide (10% of the dose used in the studies by Yeh et al.[1;2]) administered to 8 ELGANs who are intubated at 3-14 days of age. Daily doses (at the same dosage) will be administered to infants who remain intubated for a potential of 5 total doses in each patient. Subsequent groups of 8 infants each will receive 0.05 mg/kg, 0.10 mg/kg, and 0.15 mg/kg **or** 0.20 mg/kg of budesonide in calfactant. Each patient will receive up to 5 total doses, for a total of up to 32 infants in the entire trial. We will evaluate the clinical, laboratory and safety data from each group of treated infants to 28 days of age before moving to the next dosing level of budesonide.

Control Group

Case-matched premature infants who received calfactant alone in the Trial of Late SURFactant (TOLSURF)[3] will serve the comparison group using banked samples of tracheal aspirates.

4. Study Population

a. Number of Subjects

The potential study sample size is 32 total patients with 8 patients per dosing level. Each site will enroll approximately 2-3 subjects per dosing level, though there is no minimum/maximum number of subjects per site.

b. Inclusion and Exclusion Criteria

Inclusion Criteria

a. $23^{0}/_{7}$ to $27^{6}/_{7}$ weeks, inclusive, of gestational age based on center's best estimate of due date (using earliest obstetrical ultrasound, last menstrual period, examination, and other pertinent available information)
b. Day of life 3-14 from the date and time of delivery, with the date of birth being DOL 0
c. Intubated and mechanically ventilated and do not anticipate extubation in next 24 hours

Exclusion Criteria

a. Serious congenital malformations or chromosomal abnormality (as described in the manual of operating procedures)
b. Likely to be extubated in next 24 hours
c. Clinically unstable (see criteria below)
d. Infants who have received systemic steroids prior to dosing with study medication.
e. Infants who have received Indocin, Ibuprofen, or acetaminophen ≤ 96 hours prior to enrollment window ending

Definition of Clinically Unstable Infant:

1. Active Pneumothorax with chest tube
2. Active Pulmonary Hemorrhage
3. Uncontrolled hypotension requiring more than 20 mcg/kg/min dopamine, 2 inotropic agents, or > 30 mL/kg of fluid boluses
4. Acute NEC – less than 24 hours from diagnosis or surgery or isolated intestinal perforation
5. Untreated culture positive sepsis (<6h)
6. RSS >14
7. Uncontrolled hyperglycemia
8. Clinical team feels infant would not tolerate dosing procedure

Note: If patient becomes unstable during the period of dosing administration, study drug administration will be discontinued. If patient stabilizes again within 14 days of life, dosing may be continued, up to 5 total doses per subject.

If a subject is consented into the study but NOT dosed (due to instability, extubation, withdrawal of consent, or any reason), another subject may be consented to replace the subject that did not get dosed. If a patient gets treated for a PDA in the midst of SASSIE study dosing, further SASSIE doses will be discontinued and patient may be replaced for study purposes.

Data collected on the pre-screening log and during the screening visit in the event of a screen failure will be retained for separate analysis.

c. Vulnerable Populations

Neonates will be included in this research. The inclusion of neonates is important to the research because they are at risk for BPD and associated morbidities. Parent or guardian

permission will be obtained before the child will be enrolled in the study, as per respective IRB guidelines.

d. Setting

The study is an open label dose escalation trial being conducted at 4 clinical locations: Oregon Health and Science University in Portland Oregon (PI: Cindy McEvoy, MD), University of Florida in Jacksonville, Florida and Wolfson Children's Hospital in Jacksonville, (PI: Mark Hudak, MD), Florida Hospital for Children in Orlando, Florida (PI: Rajan Wadhawan, MD) and University of California in San Francisco, California (PI: Roberta Keller, MD). Each PI will be responsible for the conduct of research at their institution. Each institution will submit to their respective IRB.

e. Recruitment Methods

Subjects will be recruited from eligible inpatients within the Neonatal Intensive Care Units at the 4 sites listed above. To facilitate timely enrollment in the trial, infants < 28 $^{0}/_{7}$ weeks gestational age will be screened at age 1-3 days of age by the study physicians, research personnel, and/or nurses for eligibility. A pre-screening log will be kept. Records of potential candidates for the study will then be reviewed by one of the study investigators and/or study coordinator, and the child's condition discussed with the attending physician. If eligible, the infant's parents will be approached by study personnel to potentially obtain informed consent. This will occur in preterm infants preferably within the first 3 days of life with the expectation that if the infant meets criteria between 3 and 14 days of age, he/she will be enrolled at that time. Subjects will not be reimbursed for participation in the study.

f. Consent Process

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

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

The informed consent process will be an ongoing active process of sharing information between the investigator and the parent/legal guardian(s). If a protocol change requires a change to the consent form, parents/legal guardian(s) will be notified in a timely manner and the new informed consent form will be signed.

Assent will not be obtained as the subjects in this study are neonates.

Permission may be obtained from legally authorized representatives, described as an individual who is authorized under applicable state or local law to consent on behalf of a child to general medical care when general medical care includes participation in research.

5. Procedures Involved

a. Study medication dosing

Subjects will be dosed with budesonide given in calfactant as per the dose escalation plan. There are four dosing levels: 0.025 mg/kg, 0.050 mg/kg, 0.10 mg/kg, and 0.15 mg/kg or 0.20 mg/kg of budesonide given with calfactant as the vehicle. The calfactant dose is as recommended by the manufacturer for newborn therapy (3 ml/kg). The final product will be compounded according to the following formula:

Table 2a. Dosage Preparation of Budesonide and Calfactant (3 mLvial) for 1 kg infant

Bud Dose (mg/kg)	Bud Aliquot (ml/3 ml Calfactant)	Final Volume (ml)	Mixture Dose (ml/kg)
0.025	0.05	3.05	3.05
0.05	0.1	3.1	3.1
0.10	0.2	3.2	3.2
0.15	0.3	3.3	3.3
0.20	0.4	3.4	3.4

Table 2b. Dosage Preparation of Budesonide and Calfactant (6 mL vial) for 1 kg infant

Bud Dose (mg/kg)	Bud Aliquot (ml Budesonide /6 ml Calfactant)	Final Volume (ml)	Mixture Dose (ml/kg)
0.025	0.10	6.10	3.05
0.05	0.2	6.2	3.1
0.10	0.4	6.4	3.2
0.15	0.6	6.6	3.3
0.20	0.8	6.8	3.4

Budesonide (Pulmicort Respule 1.0 mg/2 ml); Calfactant 35 mg/ml, 3 ml/kg dose.
Add indicated aliquot of budesonide to 3 ml or 6 mL of calfactant, gently mix, pull mixture into syringe at dose/kg infant weight as shown. Note: dosing level 4 will be either 0.15 mg/kg or 0.20 mg/kg based on effectiveness of previous doses.

The research pharmacy at the respective institutions will prepare the combination of the appropriate dose of budesonide (Pulmicort nebulizing suspension, Astra Zeneca, Sweden) suspended in calfactant (Infasurf ONY INC) as per the dose escalation protocol. Under sterile conditions, the appropriate amount of budesonide will be added to the 3 or 6 ml vial of calfactant to provide the desired drug concentration. The contents will be mixed by gentle inversion of the syringe (vial) to avoid excessive bubble formation. Because both budesonide and calfactant are suspensions of lipophilic substances, effective mixing is expected to occur rapidly. The budesonide/ calfactant mixture will be prepared in the research pharmacy shortly before it is sent to the NICU for instillation. If there is an unexpected delay in instillation, the vial will be stored at 5°C for no more than 24 hours.

The study medication will be administered using standard procedures and precautions as for newborn treatment. Study drug is administered via skilled personnel with care taken to assure adequate lung inflation during the instillation based on our experience with preterm lambs.[8] 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. Vital signs, saturations, respiratory 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 over distention and oxygen exposure. Up to 5 total doses will be given at daily intervals, with dosing no sooner than 18 hours post last dose.

If the infant remains intubated 18 to 24 hours after the 1st study dosing with no plan for extubation in next 24-48 hours, a second dose of the same dosage of budesonide will be given. Up to 5 total doses may be given daily if the infant remains intubated. Retreatment intervals may change after analysis of data from the first 8 patients in terms of respiratory status and cytokine suppression after dosing. If the infant is considered clinically unstable by the physicians at the time when a dose should be given, but later stabilizes during the study eligibility period (up to 14 DOL), the infant will continue with study drug dosing as per the dosing schedule outlined above, and is still eligible for 5 total study doses after study dosing resumes.

Patients who are extubated within 24 hours of a study dosing will not receive subsequent study medication dosing. However, if an infant is re-intubated up to 14 days of life, they can restart study dosing again at the next appropriate dose and receive a total of 5 doses. Study sampling will occur according to the study procedure chart (see chart below on subsequent pages).

If the attending physician deems that the infant would benefit from intravenous or oral steroids during the 5 days of study dosing, the following must be followed: the site PI should be notified promptly, and the site PI, in turn, will report this development to the study PI, Dr. McEvoy within 72 hours. All further dosing with the study dosing protocol will be discontinued; data and sample collection and AE reporting on the infants should continue as per study protocol.

If an eligible patient is treated with Indocin, ibuprofen, or acetaminophen, enrollment for the infant will be delayed by 96 hours after the last dose. If the attending physician feels that the infant requires Indocin, ibuprofen, or acetaminophen for the treatment of a clinically significant PDA (as agreed upon via the clinical guidelines) during the 5 days of dosing, study medication administration will cease. An attempt will be made to replace the infant in the study with another for purposes of the study. In addition there should be an attempt to avoid the use of Indocin or Ibuprofen until > 48 hours after last study treatment dosing

Study dosing will be stopped if there are any safety concerns deemed significant or potentially significant by the Medical Monitor. The Medical Monitor will review SAEs and any other concerns/questions reported by the sites in detail and will consult with the DSMB, site PIs, and/or study PI as needed.

If the first 3 dose levels do not meet the criteria for effectiveness as outlined in Specific Aim 1, the final dose level (level 4) will be increased from 0.15mg/kg to 0.2mg/kg. If dose level 3 does meet criteria for effectiveness, the final dose level (level 4) will remain at 0.15mg/kg.

b. Tracheal Aspirate

Lung fluid from the infants breathing tube will be collected and tested for markers of inflammation. Tracheal aspirate (TA) samples are collected before each of the maximum of five (5) doses of budesonide: calfactant. TA's will also be drawn 12 ± 2 hours (10-14 hours) after the first dose and 24 ± 2 (22-26 hours) and 48 ± 2 hours (46-50 hours) after the fifth dose (if infant is still intubated). If extubation is planned sooner than 24 hours after any dose, a TA sample should be obtained during routine airway preparation procedures just prior to extubation. A maximum of 8 TA's can be collected if an infant receives all 5 doses of study drug and remains intubated more than 24 hours after the fifth dose.

Each tracheal aspirate sample will be centrifuged within 20 minutes of collection to provide a cell pellet and a supernatant fraction. After freezing and batch shipment to UCSF, the supernatant is centrifuged and assayed for total protein and for selected cytokines using ELISA. The TA cell pellet is used for quantitation of DNA to determine total cell number. All tracheal aspirate samples are labeled by study ID number and date/time, without other identifiers. Analyses of data from study samples as related to clinical parameters is performed by P Ballard with assistance of project manager Jeanette Asselin.

Further exploratory analyses will be done as follows. Once the dose of budesonide is identified that effectively suppresses IL8 and CCL2 , a limited set of TAs (from infants treated with that dose)will be sent to Dr. Ambalavanan's laboratory at the University of Alabama for multiplex analysis of up to 32 cytokines of interest in the development of BPD[13] as well as airway microbiome analysis to assess potential modulation of the mucosal microbiome by glucocorticoids.[14] Again, all tracheal aspirate samples are labeled by study ID number and date/time, without other identifiers.

c. Plasma Samples

To minimize sample collection volumes, dried blood spot (DBS) samples (about 0.2 ml per sample) will be obtained for the measurement of budesonide levels via the central umbilical line in place for clinical care or by heel stick. We are collaborating with investigators at the Center for Human Toxicology who have experience with DBS sampling[7] and will be performing the DBS analysis for this study. Filter paper will be labeled with the patient's study identification number, date and time of collection. Filter paper will be handled by the edges to prevent cross contamination. Using a previously published assay for budesonide by LC/MS/MS using a Thermo LCQ Advantage Max ion trap instrument equipped with a Finnigan Surveyor LC pump, Surveyor Autosamples, and a universal Ion Max source,[8] DBS levels for budesonide are linear across the range from 0.5 to100 ng/mL, and the LLOQ was 2 ng/mL.

If the infant has a central line, samples of blood will be drawn to test for budesonide levels at 15 minutes, 1 hour, and 4 hours after initial dosing for a total of 3 samples. If the infant does not have a central line, 2 blood levels will be drawn at 1 and 4 hours and we will attempt to time them with another blood draw being done for clinical care whenever possible. Trough levels will be done prior to any further doses for a potential total of 7 DBS or a total of 1.4 mL (2.8 mL/kg for a 500 gram baby over a five day period which does not represent an excessive blood loss).

d. Clinical Data Collection

Clinical data that will be collected includes patient demographics, vital signs including blood pressure measurements, laboratory data obtained for clinical care during calfactant: budesonide dosing including complete blood counts, glucose, and electrolytes and detailed respiratory support through 28 days of age, and 36±1 week and 40 ±1 week postmenstrual age (PMA), medications, significant co-morbidities, and status at discharge. See MOP for more details regarding data collection. 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 through 28 days of age include the length of ventilatory support, duration of oxygen supplementation, data on medication administration, including use of steroids, vitamin A, caffeine, antibiotics and indomethacin. We will collect vital signs, saturations, RSS, and ventilator settings prior to, at 60 minutes and 120 minutes after each dose. Survival at discharge without BPD at 36 and 40 weeks and date of discharge will also be collected. Data may be entered directly onto CRFs. Please see Table 3 below for procedures done during the study.

e. Oxygen/Flow Reduction Challenge Test

Oxygen/Flow Reduction Challenge Test is an assessment used to determine whether an infant who is receiving a small amount of supplemental oxygen or flow support at 36 weeks corrected age requires supplementation. This is an important component in determining whether an infant has BPD. Study patients who remain hospitalized at 36 (±1) weeks PMA and are stable on a small amount of supplemental oxygen or supplemental flow via nasal cannula are eligible to complete the oxygen/flow reduction challenge test. If the infant remains hospitalized at 40 (±1) weeks PMA and meets the same criteria, the test will be completed then as well. The O2/Flow Challenge Worksheet will be used when completing this test. For infants no longer in the study hospital, a member of the study team will contact the referral center and/or parents to obtain information about infant's respiratory support at 36 and 40 weeks of PMA.

Prior to starting the test, patients will be evaluated by a physician to determine stability and also to collect baseline data using a pulse oximeter. The pulse oximeter collects baseline data from the infant on their current oxygen and nasal cannula liter flow/minute support. Data is collected for 5 minutes, with the time and oxygen saturation recorded at every minute. If oxygen saturations recorded are ≥90% after 5 minutes, the infant may complete the oxygen reduction phase.

The oxygen/flow reduction challenge test will follow the current NIH recommendations for BPD. First, the FiO2 will be weaned by 0.20 every 5 minutes until either: 1) The infant is in room air (FiO2 0.21) and off nasal cannula with adequate saturations OR 2) The infant fails the oxygen/flow challenge exam with saturations < 90% for 5 continuous minutes OR saturation <80% for 15 seconds. Once the patient is placed in room air off all support, the infant will be monitored and data collected for one hour or until the criteria for failure is met.

An alternative weaning schedule can also be used if the supplemental oxygen is being delivered by nasal cannula without blender/medical air. The flow will be weaned by 50% every 10 minutes until the flow if ≤ 0.15 lpm, then the cannula will be gently removed from the nares. The wean will be discontinued if the infant fails the oxygen/flow challenge exam with saturations < 90% for 5 continuous minutes OR saturation <80% for 15 seconds. Again, once

the patient is placed in room air off all support, the infant will be monitored and data collected for one hour or until the criteria for failure is met.

Possible test results include: 1) Pass: All saturations ≥90% for one hour; 2) Fail: Saturation < 90% for 5 continuous minutes or saturation <80% for 15 seconds. At the conclusion of the oxygen/flow challenge the infant should be returned to their previous level of supplemental oxygen. The clinical care team will be notified of the results of the challenge test.

f. Pulmonary Function Testing in Patients Enrolled at Oregon Health & Science University

Non-invasive pulmonary function tests (PFTs) have been used to quantify the response of the newborn infant to a number of medications including antenatal[10-12] and postnatal corticosteroids,[9;45] and these physiologic measurement have been shown to correlate with the infant's clinical course.[9;11] Neonatal PFTs are regularly performed at OHSU and in the present study, PFTs will be done at OHSU prior to the first dose of study medication, 24 hours after the first dose, prior to extubation and then at discharge at 34-36 weeks of postmenstrual age. International criteria for testing acceptance will be followed as outlined below.[9;11;12]

PFTs will be measured with a computerized infant pulmonary function cart (SensorMedics 2600, SensorMedics Inc., Yorba Linda, CA and the Master Screen BabyBody, Jaeger/Viasys). Passive respiratory compliance (Crs) and resistance (Rrs) will be obtained with the single-breath occlusion technique[12] and functional residual capacity (FRC) with the nitrogen washout method.[10;11] In intubated patients including those requiring surfactant, testing will be performed by connecting the infant's endotracheal tube into the system via a three-way valve that also connected to the ventilator. In non-intubated patients, a face mask will be used.

For the single-breath occlusion technique, the airway will be briefly occluded at end inspiration until an airway pressure plateau is observed and the Hering Breuer reflex invoked. The linear portion of the passive flow-volume curve will be identified, and a regression line drawn to obtain the best fit. From the intercepts on the flow and volume axes, respiratory system compliance and resistance will be calculated. Acceptance criteria will include: 1) stable end expiratory baseline; 2) plateau pressure lasting >100 msec; 3) plateau pressure varying by < ± 0.125 cm H_2O; 4) acceptable flow-volume curve by visual inspection, with linear data segment identified; 5) at least ten breaths accepted with a coefficient of variation of <20%.[10-12]

For the nitrogen washout technique, calibration will be done with 2 known volumes, and a calibration line will be constructed for the system at the specific flow rate. The calibration curve will then be used to correlate the nitrogen washed out to the infant's FRC. The system will be corrected for dead space present and corrected the FRC to body temperature, pressure, and water-saturated conditions. Total FRC will be related to body weight. Acceptance criteria will include: 1) infant supine and quietly asleep; 2) test initiated at end expiration; 3) no evidence of leak on tracing of the washout; 4) consistent tracings; 5) at least 3 measurements with a coefficient of variation of < 10%.[10-12]

g. Future Contact

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

h. Adverse Event and Protocol Violation Reporting

All AE's reported or observed from the time the patient is consented into the study through the end of study participation (28 days) will be documented/reported.

Definitions

Adverse Event: An Adverse Event (AE) is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical (investigational) product, whether or not related to the medicinal (investigational) product (ICH Guidance E2A 1995).

Serious Adverse Event: An SAE is an event that:

- Results in Death
- Is immediately life threatening
- Requires inpatient hospitalization or prolongs existing hospitalization
- Results in Persistent or significant disability and/or incapacity, or
- Is a congenital anomaly/birth defect
- Is an important medical event; Note: Important medical events that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition.

Hospitalizations, which are the result of elective or previously scheduled surgery for pre-existing conditions, which have not worsened after initiation of treatment, should not be classified as SAEs. However, complication(s) resulting from a hospitalization for an elective or previously scheduled surgery that meets serious criteria must be reported as SAE(s).

Assessment of Severity

The severity of AEs/SAEs must be recorded during the course of the event including the start and stop dates for each change in severity. An event that changes in severity should be captured as a new event. Worsening of pre-treatment events, after initiation of investigational product, must be recorded as new AE(s).

The medical assessment of severity is determined by using the following definitions:

- **Mild:** A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention.

- **Moderate:** A type of AE that is usually alleviated with specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.

- **Severe:** A type of AE that significantly affects clinical status, or may require intensive therapeutic intervention.

Assessment of Causality

- **Definite:** Events that, after careful medical evaluation, are considered definitely related to the drug treatment and other conditions (concurrent illness, progression/expression of disease state, or concurrent medication reaction) do not appear to explain the event.

- **Probable:** Events that, after careful medical evaluation, are considered with a high degree of certainty to be related to the study drug. The following characteristics will apply:

    - A reasonable temporal relationship exists between the event and exposure to the study drug, and
    - The event is a known reaction to the study drug that can be explained by an alternative cause commonly occurring in the population or individual, or
    - The event is not a known reaction to the study drug but cannot be reasonably explained by an alternative cause.

- **Possible:** Events that, after careful medical evaluation, do not meet the criteria for a definite or probable relationship to the study drug, but for which a connection cannot be ruled out with certainty. The following characteristics will apply:

    - The event occurs after exposure to the study drug
    - There is a reasonable temporal relationship to the study drug, but the event is not a known reaction to the study drug and could be explained by a commonly occurring alternative cause, or
    - In the absence of a reasonable temporal relationship, the event cannot be explained by an alternative cause.

- **Unlikely:** Events that, after careful medical evaluation, do not meet the criteria for a possible or probable relationship to the study drug and for which a connection is unlikely. The following characteristics will apply:

- The event does not follow a reasonable temporal sequence from study drug dosing, or
- The event may have been produced by environmental factors, and
- There is no apparent pattern of response to the study drug.

➢ **Not related:** Events in this category will have either of the following characteristics:

- The event occurs before exposure to the study drug, or
- The event does not have a reasonable temporal relationship to study drug and can be explained by a commonly occurring alternative cause.

Event reporting

All AEs, and SAEs should be documented on the appropriate adverse event logs/forms, entered into Redcap, and reported as outlined below.

- All **SAEs** should be reported by the clinical site to the project manager (J Asselin) or the project PI (Cindy McEvoy, MD) within 1 business day.
    - The medical monitor (Dr. Sue Ann Smith, a neonatologist uninvolved in care of recruited patients) will then be notified and will review the event. After the medical monitor has reviewed the event, additional notifications will be made to the DSMB, IRB, and FDA as necessary.
    - Sites should report events to their IRB according to IRB guidelines and regulations.
    - When deemed necessary by the medical monitor, the DSMB will be notified of any significant events immediately and a meeting will take place to determine appropriate actions.
- All other AEs should be entered into Redcap within 7 days.

The DSMB will meet after each dosing group of 8 patients and will review all other AEs/SAEs at that time. The DSMB will be composed of Dr. Patricia Spitale (neonatologist), Dr. Thuy Nguyen (pediatric pharmacist), and Zunqiu Chen (biostatistician). Dr. Sue Ann Smith will be the medical monitor for the study.

Safety Monitoring

Monitoring for SAEs and AEs will be performed throughout the study period. The site will submit completed SAE CRFs to the data coordinator and/or the project PI, Dr. McEvoy, within 1 business day. Any additional background information will be collected about the event and the medical monitor will be notified. The medical monitor will review the event information and determine additional action as necessary. The medical monitor may stop the study or put the study on hold pending further review by the DSMB as the medical monitor deems necessary.

The DSMB will meet before subject recruitment begins and will subsequently meet after each group of 8 patients reaches 28 days of age to review study progress and AE/SAE reports. Additionally, they may meet as needed to review SAEs when the medical monitor deems it necessary.

Table 3. Study Procedure Table

Study Day	Pre-Study	First dose	15 min	1h	4h	12h	24h	48h	Add'l doses 2-5	Through 28 days	36 wks PMA[f]	40 wks PMA[f]	24 months of age
Informed consent	X												
Dose with Budesonide in calfactant[a]		X							X				
Tracheal Aspirate[b]		X				X	X	X	X				
Dried blood spots from central line[c]			X	X	X				X[d]				
Clinical data collection		X	X	X	X	X	X	X	X	X			
PFT[e]		X					X				X		
AE and SAE collection		X	X	X	X	X	X	X	X	X			
Respiratory status											X	X	
Medical record review													X

a) If remains intubated and mechanically ventilated may get up to 5 total doses 24-48 hours apart

b) For the first dose, TA is obtained at 0 h, 12 h, and prior to a second dose. When multiple doses occur back to back, TA is collected before each dose and at 24 and 48 h after the final dose.

c) If no central line, samples will be done at 1h and 4h

d) If further daily doses given a single daily sample will be done before dosing
e) PFTs will be done **at OHSU only**. Tests will be performed prior to the first dose of study medication, 24 hours after the first dose, prior to extubation and then at discharge at 34-36 weeks of postmenstrual age
f) PMA is postmenstrual age

6. Data and Specimens

a. Handling of Data and Specimens

Tracheal Aspirate (TA) Samples: Each TA sample is centrifuged within 20 minutes after collection to provide a cell pellet and a supernatant fraction. Both the supernatant and cell pellet are frozen until shipment to UCSF laboratory via Fed Ex utilizing dry ice to keep samples frozen. After shipment to UCSF, the supernatant fraction is assayed for selected cytokines using ELISA . The TA cell pellet is used for isolation of DNA to quantitate cell content and to develop a repository for genetic studies of gene variants.

All tracheal aspirate samples will be labeled by study ID number and date/time, without other identifiers. The study samples will be 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 will be performed with assistance from the project manager. Should any portion of samples remain, we will 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).

Dried Blood Spot (DBS) Samples: DBS samples will be labelled with the subject study ID and the date and time the sample was collected. DBS samples will be placed in DBS envelope and kept in ambient temperature in a secure location until samples can be batched and mailed to the University of Utah's Center for Human Toxicology Laboratory. Promptly upon receipt of the samples, they will be catalogued and stored until they are analyzed via LC/MS/MS. After the budesonide concentrations have been established via LC/MS/MS, the data will be used to construct non-compartmental and population pharmacokinetic models. These models will be constructed according to FDA guidelines and industry-standard best practices and will be used to quantify the extent of budesonide exposure.[46]

Once all testing has been completed, any leftover samples will be shipped to Dr. Cindy McEvoy at OHSU.

b. Sharing of Results with Subjects

TA and blood spot results will not be shared with subjects or site investigators. Each tracheal aspirate sample and blood spot will be coded with subject study ID only. Patient name, initials, birth date and other potential identifiers are known only to investigators at each study site and this information will not be available to Dr. P. Ballard or other laboratory personnel.

7. Data Analysis

a. **Primary outcome/objective**

Efficacy will be measured and summarized within each dosing level. Dose escalation will be defined as effective at the dose level in which 5 of 8 infants achieve:

- At least 1 of the following:
    1) Extubation within 72 hours of first dose or after ≤ 3 doses-without re-intubation before 28 days of age
    2) RSS on nasal continuous positive airway pressure (NCPAP) < 1.5 or on nasal cannula Fi02 <25% at ≤ 2L/min at 28 days of age, persisting for at least 72 hours.
    3) Cumulative supplemental oxygen < 4.2 from time of enrollment to 28 days of age[4] (see calculation below)
    4) No respiratory support at 28 days, including no supplemental oxygen by nasal cannula
- AND a ≥ 50% suppression of the tracheal aspirate interleukin-8 (IL-8) or CCL2 (chemokine ligand 2) at 24-72 hours (or prior to extubation if occurs at <24 hours after dosing) after the initial dose in 5 of 8 of the infants (this may be a different combination of 5 patients than those who met the above criteria).

Note: If a patient is re-intubated before 28 days of age for non-respiratory reasons (e.g. required for surgery), these additional days of respiratory support will not be included when analyzing the primary outcome.

As the design of this study is for dose escalation, analyses will likely be under-powered to detect significant differences, yet these will provide valuable data for future studies.

Cytokine values will be compared between infants in this study and a matched comparison group from the TOLSURF study.[3] If 32 patients are enrolled and 32 matched patients are used, this study will have 80% power to detect a significant ($\alpha<0.05$) change of 71 pg/ml/mg urea (estimated SD 100) in IL-8 (as compared to zero) from before the first dose to 12 hours post-dose. This is based on a previous finding of a significant difference ($p=0.03$) of 145 pg/ml/mg urea IL-8 between treated and untreated infants at 12 hours after treatment with budesonide. Likewise, this study will have 80% power to detect a significant ($\alpha<0.05$) change of 0.14 in the ratio CCL2 secretion relative to pre-dosing at 24 hours after the first dose. This is based on a significant finding of a 90% reduction in CCL2 as compared to controls after budesonide treatment of lung explants.

Change in CCL2 and IL8 within patients will also be assessed via statistical analysis. If 32 patients are enrolled, this study will have 80% power to detect a significant ($\alpha<0.05$) change of 73 pg/ml/mg urea (estimated SD 150) in IL-8 (as compared to zero) from before the first dose to 12 hours post-dose. This is based on a previous finding of a significant difference ($p=0.03$) of 145 pg/ml/mg urea IL-8 between treated and untreated infants at 12 hours after treatment with budesonide. Likewise, this study will have 80% power to detect a significant ($\alpha<0.05$) change of 0.1 in the ratio CCL2 secretion relative to pre-dosing at 24 hours after the first dose. This is based on a significant finding of a 90% reduction in CCL2 as compared to controls after budesonide treatment of lung explants.

Comparisons of major co-morbidities of prematurity (PDA/NEC/IVH/sepsis/ ROP) between treated infants in this study and the matched comparison group will be descriptive and include comparisons of the co-morbidities and overall outcomes between the different dosing levels and compared using Fisher's exact test.

b. Secondary outcomes

The secondary objectives will quantify safety criteria including clinical parameters that may be associated with potentially elevated systemic glucocorticoid concentrations. Serial budesonide levels will also be collected to perform pharmacokinetic modeling.

c. Clinical parameters including blood pressure, glucose, sodium and potassium levels

Clinical parameters potentially consistent with elevated glucocorticoid concentrations will be collected and analyzed descriptively within each infant, dosing group, and across the total study population. Comparisons of clinical parameters (continuous and normal/abnormal) will be made between dosing levels. These clinical parameters will be collected after the first dose of the study drug and up to 7 days after the final dose.

d. Budesonide pharmacokinetic modeling

Blood concentrations of budesonide will be examined over time (15 minutes, 1 hour, and 4 hours after each dose) to evaluate the pharmacokinetics of the budesonide at each dosing level. Based on preliminary data in lambs, we could expect a Cmin of 1.9 ng/mL at 9 hours post-dosing. Non-compartmental and population pharmacokinetic analyses will be performed to establish the extent of budesonide exposure. Commonly reported pharmacokinetic parameters will be calculated including: clearance, volume of distribution, area under the curve (AUC), half-life, peak concentration (Cmax), and trough concentration (Cmin).

e. Clinical respiratory status at 28 days of age

Using descriptive statistics and basic inferential statistics RSS at 28 days of age and BPD status at 36 and 40 weeks will be compared between dosing groups and to case matched patients from the TOLSURF study.[3] For normally distributed data an ANOVA will be conducted, and for non-parametric data a Kruskal-Wallis ANOVA will be conducted. Any pairwise comparisons will be controlled for using a multiple testing correction to protect against Type I error. As this is a secondary objective, analyses will likely be underpowered to detect significant differences, yet these analyses will provide valuable data for future studies. The difference in change in RSS between the first day of treatment and day 28 will also be evaluated between groups using multiple paired t-tests and a correction for multiple testing to control Type I error. In addition, the decrease in RSS over the period of medication dosing will be compared/associated with the measured plasma concentrations of budesonide. The incidence of BPD at 36 and 40 weeks will be compared to historical case matched infants from the TOLSURF study.[3]

Individual patient's decrease in RSS at 28 days of age will be correlated with their measured budesonide levels at each level after the first dose. If all 32 patients are enrolled, the study will have 80% power to detect a significant ($\alpha<0.05$) correlation between RSS and budesonide of $\rho \geq \pm 0.48$. If 16 patients are enrolled, the study will have 80% power to detect a significant correlation between RSS and budesonide of $\rho \geq \pm 0.65$.

f. Safety Analyses

A review of all adverse events will be conducted at a minimum after each set of 8 patients by the DSMB.

g. Exploratory Outcomes

Serial PFT measurements within each patient will be analyzed using ANOVA for repeated measures. The analyses of samples forwarded to Dr. Ambalavanan's laboratory will primarily be descriptive and provide new findings on the scope and differential levels of cytokine suppression in infants treated with budesonide suspended in surfactant as well as potential modulation of the mucosal microbiome.

8. Privacy, Confidentiality, and Data Security

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

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

9. Provisions to Monitor the Data to Ensure the Safety of Subjects

We will monitor the accuracy of data entry by the sites, internally, by regular review of the CRF completion in REDCAP by Jeanette Asselin the project manager (PM). Ms. Asselin will communicate regularly with each study site (coordinator and respiratory staff) and will resolve any data concerns or issues on those calls. She will perform a site visit to each study site as well as external monitoring. All data will be monitored for meeting entry criteria, adherence to protocol, primary and secondary outcomes, and adverse event reporting

10. Risks and Benefits

a. Risks to Subjects

<u>Surfactant</u>

The most common side effects reported when premature infants are given surfactant are: decreases in their oxygen saturation, plugging of their breathing tube, decreases in heart rate, reflux of surfactant into the breathing tube, and the need for extra mechanical breaths to be given from the breathing machine. There is also a chance that during this procedure that the infant may require reintubation. These events are generally brief and not associated with serious complications or death. The surfactant is given by skilled care givers in the NICU who can respond to all of these potential side effects.

<u>Budesonide</u>

When the steroid is administered in high doses and is found in the infant's blood stream (systemic circulation), potential adverse effects include: decrease in growth, hyperglycemia, hypertension, a negative effect on brain development or ability to respond to stress. Infants who were given high doses either intravenously or by mouth, and given this medication over 42 days of treatment with a slow decrease each day were more likely to have cerebral palsy at follow-up. In this study, we will start at 0.025 mg/kg of budesonide given onto the surface of the lung with surfactant and do not expect much to be absorbed into the bloodstream. This is 1/10 of the dose used in previous studies of about 400 total premature babies. We will closely monitor the amount of steroid being absorbed into the infants' blood. Previous studies in babies giving 10 x the dose of steroid in surfactant did not show any adverse developmental outcomes at follow-up at 2-3 years of age.

Tracheal Aspirate

This will be collected at the time of routine suctioning as much as possible. The discomfort from this procedure is minimal, although can result in transient decrease in oxygen saturation and occasionally dislodgement of the endotracheal tube, requiring reintubation. .

Plasma Samples

Blood draws are common from central lines that premature babies often have in place during the first 1-2 weeks of life. Collecting study samples from the central line will not cause any additional pain. If a central line is not available, blood samples will be collected via heel prick. We will time study blood draws with testing done for clinical care as much as possible. The maximum total blood collected for pharmacokinetic analysis is 7 total samples of 0.2 ml or a total of 1.4 ml. This would be 2.8 ml/kg for a 500 gm newborn over a five day period which does not represent an excessive blood loss.

Pulmonary Function Tests:

These tests are non-invasive, well tolerated, and can be performed in intubated and extubated patients. [9-12;45] They are routinely be performed in the neonatal intensive care unit at OHSU. There are no known specific risks of the lung function test. If the infant is on a ventilator there is the risk the ETT will become dislodged during the testing but this risk will be minimized by continuous and careful monitoring by the bedside skilled NICU personnel during testing. During the testing there may be small fluctuations in the infant's oxygen saturation or the infant may become restless during testing. This will be monitored closely and appropriate actions taken if needed.

Confidentiality

There is a risk to confidentiality. This risk will be minimized by storing the subjects personal and medical information collected as part of this study in a secure location as detailed in section 8 above.

b. Potential Benefits to Subjects

The subject may or may not benefit by taking part in this study. There is no guarantee that the infant will receive direct benefit from his/her participation in this study. The benefits of being in this study may be a chance that the study medication may decrease the inflammation in the lungs and help decrease their risk of developing BPD. Participation may provide information that may benefit other premature babies by helping to determine which dose of steroid is best to decrease markers of inflammation. Still, the infant may get no direct benefit from this study.

11. Drugs or Devices

As these 2 FDA approved medications will be given in combination and given intratracheally, the study medication is investigational (IND number 128102). This study will comply with all FDA requirements, IRB requirements, and ICH-GCP guidance. We will follow applicable Research Pharmacy policies and procedures and will provide each research pharmacy with a manual for the study drug.

12. Multi-Site Coordination

Prior to study initiation, study template documents including template consent forms and CRF forms will be provided to the outside participating sites. The PIs at each of the participating sites will submit the protocol, consent, and other pertinent items to their respective IRBs. When approval is obtained, each recruitment site PI will forward the IRB approval letter and approved consent to Dr. McEvoy at OHSU. These items will be submitted as a modification to the OHSU submission for this study. Once the OHSU IRB approves these modifications the respective sites will be notified that the research can be initiated at that site.

Prior to beginning the study, all sites will have a site initiation visit by the Project Manager (PM). At that visit, the study protocol and procedures will be reviewed, with site sponsor, study coordinator, study respiratory staff, research pharmacy and other medical staff. A manual of operations will be provided and reviewed. PM will meet separately with study coordinator to review all regulatory and study procedures. Appropriate recording of clinical data as specified by FDA Regulations for Good Clinical Practice will be reviewed with study personnel.

During subject enrollment, the PM will communicate regularly with site coordinators to review site screening, enrollment, and subject status and data completion. When a dosing group has been enrolled (8 infants) she will communicate when study enrollment is on hold and when enrollment can be restarted.

The project manager for the study is J Asselin at UCSF Benioff Children's Hospital Oakland. Sites will complete data on case report forms (CRFs) and then enter data into a secure REDCap database housed on the UCSF server. Data will be assessed for quality via automated and manual processes. Queries (data discrepancies) will be generated to identify potential errors in the study data and to resolve them in a timely manner. Copies of the original site CRFs will be faxed or pdf to DCC on a scheduled basis for comparison with

REDCap entries. Original CRF 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 entry into REDCap or faxing to the DCC. Should any questions or changes arise during this review, all queries will be referred back to the site investigator for resolution prior to faxing the forms to the DCC.

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