AEROSOLIZED SURVANTA IN NEONATAL RESPIRATORY DISTRESS SYNDROME: PHASE I/II STUDY (IND 115430)

NCT02294630

FINAL STUDY PROTOCOL & STATISTICAL ANALYSIS PLAN

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RESEARCH STRATEGY

1.1. Research Design:

1.1.1. Study Design:

This is a proposal for the Phase II portion of our unblinded Phase I/II trial[1,2]. It is a safety, feasibility and dose finding study of AS for the treatment of RDS in spontaneously breathing PT neonates receiving NIV. To remove selection bias and control temporal trends, infants will be randomized to 4 parallel fixed dosing schedule groups[1,2]. This Phase II study will identify 1 or 2 optimal doses, and provide additional data for safety, feasibility and early indication of efficacy that will be the basis for designing a definitive Phase III trial. Optimal dose(s) will be identified based on evidence of a significant positive trend with increasing dose, even if no two groups are significantly different[3,4]. Equitable representation of GAs within each dose group will be achieved by stratified randomization. Equitable representation of two FDA-approved nebulizers will be achieved by using block sizes of 8. It is expected that choice of nebulizer device will not affect efficacy but may influence caregiver preference based on institutional practice and ease of administration (feasibility).

1.1.2. Inclusion Criteria:

1. Infants admitted to the NICU at Hutzel Women's Hospital (HWH)/Children's Hospital of Michigan (CHM)
2. Gestational age of $24^{0/7}$-$36^{6/7}$ weeks
3. Postnatal age ≤ 24 hours
4. Clinical diagnosis of RDS based on (i) presence of at least two of the four classic symptoms (need of supplemental oxygen, tachypnea, intercostal retractions or grunting), *and* (ii) exclusion of other causes of respiratory failure (Section 3.6.4.1) *and* (iii) Clinician intent to administer surfactant if infant requires intubation
5. Respiratory support with NIV (CPAP or NIPPV or HFNC) with FiO2 ≥25% or PEEP ≥ 4 cmH20 or HFNC rate ≥ 2 LPM for ≤8 hours
6. Written informed consent from parent/guardian

1.1.3. Exclusion Criteria:

1. Previous receipt of surfactant
2. Infants with respiratory distress who are unstable and require immediate intubation (Section 3.6.5)
3. Active air leak syndrome (e.g. pneumothorax, pneumomediastinum)
4. Lethal congenital malformations; death anticipated within first 3 days of life; decision to withhold support
5. Serious abdominal, cardiac, airway or respiratory malformations including tracheal esophageal fistula, intestinal atresia, omphalocele, gastroschisis, pulmonary hypoplasia, or diaphragmatic hernia
6. Neuromuscular disorder resulting in respiratory compromise

1.1.4. Study Procedures:

Table 9: Time & Event Table:

Assessment	Screening	Baseline	Treatment	Post-intervention Events					
Study Time Frame	0-24 hrs	0-24 hrs	0-24 hrs	0-72 hrs post intervention	D7	D14	D28	36 wks corrected GA	Discharge
Informed consent	x								
History	x								
Vital Signs	x	x	x	x					
CRIB score		x							
RD score		x	x	x					
Nasal Trauma Score		x	x	x					
Blood gas	x	x	x	x					
Cerebral oximetry		x	x	x					
Gastric aspirate		x		x					
Effectiveness:									
Primary outcome				x					
Secondary outcome				x	x	x	x	x	x
Safety:									
Adverse events			x	x					

Feasibility:				x					

CRIB-Clinical Risk Index for Babies; RD – respiratory distress; D7 = 7 days, D14 = 14 days, D28 = 28 days

Study procedures are described below and their time frame is summarized in Table 9; greater detail is provided in the *drafts* of the standard operating procedures (Appendix VII) and study forms (Appendix VIII).

1.1.4.1. Ascertainment of Study Eligibility:

Research staff will screen admission databases at HWH and CHM NICU for infants $24^{0/7}$ to $36^{6/7}$ weeks GA admitted at ≤ 24 hours of age for study eligibility and maintain a screening log. Informed parental consent will be sought for study participation of potentially eligible infants after a member of the clinical team obtains permission for research staff to approach the parent(s)(Appendix V). To minimize treatment delays, attempts will be made to obtain consent antenatally or soon after admission. Once the clinical team initiates NIV in a potentially eligible infant, the research team will determine that all inclusion criteria have been met, none of the exclusion criteria are present and fill out an Eligibility form. The infant will be assigned the next available pre-printed randomization number from the randomization log for the appropriate GA stratum. Research staff will determine the treatment assignment corresponding to the randomization number by calling the research pharmacy staff that is available 24/7. As confirmation of randomization, an order form documenting the randomization will be placed in the infant's electronic medical record (Appendix VII [Study Flow, Table A1]).

Diagnosis of RDS will be based on[5] (i) presence of at least two of four classic symptoms (need of supplemental O2, tachypnea, intercostal retractions or grunting), *and* (ii) exclusion of other causes of respiratory failure, *and* (iii) Clinical team plan to administer surfactant if infant requires intubation. A chest x-ray (CXR) is not mandatory prior to enrollment; however, if obtained clinically, severity of RDS will be graded and results recorded (Appendix VII [Table A4])[6]. We have not mandated a radiologic diagnosis of RDS because classic radiologic findings may evolve over the first 12-24 hours and be modified by early NIV; furthermore, the need for a CXR may unnecessarily delay study intervention[7,8].

1.1.4.2. Timing of Aerosolized Surfactant:

Enrolled infants will receive AS at ≤24 hours of age *&* within 8 hours of initiating NIV as several studies have shown that earlier administration of surfactant to PT babies with RDS improves outcomes.

1.1.4.3. Study Intervention – Aerosolized Surfactant Preparation and Dose:

For this study, Survanta (200 mg PL/8 ml) (Beractant, Abbvie Inc.) will be used at two doses – 100 mg/kg and 200 mg/kg and two dilutions (1:1 and 1:2) resulting in 4 dose schedule groups.

Survanta has been chosen as the surfactant for this study as this is the surfactant used exclusively at our institution; it is also the most commonly used surfactant with the longest duration of use in the US[9,10]. *We will validate aerosolized use of other surfactants in subsequent studies*. Many studies have compared Survanta with other animal derived surfactants, however, consensus regarding the best preparation has yet to be determined[10]. Although several relatively small randomized controlled trials (RCTs) showed significant reductions in mortality and need for redosing with high-dose (200 mg/kg) but not low-dose (100 mg/kg) poractant alfa vs. beractant (100 mg/kg)[11,12]; three large recent retrospective cohort studies of surfactant use in the US (n=51,282 and 14,173) and Australia (n=515) published in 2013 failed to show significant differences in outcome between poractant and beractant in PT neonates with RDS[9,13,14]. It is speculated that previously described differences in mortality between surfactants may relate to site variation in outcomes[9].

Previous studies have indicated that with surfactant *instillation* a minimum of 19 mg lipid/kg natural surfactant was required to improve gas exchange and 53 mg lipid/kg resulted in greater improvement in pressure-volume curves in PT lambs[15]. Consequently, an empiric dose of 50 mg/kg has been accepted as the minimal amount of surfactant required to effectively treat neonates with RDS although most centers use 100 mg/kg[16], a dose that exceeds ten-fold the amount necessary to establish a monolayer. On the other hand, pharmacokinetic and clinical data suggest that 200 mg PL/kg of instilled surfactant has a longer half-life and a better acute response compared to 100 mg/kg[17,18]. Therefore both these doses will be tested in this Phase I/II trial. Several investigators have shown that even quite low amounts of inhaled surfactant might be more effective in improving gas exchange than large amounts of instilled substance[19-23].

Initial reports evaluating *aerosolized* surfactant in lung injury demonstrated significant improvements in oxygenation with as little as 0.5 -2 mg/kg surfactant deposited in lung tissue[19]. This is lower than the endogenous surfactant pool size of 3-4 mg/kg that is required to increase compliance in PT rabbits[24] and is comparable to the theoretical dose of 3 mg/kg that would be necessary to form a monomolecular film on the alveolar surface[25,26]. As surfactant delivery techniques have improved, the deposition efficiency has increased. In one study, 31% of the total quantity of surfactant aerosolized into the system was deposited in lung tissue

representing a lung dose of 4.5 mg lipid/kg[27]. Furthermore, a dose-response relationship existed for AS in this model. There is *in vitro* evidence that even with lower alveolar delivery with aerosolized as compared to instilled surfactant, surfactant retains its anti-inflammatory properties and actually decreases leukocyte infiltration in the alveolus and the interstitium and improves oxygenation[28]. Our Preliminary data and reports in literature suggest that diluted surfactant solutions are most favorable for aerosolisation (Section 3.4.1 & 3.4.2)[19,29-32].

Based on the above, we will evaluate two doses and dilutions of Survanta:

1. Dosing Schedule I: Survanta 4 ml (or 100 mg PL)/kg + equal volume of NS
2. Dosing Schedule II: Survanta 4 ml (or 100 mg PL)/kg + 2x volume of NS
3. Dosing Schedule III: Survanta 8 ml (or 200 mg PL)/kg + equal volume of NS
4. Dosing Schedule IV: Survanta 8 ml (or 200 mg PL)/kg + 2x volume of NS

1.1.4.4. Administration of Aerosolized Survanta:

a) Aerosol Generating Device:

Our goal is to study safety and feasibility of AS; optimal dose and dilution; and to get an estimate of efficacy of AS using existing modes of NIV and aerosol delivery. We do not mandate the mode/manufacturer of NIV or the aerosol generating device used in the study as there is no evidence of significant differences in outcomes among various commercial devices approved for use in infants; we will record the mode of NIV and nebulizer type to be able to evaluate safety, feasibility and associations with response. The MH jet and VM nebulizers are FDA-approved for use in infants, and have been shown to be efficacious in delivery of AS; this is supported by our preliminary data. The operating characteristics of the nebulizers are different – the VM nebulizer puts out more aerosol in a shorter period of time which may result in a quicker response and may be better tolerated by the patient and bedside caregivers; however this may be associated with increased condensation in the NIV circuit and delivery of only part of the surfactant placed in the reservoir. In addition the VM nebulizer has a tendency to stop because of clogging of pores by viscous surfactant. This can be remedied by tapping the nebulizer or using more dilute surfactant. In contrast to the VM nebulizer, the MH jet nebulizes the surfactant placed in the nebulizer slowly, resulting in a lower output compared to the VM nebulizer; less condensation in NIV circuit and a more prolonged period of aerosol delivery. This may lead to slower onset of action and also impact its acceptance by bedside nurses and respiratory therapists.

b) Non-Invasive Ventilator Circuit:

Non-invasive respiratory support, defined as any form of respiratory support that is not delivered via an endotracheal tube, will include NCPAP, NIPPV, and HFNC for this study. *Studies have shown that the commercial system of delivering NIV is of little importance, short binasal prongs are a better interface than single longer prongs, and that there is no difference between NIPPV or HFNC compared to CPAP [7,33-41].*

Although four small RCTs (n = 41 to 64) have reported consistent superiority of NIPPV compared with NCPAP in reducing re-intubation rates after extubation in PT infants, these findings did not correlate with improvements in long term respiratory outcome of BPD[42-46] and are not substantiated by more recent studies[33,39]. When used as the primary mode of respiratory support in PT (24-34 weeks GA) infants with RDS, two small RCTs (n=76 & 84) showed lower intubation rates with NIPPV compared to NCPAP whereas two other RCTs (n=40 & 200) showed no difference in re-intubation rates; three of these four RCTs showed no difference in rates of BPD and/or death[46-50]. In the recently published, largest RCT (n=1009) comparing NIPPV with NCPAP in PT neonates <30 weeks GA, there was no difference in the need for re-intubation, BPD, and other clinically important outcomes between the two modes of NIV[33]. In preplanned subgroup analyses, there was no difference in these outcomes when either NIPPV or NCPAP was used as the primary mode of respiratory support or following extubation. An updated meta-analysis evaluating NIPPV vs NCPAP post-extubation in PT infants *showed no advantage of NIPPV over NCPAP* for either BPD or death[39].

Similarly, HFNC has been used increasingly as a form of NIV in PT infants despite concerns about airway pressures generated[51]. Recently it has been shown that pharyngeal pressures generated by two frequently used HFNC systems ranged from 2.2 to 4.9 cmH2O at flow rates of 2-8 LPM with an increase of 0.4-0.5 cmH2O for each LPM flow (r^2=0.97-0.99)[52]. Moreover, four recent studies have shown comparability of efficacy of HFNC and NCPAP/NIPPV in PT neonates with the potential of reduced nasal trauma with HFNC[34,36,38,40,53]. In two RCTs, compared to NCPAP, HFNC used for post-extubation respiratory support in PT infants <32 weeks GA (n=132 & 303) resulted in similar rates of extubation failure with significantly reduced nasal trauma scores[36,40,53]. Another smaller RCT (n=49) reported that HFNC is as effective as NIPPV in preventing intubation in PT neonates <35 weeks GA when used as the primary treatment for RDS [38]. A 4th large

RCT (n=432) demonstrated *similar efficacy and safety of HFNC as NCPAP* when applied immediately post-extubation or early as initial NIV support in neonates ≥28 weeks GA[34].

At our institution, as demonstrated by our preliminary data (Section 3.4.5), NIPPV and NCPAP are the modes of NIV used for primary respiratory support in infants ≤ 32 weeks GA; and NCPAP and HFNC as primary modes of respiratory support in PT infants >32 weeks GA. A common ventilator circuit is used for NIPPV and NCPAP with short binasal prongs as the patient interface (INCA®). For HFNC, the Fisher & Paykel heated humidified system is used with short binasal prongs.

The neonatal ventilator circuit will have heated and humidified gas flow as is standard of clinical care. The *MiniHeart Lo-Flo jet nebulizer (WestMed®)* will be placed in the inspiratory limb of the ventilator circuit close to the patient interface wherever there is a natural break in the circuit. The gas flow in the nebulizer will be ≤2LPM and will have the same FiO2 as the NIV circuit. The output of this nebulizer is 8 ml/hr at a flow rate of 2 LPM. After the flow from the nebulizer is introduced, the ventilator parameters and or gas flow through the ventilator will be adjusted to keep pressures constant. Every attempt will be made not to change ventilator settings during nebulization unless patient's well-being mandates it. The *AeroNeb Solo nebulizer (Aerogen®)* will be placed in the ventilator circuit close to the patient interface wherever there is a natural break. Although in pediatric and adult MV, vibrating mesh nebulizer placement proximal to the humidifier was associated with increased aerosol delivery[54], during NIV in pediatric and infant models, aerosol delivery was greater if the nebulizer device was placed closer to the patient interface[55,56].

Surfactant dose determined from the Dosing Schedule Table (Appendix IV) will be diluted with saline, mixed by a gentle swirling motion and placed in the nebulizer chamber and aerosolized till the nebulizer runs dry. If the volume is in excess of the capacity of the nebulizer reservoir, the reservoir will be replenished as the level of medication drops in the reservoir by research staff at the bedside or using a syringe pump to deliver the dose depending upon availability. Inspiratory and expiratory tubes will be kept at a lower level than the nasal prongs to prevent reflux of condensed fluid from these tubes into the nasal prongs[30,57].

c) Patient Interface:

Only short binasal prongs will be used for aerosol delivery in the NIV circuit. Since the ventilator circuit for NCPAP and NIPPV is identical, only one type of neonatal nasal prongs (INCA®) will be used. For the HFNC circuit, only the neonatal Fisher & Paykel short binasal prongs will be used. Nasal cannulas will be applied with recommendations that the prong outer diameter occupy ~50% of the nares internal diameter to allow free egress of flow around the cannula[34].

d) Number of doses of Aerosolized Surfactant & Intervals between Doses:

Infants will be eligible to receive a 2nd dose of AS 4 hours after the 1st dose of AS if there is continued need for NIV with FiO2 ≥25% *or* PEEP ≥ 4 cmH20 *or* HFNC rate ≥ 2 LPM.

Since most PT infants with RDS receive ≤2 doses of intratracheal surfactant as standard of care, we chose 2 doses of AS as being the minimum number to assess efficacy without inordinately delaying standard of care treatment with intratracheal surfactant[58]. Early trials of intratracheal surfactant used dosing intervals ranging from 1 to 12 hours based on ventilator settings. Since pulmonary delivery following aerosolisation is not precisely quantified, we will administer the 2nd dose 4 hours after the 1st if the infant meets criteria.

e) Duration of nebulization:

Time to nebulize each dose will depend upon volume of diluted surfactant, type of nebulizer, and ambient humidity and temperature. It is expected that for each dose nebulization will be <1 hour for the smallest infants and <4-5 hours in the largest infants. This Phase II study will provide critical information regarding time for nebulization of various doses for PT babies and will be crucial in determining tolerance of intervention both by the patient and clinical bedside caregivers. This will be one factor in selecting the optimal dose and dilution of AS for the definitive Phase III trial.

1.1.4.5. Gestational Age Group Strata:

Enrolled infants will be stratified by three GA strata ($24^{0/7}$-$28^{6/7}$; $29^{0/7}$ - $32^{6/7}$, $33^{0/7}$ - $36^{6/7}$) because need for intubation and MV has been reported to be higher in more immature infants along with higher co-morbidities of prematurity and mortality. Infants <24 weeks will be excluded as most are likely to receive intubation. [59,60]

1.1.4.6. Randomization Procedures: Assignment to Surfactant dosing schedule:

An *a priori* computer-generated *stratified block randomization* list will be used to assign patients to each of the 4 parallel fixed dosing schedules (allocation ratio 1:1:1:1, total subjects = 120). To ensure randomization of equal number of subjects to both nebulizers, block size of 8 will be used. To ensure equitable representation of gestational ages, GA stratum-specific randomization lists will be generated. Stratified block randomization will ensure balanced and independent distribution of participants within each dose group, avoid systematic differences between dose groups with respect to known or unknown confounding baseline variables, remove selection bias and control temporal trends[2,62-66]. The balance resulting from blocked stratification can improve the power of trials by reducing unwanted variation, reduce Type I and II error rates, increase efficiency, and improve subgroup analyses.

Table 10: Stratified Block Randomization of Sample

GA Group (Strata)	Nebulizer Device (Blocks)	Dosing Schedule			
		I	II	III	IV
24 0/7 – 28 6/7 (Extremely PT)[61]	MiniHeart	5	5	5	5
	AeroNeb	5	5	5	5
29 0/7 – 32 6/7 (Very PT)	MiniHeart	5	5	5	5
	AeroNeb	5	5	5	5
33 0/7 – 36 6/7 (Moderately PT)	MiniHeart	5	5	5	5
	AeroNeb	5	5	5	5
	Total	**30**	**30**	**30**	**30**

The structure of Table 10 shows breakdown of the sample by confounding variables in rows (GA [stratifying factor] & nebulizer type [blocking factor]) with columns representing treatment groups. Therneau showed that balance in covariates begins to fail when the number of blocks approaches half the sample size (n/2)[64,67]. Therefore studies using stratified assignment should not attempt to balance on more than 2 to 3 important factors. In this study we have balanced 2 important factors (GA, nebulizer type); the resulting number of blocks (3 GA strata x 2 nebulizers x 4 dosing schedule groups=24) is <n/2 (120/2=60).

Multiple births, common in this premature population, will be randomized independently and not as a unit as the latter can lead to bias in the randomization process.

1.1.4.7. Allocation Concealment:

The randomization list with treatment assignments corresponding to randomization numbers will be generated by the study statistician not involved in screening or enrollment of study subjects. The research pharmacist, also not involved in screening or enrollment, will receive the list and keep it concealed. For each infant enrolled, the research staff will obtain the treatment assignment corresponding to the infant's randomization number from the research pharmacist by phone thus eliminating allocation bias[68].

1.1.4.8. Blinding:

All enrolled infants will receive active medication; only the research staff and pharmacist will be aware of the infants' assigned group. Study medication will be reconstituted by research staff in a designated private space in the NICU. The clinical caregivers and subjects will be blinded to dose and dilution, although an experienced clinical caregiver may be able to guess the dose and dilution. Research staff monitoring the enrolled infants during study intervention will collect objective data thus minimizing bias. It is not possible to blind nebulizers as they are of very different configurations and covering them will interfere with monitoring during clinical care. Infants will remain on their allocated nebulizer device for the duration of the study.

1.1.4.9. Clinical Care of Enrolled Infants:

The attending neonatologist and clinical team will make all decisions for initiation of NIV support and need for intubation and/or intratracheal surfactant instillation. Consensus guidelines have been agreed upon by clinical staff based on previously published studies for consistency[7,33,34,69-74].

Suggested initial and maximum settings for NIV support are provided although clinicians may individualize care (Table 11)[33,34,74]. Escalation of NIV will be recommended for increasing FiO2 (>10% from baseline), partial pressure of CO2 (PCO2, >10 mmHg from baseline), or clinical respiratory distress. Weaning will be recommended for SpO2 and PCO2 maintained at 90-95% and <65 mmHg respectively for 4 hours in the absence of significant clinical signs of respiratory distress[34].

Table 11: Suggested initial and maximum settings for NIV support

Settings	NIPPV		NCPAP		HFNC	
	Initial	Max	Initial	Max	Initial	Max
rate bpm	10	40				
PIP (cm H2O)	10 above PEEP	18				
PEEP (cm H2O)	5-6	8	5-6	8		

FiO2 % to maintain SpO2	90-95%	90-95%	90-95%	90-95%	90-95%	90-95%
Ti Seconds	0.3-0.5	0.3-0.5				
Flow LPM	8-12	8-12	8-12	8-12	2-3	5-6

In our unit, infants ≤32 weeks GA with RDS being treated with NIV receive *caffeine* shortly after starting NIV as recommended/reported in the literature[7,74]. Enrolled infants will receive the 1st dose of caffeine prior to study intervention as standard clinical care in this study. This will prevent confounding of assessment of efficacy of study intervention both within and between subjects by caffeine effect. Caffeine therapy will consist of an intravenous loading dose of 20 mg/kg caffeine citrate/kg body weight followed by a daily maintenance dose of 5 mg/kg; duration of treatment will be determined by the clinical team.

Recommended criteria for intubation will be same as defined under Exit criteria (Section 3.6.5). If in an enrolled infant respiratory distress progresses and criteria for intubation are met prior to the institution of nebulized surfactant, the infant will exit the study and rescue therapy will be instituted immediately. *Recommended criteria for adjustments to ventilator settings* on conventional and high frequency ventilation include maintaining PaO2 50 to 70 mmHg, or oxygen saturation 90% - 95%, and PaCO2 <65 mmHg.

Infants who are enrolled in the study and subsequently require intubation may receive *intratracheally instilled surfactant* at the discretion of the clinical team. It is not necessary to wait 6 hours from last dose of AS to administer the first dose of intratracheal surfactant if other clinical criteria are met[75]. *Criteria for subsequent clinical intratracheal surfactant doses* (drawn from a clinical stock of Survanta) will include need for MV with FiO2 >0.30 and mean airway pressure >7 cm H2O for ≥20 min at ≥6 hours from last dose[69,76].

It will be recommended that extubation be attempted within 24 hours after the infant meets all of the following criteria: PaCO2 <65 mm Hg, pH >7.20, SpO2 90-95% with an FiO2 < 0.50, mean airway pressure <10 cm of water, ventilator rate < 20 breaths per minute, an amplitude of less than twice the mean airway pressure if high-frequency ventilation is being used, and hemodynamic stability[75].

All PT neonates with respiratory distress requiring NIV receive a *chest radiograph* (CXR) as standard clinical care; a CXR is not mandatory prior to enrollment for this study. *Echocardiography* and c*ranial sonography,* will be performed at the discretion of the clinical team based on infants GA and clinical status.

For PT infants with RDS to have the best outcome it is essential that they have *optimal supportive care*, including delivery room care, maintenance of normal body temperature, proper fluid management, nutritional support, circulatory support to maintain blood pressure & tissue perfusion, and skin-to-skin contact and kangaroo care[7]. Clinical protocols in place for the care of preterm infants in our unit will be followed.

1.1.4.10. Monitoring During & After Aerosol Administration:

Every attempt will be made to administer AS with the infant in stable condition, with heart rate >120 bpm, and oxygen saturation (SpO2) >85%. Prior to or concurrent with enrollment, capillary/arterial blood gas (CBG/ABG) analysis, vital signs including pulse oximetry (SpO2), respiratory support & receipt of medications such as caffeine, pressors or sedation will be recorded. Newborn respiratory distress score used in the Acute Care of At-Risk Newborns (ACoRN) program (adapted from Downes) will be used to objectively evaluate changes in respiratory distress during study intervention[77,78] (Appendix VII [Table A5])). Severity of illness will be determined using the CRIB (Clinical Risk Index for Babies) score[79,80] (Appendix VII [Table A6])).

Adverse events (AEs) during study aerosol administration will be recorded including: desaturations, apnea, bradycardia, tachycardia, air leaks, need for resuscitation, occurrence of coughing/gagging, surfactant reflux from the nose/mouth, need for positive pressure inflations, residual surfactant in nasal prongs or nebulizer chamber, and rainout in the ventilator tubing (Appendix VII [Tables A8-A9]). We will take pictures of components of the ventilator circuit without taking a picture of the subject – but this will not be mandatory. 60 ±30 minutes after end of nebulization, a CBG/ABG will be obtained and SpO2 values noted. It will be requested that clinical team make no changes to respiratory support from the start of aerosol till the CBG/ABG 60±30 minutes after end of nebulization has been obtained. However, if for patient well-being these changes are deemed necessary during this time by the clinical team, a note will be made in the research records.

Nasal trauma will be scored prior to AS, at end of AS and at 24 hours after AS[53,81] (Appendix VII [Table A7]). At each time point, the nasal prongs will be briefly slid out of the nares and scores assigned for redness, bleeding, ulceration, or skin breakdown (Nasal Trauma Score) by research staff. In addition at the conclusion of the study intervention, the bedside nurse and respiratory therapist will complete a *Feasibility Questionnaire* to determine impact of study intervention on clinical care (Appendix VIII).

Ventilator parameters and vital signs will be monitored every 15 min during administration of aerosol, 30 min after completion of aerosol, every hourly till 4 hours and every 4 to 8 hour till 72h after completion of aerosol. CBG/ABGs are generally followed every 4 to 6 h for the first 24 h of life and every 4 to 12 h for the next 48 h, as clinically indicated and will be recorded. Cerebral oxygenation will be monitored before, during and after AS in all subjects. Demographic and clinical data will be collected prospectively. We will also record respiratory support at 3d, 7d, 14d, 28d and 36 weeks corrected GA; clinical course; need for medications (caffeine, pressors, sedation, indomethacin/ibuprofen, diuretics, postnatal steroids, antibiotics course, blood transfusions); morbidities; outcomes at discharge; and laboratory and imaging results.

1.1.4.11. Cerebral Oximetry Using Near Infrared Spectroscopy (NIRS):

Participation in this part of the research protocol will be mandatory. Cerebral oxygen saturation (RCO2) will be monitored before, during and after administration of AS using NIRS. Monitoring will be started once consent is obtained and will continue for 6 hours after end of last dose of AS. RCO2 reflects oxygen saturation in veins (70–80%), capillaries (5%) and arteries (15–25%), and can be used as a surrogate for oxygen saturation in jugular venous blood[82,83]. A near infrared spectrometer (Covidien®) will be used. A neonatal transducer, containing a light-emitting diode and two distant sensors, will be placed on the forehead in the midline. RCO2 will be calculated from differential signals obtained from the two sensors, expressed as the venous-weighted percentage of oxygenated hemoglobin (oxygenated hemoglobin/total hemoglobin)[84]. To investigate the balance between O2 delivery and consumption, relative cerebral fractional tissue oxygen extraction (cFTOE) will be calculated as: (SaO2 - RCO2)/SaO2 where SaO2=arterial oxygen saturation[85].

1.1.4.12. Gastric Aspirates for quantitative PC analysis using LC-MS:

Parent(s) will be given an option for their infant's participation in this part of the study involving assessment of surfactant activity at baseline and end of AS in gastric aspirate samples. Surfactant activity will be determined by quantifying PC using LC-MS at the Lipidomics core at Wayne State University (Support letter from Dr. Maddipati, Director Lipidomics Core attached). Baseline values will help us predict if an infant will respond to AS and levels after AS will help to assess efficacy of delivery and response to AS.

Gastric aspirates will be collected from eligible infants prior to starting AS and at the end of AS using a using an appropriate size nasogastric tube typically in place for routine clinical care. All samples will be assigned coded labels, frozen at –20°C and transported to the Lipidomics Core in batches for analyses.

1.1.5. Historical Controls:

The Phase I/II Clinical Trial under this protocol does not have a placebo group; all randomized subjects receive active treatment. Preterm infants 24 0/7 - 36 6/7 weeks gestation that were admitted to the Hutzel Women's hospital NICU during the enrollment period (Dec 10, 2014 to Aug 31, 2017) will serve as historical controls to be able to compare intubation rates in infants enrolled and those not enrolled in the study. This will also allow comparison of other in-hospital outcomes, give an idea of numbers of infants available for the future Phase III clinical trial and the optimal time for administering study intervention.

1.1.6. Exit Criteria/Treatment Failure Criteria:

It will be suggested that infants be intubated and receive MV if they met 2 or more of 5 failure criteria:

i). worsening clinical signs of respiratory distress (increasing tachypnea; expiratory grunting; intercostal, subcostal, and/or sternal recession);
ii). apnea treated with positive pressure ventilation (PPV) by mask on 2 or more occasions in 1 hour;
iii). FIO2 >0.5 to maintain pulse oxygen saturations 90%-95% for >30 minutes;
iv). pH <7.2 on 2 arterial or capillary blood gases taken >30 minutes apart; and
v). partial pressure of CO2 (PCO2) of >65 mm Hg on 2 CBG/ABGs taken 30 minutes apart.

1.1.7. Primary outcome:

1.1.7.1. Safety:

Safety will be assessed by monitoring for oxygen desaturations, apnea, bradycardia/tachycardia, hypotension, air leaks, need for resuscitation, nasal trauma score, agitation, blockage of nasal prongs or NIV circuit, and cerebral oxygen saturation prior to, during and after study drug administration (Adverse Event [AE] Monitoring Form, Appendix VII [Table A8, A9]). Other safety measures that will be monitored include increased secretions requiring suctioning, coughing/gagging, surfactant reflux from nose/mouth, rainout in ventilator tubing, dislodged nasal prongs, abdominal distension, and nebulizer malfunction. It is expected that need for intubation is likley to be higher in the lower GA group strata.

1.1.7.2. Feasibility:

We define feasibility for this pilot RCT as the ability to:

i). Safely deliver AS to PT infants with RDS without interrupting clinical care because of clogged nasal prongs or ventilator alarms related to inline nebulization. At the end of AS a Feasibility Questionnaire will be administered to bedside nurses *&* respiratory therapists to evaluate this (Appendix VIII).

ii). Secure cooperation of clinical bedside nurses and respiratory therapists in administering aerosol.

iii). Identify and randomize a sufficient number of patients within a reasonable study period. This includes ability to identify eligible infants, obtain informed consent, and prepare and deliver aerosolized study medication in the defined time frame.

1.1.7.3. Dose Finding:

We will randomize infants to receive one of 4 fixed dosing schedules. Data collected during this Phase I/II trial will be critical in selecting the optimal dosing schedule for the definitive Phase III trial. Optimal dosing schedule will be determined by preliminary evidence of efficacy, lack of adverse effects, and tolerance by patients and bedside clinical caregivers.

1.1.7.4. Efficacy:

The primary efficacy outcome for this Phase I/II trial is the need for need for intubation and MV within 72 hours of study intervention (treatment failure). This will be compared to a historical control rate of 0.5 for need for intubation in PT infants 24-36 weeks GA undergoing NIV. Treatment failure criteria are defined *a priori* in Section 3.6.5. We chose treatment failure within 72 hours of randomization as our primary efficacy outcome as failure within that time would likely be due to RDS, whereas failure after this time would more likely be due to secondary causes like sepsis, PDA, pneumonia, NEC, IVH, or apnea of prematurity.

1.1.8. Secondary Efficacy Outcomes:

1. CBG/ABG, pulse oximetry, vital signs at 60±30 minutes after end of AS
2. Number of doses of surfactant – aerosolized & intratracheal
3. Respiratory support at 24, 48 & 72 hours, 7 days (D7), D14, D28, 36 weeks corrected GA and Discharge.
4. Pneumothorax, pneumomediastinum or other air leak
5. Changes in cerebral oxygenation as evaluated by NIRS
6. Changes in surfactant activity in gastric aspirates
7. Cumulative duration of NIV and invasive ventilation on D3, D7, D14, D28, 36 weeks CGA and discharge
8. Duration of supplemental oxygen, intensive care, hospital stay
9. Age at start of feeds, feeding progression, age at full enteral feeds
10. Need for blood transfusions
11. Growth parameters at 7 days, 28 days, 36 weeks corrected GA and discharge
12. Morbidities associated with prematurity: PDA, IVH (Grade III-IV), NEC, periventricular leukomalacia, ventriculomegaly, ROP (stage>3 or requiring treatment), and BPD. Two definitions of BPD will be used:
    a) Physiological definition: receipt of > 30% supplemental oxygen at 36 weeks or the need for positive-pressure support or, in the case of infants requiring < 30% oxygen, the need for any supplemental oxygen at 36 weeks after an attempt at withdrawal of oxygen[86].
    b) Receipt of any supplemental oxygen at 36 weeks.
13. Survival to hospital discharge
14. Survival to discharge without severe morbidity, defined as any of the following: BPD, late onset sepsis, ROP, or serious brain abnormality (IVH, periventricular leukomalacia, or ventriculomegaly)

1.1.9. Monitoring for Adverse Events (AEs):

Adverse events (AEs) that will be monitored during the study and a toxicity grading scale for potentially serious and life-threatening AEs is defined in Appendix VII [Tables A8, A9][87].

1. Plugging of nasal prongs, increased secretions requiring suctioning, occurrence of coughing/gagging, surfactant reflux from the nose/mouth, residual surfactant in nasal prongs or nebulizer chamber, rainout in ventilator tubing, dislodged prongs, abdominal distension, and agitation
2. Nebulizer malfunction – clogged, no visible mist, disconnected ventilator tubing
3. Bradycardia, tachycardia or hypotension requiring treatment during or within 6 hours after study intervention
4. Desaturations and/or apnea during or within 6 hours after study intervention
5. Need for resuscitation (positive pressure ventilation, chest compression or cardiac medications) during or within 6 hours after intervention
6. Air leak (pneumothorax, pneumomediastinum) during or within 6 hours after study intervention

7. Nasal trauma score (Appendix VII [Table A4]) before AS, at end of AS and 24 hours after end of AS
8. Death

1.1.10. Sample Size:

In this safety, feasibility and dose finding Phase II trial, 30 infants will be assigned to each of the 4 dose schedules[4,88]. The aim of phase II trials is to explore different doses, evaluate safety & tolerability, and obtain early indication of efficacy[88]. The dose-safety relationship is more important than the dose-efficacy one as efficacy is irrelevant if the dose is unsafe[89]. Phase II trials are generally inadequate with respect to size, outcome, precision, and control of bias to provide formal proof of efficacy[3,90]. Rather these trials provide baseline data, potential primary outcome and effect size justifying the larger, longer, and more expensive Phase III trial. *Recognizing that our Phase II trial is not powered for efficacy, sample size calculations based on precision of the estimate of the primary outcome & the null hypothesis and error rate are presented below.*

Sample Size Based on Precision for the Primary Efficacy Outcome:

The efficacy goal of Phase II studies is typically to estimate a clinical endpoint with a specified precision. The primary efficacy outcome in this study is the need for intubation & MV within 72 hours of AS. Using the normal approximation to the binomial as described by Piantadosi[88] ($w = Z_\alpha \times \sqrt{p(1-p)/n}$; or more simply $n=1/w2$ for estimated proportions (p) ranging from 0.2 to 0.8, where Z_α is the quantile from the normal distribution corresponding to the two-sided probability, n=sample size, w=width of the 95% confidence interval [CI] or precision), a sample size of 30 per dosing schedule group will allow evaluation of a failure rate of 0.25 - 0.4 with 95% CI of ±0.15 to ±0.17 (15 to 17% precision) (Table 12). The precision of this estimate is increased if we collapse the dilution groups corresponding to dose of 100 mg/kg & 200 mg/kg (<12%) or consider our entire sample of 120 subjects all of whom receive the study intervention (<9%).

Table 12: Sample Size & Precision for various proportions of primary efficacy outcome:

Sample Size	Rate	Precision for Estimating Outcome		
		95% CI		Precision
		Lower	Upper	
30 (for each dosing Schedule i.e. I, II, III, IV)	0.25	0.13	0.43	±15%
	0.3	0.17	0.48	±15%
	0.35	0.21	0.53	±16%
	0.40	0.25	0.58	±17%
60 (for each dose group 100/kg vs. 200 /kg)	0.25	0.16	0.37	±11%
	0.3	0.2	0.45	±12%
	0.35	0.24	0.48	±12%
	0.40	0.29	0.53	±12%
120 (for the whole group)	0.25	0.18	0.33	±8%
	0.3	0.23	0.39	±8%
	0.35	0.27	0.44	±8%
	0.40	0.32	0.49	±9%

Type I error of 0.05, two-tailed test

Sample Size Based on the Null Hypothesis& Error Rates:

We will also compare the primary efficacy outcome to that of historical controls. The eligibility criteria, baseline factors, clinical management protocols, criteria for NIV and MV, & outcomes in our study are similar to those of recently published clinical trials. The use of historic controls will decrease sample size, shorten duration, and enhance efficiency of our Phase II study; definitely an advantage for testing four dosing regimens in this orphan population[1,90-94]. Additionally, since AS has been shown to be potentially beneficial, this will allow all patients to receive study medication early and non-invasively thus enhancing study feasibility.

The rate of intubation following NIV for RDS among 24-36 weeks GA PT infants is ~50%[75,95]. We estimated a 50% relative risk reduction to be clinically significant and plausible based on our preliminary data & reports in literature. Studies of MIST during NIV in PT infants 24- 36 weeks GA with RDS show an absolute risk reduction of the need for MV of 10-50% and a relative risk reduction of 21-63% (RR 0.67, 95% CI 0.57, 0.79) with a lower treatment threshold (FiO2≤0.45) conferring a greater advantage (RR 0.72, 95% CI 0.59, 0.87) compared to a higher threshold (FiO2>0.45) (RR 0.55, 95% CI 0.40, 0.77)[5,33,75,92,93,95-104].

A sample size of 30 subjects within each dose schedule group will have 81.8% power to detect a relative risk reduction of 50% (absolute risk reduction 25%) in the primary outcome of the need for intubation in 72 hours compared to a historical control value of 0.5 in a two-tailed type I error rate of 0.05 (Table 13).

Collapsing dilution groups corresponding to dose of 100 mg/kg & 200 mg/kg or considering our entire sample of 120 subjects all of whom receive AS will allow us to detect a smaller effect size with greater power.

An alternative strategy for sample size of Phase II trials is to reduce the level of rigor required by increasing the Type I error rate to as large as 25%[90,105]. This increases the risk of erroneously concluding that the treatment is worthy of further investigation but does not increase the risk of missing an efficacious treatment. This approach would allow us to evaluate the effect sizes in Table 13 with smaller sample sizes.

Table 13: Power for a Test of the Null Hypothesis assuming a Historical Control Value of 0.5

Sample Size	Rate	Absolute RR	Relative RR	Power
30 (for each dosing Schedule I, II, III, IV)	0.25	.25	.50	81.8%
60 (for each dose group 100/kg vs 200 /kg)	0.25	.25	.50	98.2%
	0.3	.2	.40	89.0%
120 (for the whole group)	0.25	.25	.50	99.9%
	0.3	.20	.40	99.5%
	0.35	.15	.30	91.6%

Power is for a two sided test with Type I error of 0.05

After enrollment of 32 infants in the Phase II study, it was recognized that there was difficulty in recruiting infants in the lowest GA stratum. The data monitoring committee (DMC) recommended:

a. Re-distribution of sample size for the study (n=120) between the two higher GA strata (60 infants per stratum compared to previous sample size of 40 infants per stratum). This will allow retention of power of the sample size calculations in the original proposal for analysis in the margins.

b. Aim to enroll additional 10-20 at least (maximum 40) patients in the lowest GA stratum to address feasibility of study intervention in this group and generate hypothesis for effect size estimate for efficacy in this stratum.

On July 21, 2016, we submitted a clarification amendment to the IRB requesting continued enrollment in Stratum I upto a maximum of 40 subjects as originally proposed till the enrollment targets for the higher two GA strata were met (approved 07-29-2016). This was based on our enrollment patterns, making it seem unlikely that we would reach our enrollment targets ahead of the timeline (30-36 months for study accrual) in the three GA strata.

In Dec 2016, we attained accrual goals for the two higher GA strata. We observed a change in enrollment patterns in the last 5 months of 2016 - we were able to enroll more infants overall and specifically more infants in Stratum I. Since we had several more months available for patient accrual on this grant, we requested an amendment allowing us to continue enrollment in the lowest stratum till Dec 09, 2017 for a maximum of 40 subjects in this stratum, whichever comes earlier. Since Stratum I subjects are most likely to benefit from this intervention, having as much data as possible about feasibility and efficacy in this GA group will be valuable in designing the future definitive Phase III randomized controlled trial.

This amendment was discussed with the FDA grant officer who recommended (02-17-2017) proceeding with amendment submission to IRB and IND review Division if the DMC concurs.

The DMC met on 02-22-2017 and recommended continuing subject accrual in the lowest GA Stratum (I) till Dec 2017. In addition, the DMC recommended continuing subject accrual in the two higher GA Strata subject to availability of resources; enrollment in excess will increase power and confidence in the results.

We proposed increasing the sample size by 40 to account for additional infants to be enrolled in the two higher GA strata till Dec 2017 or when 40 subjects have been accrued in Stratum I, whicever comes earlier. In addition wee requested that the sample size for the study be increased by 20 to accommodate for current and future screen failures (estimating a maximum of 10 screen failures in the future). These two changes will increase the total sample size of the Phase II portion of the study to 220 subjects, and overall sample size of the study including the 17 subjects enrolled in the Phase I portion of the study will be 237.

1.1.11. Patient Population Available for the Single Center Phase II study:

This study will be performed primarily at HWH, a level III neonatal intensive care unit (NICU) and high risk maternal referral center. Numbers of infants $24^{0/7}$ – $36^{6/7}$ weeks GA treated for RDS are estimated from the data presented in Section 3.3.3. The goal is to enroll 120 subjects over 3 years. Given the statistics for the past 2 years an adequate population of infants is available even after considering a continued trend for declining birth rate, 10% need for intubation in delivery room and a conservative estimate of consent rate of 50%;.

Table 14: Patient Population Available for Study:

GA	Admissions at HWH			Estimates of infants requiring treatment for RDS (%)*		Estimates of Projected # of infants requiring treatment for RDS at HWH (n)			
						Over 2 years		Over 3 Years	
	2011	2012	Total	Lower	Higher	Lower	Higher	Lower	Higher
24-28	76	76	152	0.38	1.00	58	152	87	228
29-32	120	103	223	0.27	0.62	60	138	90	207
33-36	462	462	924	0.10	0.18	88	166	132	249
Total	**658**	**641**	**1299**			**206**	**457**	**309**	**685**

* based on Table 2; shaded rows represent population of interest for this study

1.1.12. Statistical Analyses:

Data will be analyzed according to the intention-to-treat principle using SAS and PASW (IBM Inc.). We will report descriptive statistics for demographic, clinical and respiratory support data at baseline and over time for the entire sample and for the 4 dosing schedule groups (Appendix IX [Table M1, M2]). In addition, three subgroup analyses are planned – according to dose regardless of dilution (100 vs. 200 mg/kg), GA strata, nebulizer device using logistic regression incorporating an additional treatment-by-subgroup interaction term.

Analysis plan to address Aim #1 – Safety and Feasibility Assessment: Safety will be assessed by quantifying and comparing the AEs and their severity for the total sample and across the dosage/dilution groups and two types of nebulizers. Indicators include cardiorespiratory responses, blockage of NIV circuit and cerebral oxygen saturation (Appendix IX [Table M5]). Descriptive statistics (mean, standard deviation, proportion, inter-quarter range [IQR]) will be used to quantify these indicators; *confidence intervals will be preferred to hypothesis testing* as considerable imprecision is expected arising from low frequencies of these AEs[3]. ANOVA or *t*-test (continuous measures) and χ^2 test (categorical variables) will be used for comparative analyses though we may not find statistically significant differences. Paired *t*-test will be used to compare heart rate, respiratory rate, blood pressure, SpO2, PCO2, cerebral oxygenation (RCO2, cFTOE), ACoRN score and nasal exam score before and after AS. These parameters will also be compared between infants who get intubated within 72 hours of study intervention and those who do not; and before and after intubation within subjects that get intubated. Feasibility will be assessed by quantifying and comparing the percentage of infants who tolerate the treatment procedure and responses of the clinical nurses and respiratory therapists on the feasibility questionnaire (Appendix IX [Table M6]). Because of the high inherent variability of these factors and small effect sizes, we do not expect to show significant differences between groups given our sample size. However this information will be useful in making the final decision to proceed with a Phase III RCT.

Analysis plan to address Aim #2: - Determination of Optimal Dilution and Dosage of AS: The optimum dose is one that produces low toxicity while nearly maximizing the biological response[89]. The optimal dosage will be determined by comparing levels of adverse responses, feasibility items and primary and secondary efficacy treatment outcomes (rates of intubation, diagnosis of PDA, need for blood transfusions, postnatal growth, duration of respiratory support, length of hospital stay, and incidence of complications of prematurity including IVH, sepsis, pneumonia, BPD, NEC, ROP, and death) across the four dosing schedules (Appendix IX, Mock Tables). The optimal dosage/dilution will be the one with the minimum level of adverse responses and highest clinical treatment outcomes.

Analysis plan to address Aim #3 - Short-term efficacy of AS: The primary outcome variable for short-term efficacy is the need for intubation & MV within 72 hours of AS. We will estimate the unconditional probability of benefit for the four dosing schedule groups (I, II, III, IV), the 2 dose groups (100 vs. 200 mg/kg) and the entire study cohort of 120 subjects. We will also compare with reported rates of intubation in historical controls (50%). A special χ^2 test to compare sample proportion with population proportion will be conducted to test the study hypothesis that AS is efficacious. In the comparison analysis, different dosages/dilutions will also be considered as weight using the "optimal" dosage/dilution obtained from Aim #2 as reference. A significantly lower rate of intubation in infants treated with AS in this study as compared to the reported rate of ~0.5 at $p<0.05$ level (two-sided) will be used as evidence supporting the efficacy of AS. However, it is likely we may not

have power to achieve statistically significant results. Nevertheless these preliminary efficacy data will be crucial in determining outcome measures and effect size for the definitive placebo-controlled Phase III trial.

A number of secondary outcomes will be assessed using the same methodology. Vital signs, SpO2, FiO2, NIV parameters (pressure, rate, flow) and ABG/CBG components (pH, PO2, PCO2) will be evaluated before and after study intervention (paired *t*-test) and over time (Longitudinal data analysis). Other secondary outcomes include change in surfactant activity in gastric aspirates, changes in cerebral oxygenation, diagnosis of PDA, need for blood transfusions, postnatal growth, duration of respiratory support, length of hospital stay, and incidence of complications of prematurity including IVH, sepsis, NEC, BPD, pneumonia, ROP, and death. We will describe and compare primary and secondary outcome data overall and by dosing schedule, and in three pre-planned subgroup analyses – by dose regardless of dilution (100 mg/kg vs 200 mg/kg), GA group stratum and nebulizer type, recognizing that we may observe trends of differences between groups without having power to determine statistical significance. We will report dichotomous secondary outcome data as proportions and compare the groups using χ^2 and nonparametric tests. We will report secondary continuous outcome data as mean (SD) for normally distributed data and median (interquartile range [IQR]) for skewed data, and will compare the groups by using parametric (*t*-test, ANOVA) and nonparametric tests (Mann-Whitney U test, Wilcoxon's test) as appropriate. Pairwise group comparisons of the four dosing schedule groups will also be performed, although we may lack power to detect significant differences. In a final posthoc analysis, we will compare infants who received intubation within 72 hours to those who did not to understand risk factors for intubation and their modification by study intervention. This analysis will be presented for the entire group and by dosing schedule group. In *adjusted analyses*, dosing schedule, GA group, nebulizer type, mode of NIV, antenatal steroids, and presence of PDA or IVH will be included as covariates recognizing that these analyses are exploratory and need to be interpreted cautiously. We will consider a p value of <0.05 for statistical significance. Mock tables for the planned analyses are presented in Appendix IX.

Recommendations for statistical analyses by the Data Monitoring Committee (DMC) during the conduct of the trial:

1. Additional factors associated with intubation in enrolled infants – clinical team, time of day/shift, clinical variables (e.g. sepsis, NEC)
2. Performing a survival curve analysis to present timing of intubation in infants who were intubated
3. Control for mode of ventilation at baseline and exploring a GA cutoff at which this intervention may be most beneficial.
4. Detailed caffeine data in relation to AS to be provided
5. DMC requested data for intubation rates in the three GA strata outside this clinical trial setting (as reported in the literature and at Hutzel Women's Hospital by initiating an observational study) as there is no placebo group in this analysis
6. Handling of infants that exited study prior to study intervention
    a. DMC recommended performing a dual level analysis
        i. The primary "intent to treat analysis" should include all enrolled subjects.
        ii. A secondary "experimental analysis" should also be presented that only includes subjects that received study intervention.

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