A Randomized Trial of Targeted Temperature Management with Whole Body Hypothermia for Moderate and Sever Neonatal Encephalopathy in Premature Infants 33-35 Weeks Gestational Age - A Bayesian Study

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A RANDOMIZED TRIAL OF TARGETED TEMPERATURE MANAGEMENT WITH WHOLE BODY HYPOTHERMIA FOR MODERATE AND SEVERE NEONATAL ENCEPHALOPATHY IN PREMATURE INFANTS 33-35 WEEKS GESTATIONAL AGE – A BAYESIAN STUDY

Short title: Preemie Hypothermia

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Objective: Determine whether whole body hypothermia for 72 hrs in preterm infants 33-35 wks gestational age (GA) and ≥1500 grams birth weight who present at <6 hrs postnatal age with moderate to severe neonatal encephalopathy (NE), formerly called hypoxic-ischemic encephalopathy (HIE), is safe and will reduce death or moderate/severe disability at 18-22 months corrected age.

Study Design: A staged prospective, randomized multicenter trial with qualifying infants randomized 1:1 to whole body hypothermia (33.5 °C esophageal temperature [Tes]) for 72 hours or normothermia (with steps to avoid hyperthermia [>37.3°C Tes]). Treatment assignment will be unmasked.

Eligibility criteria: Infants 33 0/7 – 35 6/7 wk GA (best obstetric estimate) and ≥1500 grams birth weight with pH (cord or <1 hour neonatal) ≤7.0 or base deficit ≥16 mEq/L **or** an acute perinatal event and either a 10 minute Apgar score ≤5 or ventilation initiated at birth and continued for ≥10 minutes. All such infants must have seizures or signs of moderate or severe encephalopathy (with abnormal level of consciousness as one of the signs) at ≤6 hours of age at enrollment.

Study intervention: Infants will be randomized to either whole body cooling (Tes 33.5°C) or normothermia (Tes 36.5 – 37.3°C, with steps to prevent Tes >37.3°C) for 72 hours. Hypothermia will be attained with the Cincinnati Sub-Zero Hyper/Hypothermia Device. Surveillance for safety and adverse events within 108 hours of baseline (insertion of esophageal probe) will be compared using Bayesian techniques. This will include cranial ultrasound no later than 24 hours after baseline. The first interim DSMC analysis will occur after the first 20 infants are enrolled for 108 hours. All survivors will undergo brain MRI .at 10-17 days of postnatal age We will strongly encourage that the MRI be obtained at 10-17 days of postnatal age or prior to NICU discharge/transfer to non-NRN center (if <10-17 days of postnatal age) but will include MRI studies obtained at other times post-hypothermia/control intervention as well, and undergo standard interdisciplinary neurodevelopmental assessment at 18-22 months corrected age.

Primary outcome: Death or moderate/severe disability at 18-22 months of age, using standard Neonatal Research Network (NRN) criteria.

Sample size: Since data are insufficient to predict enrollment, a Bayesian approach is planned. If safety and efficacy/futility reviews permit continuation for 2 years after 100% of NRN centers have been IRB-approved and screening, subject accrual will be reviewed. If <50 subjects have been enrolled, the subcommittee will discuss trial continuation on the basis of expected enrollment over the next 1 to 3 years and communicate that discussion to the Data Safety Monitoring Committee (DSMC). Extension (for an additional 1-3 years of recruitment) or termination of the trial will be evaluated and approved/rejected at this two-year review, based on projected ability to enroll a maximum of 168 infants.

Duration of Study: The duration of the study will be projected at 2 years after all centers have been screening. Follow-up of survivors for interdisciplinary NRN neurodevelopmental assessment at 18-22 months corrected age is anticipated after the final subject is enrolled.

1.0 STATEMENT OF THE PROBLEM

Neonatal encephalopathy (NE) caused by perinatal hypoxic-ischemic injury is a final common pathway of multiple perinatal pathologic conditions (e.g., uteroplacental dysfunction, cord and/or placental accidents, acute blood loss, infection, maternal hemodynamic compromise and others). It may afflict fetuses and newborn infants at all gestational ages and is a major determinant of death and quality of survival. Although many interventions to attenuate perinatal hypoxic-ischemic brain injury have been proposed and tested, only brain hypothermia (induced systemically or by focal cooling of the head) has been demonstrated by multiple investigators to offer significant benefit in neonatal humans ≥36 weeks gestation who undergo cooling for 72 hours beginning at <6 hours of age.[1-5,8] While brain cooling is far from a panacea for such infants, NRN investigators demonstrated an absolute reduction of 18% (relative risk reduction 0.28) in the incidence of death or disability at 18-22 months in a large randomized clinical trial of systemic hypothermia.[1] No interaction between gestational age and treatment effect was found. Simbruner et al reported an absolute reduction of 32% (relative risk reduction 0.39) in death or severe disability in a randomized, controlled European trial of whole body hypothermia in term infants with clinical and electrophysiological evidence of NE.[4] Chinese investigators demonstrated an 18% reduction of death or severe disability at 18 months in a trial of head cooling combined with body cooling.[5] Other investigators did not find significant reductions in death or moderate/severe disability with head cooling, but did find a decrease in death and disability among infants with less severe amplitude-integrated EEG (aEEG) changes[2] as well as decreased cerebral palsy and improved developmental scores in survivors following systemic cooling.[3] While not all differences attributable to brain cooling have been statistically significant, most positive outcomes have been associated with cooling. Although infants <36 weeks gestation may also suffer from perinatal hypoxic-ischemic injury, systematic assessment of the safety and utility of brain cooling in such premature infants has not been performed.

2.0 BACKGROUND AND SIGNIFICANCE

Most clinical studies of human NE and potential interventions have targeted infants ≥36 wks GA. Although many interventions have been suggested and assessed for prevention or palliation of NE, the only one currently supported by rigorous clinical evidence to improve outcome in human newborns has been hypothermia (targeting core temperature of 33.5 to 35.0°C. - with or without head cooling) implemented at ≤6 hrs of postnatal age and maintained for 72 hrs in infants ≥36 wks GA with moderate or severe NE.[1-5] Translation of these findings to infants who are more premature has been suggested as a subject for further scrutiny by some[6,7] and actually implemented by others.[8-17]

Reports of 'brain cooling' include infants of lower GA, but the incidence of such infants and their outcomes is unclear. One large randomized trial (ICE trial) included infants of 35 wks GA and has been published, but the number of infants at that GA and their outcomes have yet to be reported.[8] Eicher et al also included infants at 35 wks GA, but again the number of such infants and their outcomes (assessed at 12 months postnatal age in most survivors) were not specifically reported.[9,10] In some comparative series, GA restrictions are not reported.[11,12] Azzopardi et al included one infant who underwent hypothermia for NE at 34 wks GA and was reported to have normal neurodevelopmental status at 18 postnatal months (including Griffith's Developmental Quotient of 91).[13] In another study, Azzopardi et al reported a series of 120 asphyxiated infants treated with hypothermia outside of a clinical trial and included infants down to 34 wks GA; the number of infants <36 wks GA and their outcomes were not reported.[14] Zanelli et al and Kilani both report two infants each who underwent cooling at 34 wks GA, although outcomes were tracked only to NICU discharge and were not reported by GA.[15,16] Kilani reported an additional two infants <34 wks GA who met NE criteria but were excluded. Recently, Hall reported the use of hypothermia in 15 very preterm infants (mean GA 27 wks) with severe necrotizing enterocolitis and multi-organ dysfunction.[17]

Multiple other infants with GA <36 wks (including some ≤32 wks) have undergone therapeutic hypothermia for NE per observers across North America but have not been reported in the scientific literature. At least 10 such infants in the state of Utah (one from a then NRN site) are known to have undergone cooling since July 2008, albeit with neurodevelopmental status post-discharge unknown in most cases. Further, verbal communication with 9 centers in the Pacific Northwest and Intermountain West that offer non-randomized hypothermia for neonatal HIE revealed 8 of the 9 centers to have cooled a total of 15 infants <36 wks GA (5 not realized to be <36 wks until after cooling had been started) between November, 2011 and March, 2012. All 9 of these centers also reported knowledge of other infants <36 wks with NE who did not receive hypothermia for a variety of logistic reasons or who underwent cooling elsewhere. A presentation from the Vermont-Oxford Network Neonatal Encephalopathy registry (RH Pfister et al. PAS meeting 2010, Abstract 2632) reported that 12 of 495 infants (2.4%) who underwent therapeutic hypothermia for NE were <36 wks GA, although outcomes were not discussed. It was not reported how many other infants <36 wks GA had significant NE. It is clear that hypothermia for infants <36 weeks GA is 'creeping' into practice based on extrapolation from experience in more mature infants, with virtually no data to determine whether benefit outweighs risk.

2.1 How frequent is NE in infants 33-35 wks GA? The frequency of potentially qualifying infants has not been reported in any population-based source or network with a comprehensive referral area. Salhab and Perlman retrospectively observed that 62 of 5533 infants born between 31-36 wks GA over 10 years at a single institution (Parkland Memorial Hospital, Dallas, TX) had an umbilical arterial cord gas with a pH<7.00 with 7 of these having moderate or severe NE per Sarnat criteria (7/5533 = 1.3/1000).[18] Schmidt and Walsh noted an incidence of 8/1000 (12 of roughly 1500 infants born at 32-6 wks GA at a single center over a 6 year period), with NE detected retrospectively based on a cut point 5 minute Apgar <5 with subsequent requirement for acid-base criteria and

neurologic abnormalities).[19] A two-center feasibility trial of hypothermia for NE in infants 32-6 weeks GA was conducted by Schmidt and Walsh (ClinicalTrials.gov NCT00620711: Pilot study of head cooling in preterm infants with hypoxic ischemic encephalopathy. PI: WF Walsh), but only 5 patients were enrolled in 3 years. In an abstract submitted to the 2010 PAS meeting, Chalak et al retrospectively reviewed infants at 33-35 wks GA from 2005-2008 at Parkland Memorial and applied biochemical, clinical and neurologic criteria to identify those who met criteria for cooling used in those ≥36 wks GA.[20] They identified 7 qualifying infants among a total of 1305 at 33-35 wks GA (5/1000). These reports suggest a rate between 1-8/1000 for significant NE in the targeted GA, although it is unclear if the criteria applied for NE are appropriate for this GA range. A retrospective review of the 3 Utah NRN NICUs for a recent 12 month period indicated admission of 8 infants 33-35 wks GA who would have otherwise met criteria for cooling (8/380 = 21/1000). In a more recent 6 months (ending August 31, 2010), review of the 3 Utah NRN NICUs revealed a similar rate of admission for such infants (5/197 = 25/1000), with one such infant having undergone systemic cooling. We remain unable to determine how many similar infants were denied transfer for cooling or for whom transfer was not sought due to knowledge of our GA criteria.

A survey of 16 NRN centers indicated that in calendar year 2009, 3 infants <36 wks GA received hypothermia for NE, while 7 centers reported having received a total of 9 inquiries about cooling for such infants. Of the 16 centers, 13 were able to provide the number of infants admitted who were 33-35 wks GA (total 3801: 292 per center; extrapolation to all 16 centers: 4672). Use of the range of frequency of moderate or severe NE at this GA from the data cited in the previous paragraph suggests that as few as 5 or as many as 98 per year might be eligible for this proposed study, based on the current number of infants in this GA range in the NRN. A figure between these two extremes (e.g., 30-45 per year) appears likely. It is unclear if the requirement for abnormal level of consciousness as part of the qualifying neurologic exam will significantly alter the number of potentially eligible subject, but it is likely that infants with developmental hypotonia, poor suck and other such features without encephalopathy would not be inappropriately qualifying for enrollment. The number of infants outside the NRN who may qualify and be referred to NRN centers in the context of a publicized trial is unclear, although data presented earlier suggest that such infants do exist.

2.2 Potential adverse outcomes of induced hypothermia in infants 33-35 wks GA

Landmark early trials found increased mortality and morbidity with use of a 'hypothermic incubator' in infants <1500 grams birth weight.[21] The body temperature in those trials was maintained in a range that increases oxygen consumption and caloric expenditure for prolonged periods, as opposed to recent trials of hypothermia (including the current proposal) which maintain core temperature in a range associated with decreased oxygen consumption and caloric expenditure for a much more limited time period. Reported adverse effects of therapeutic hypothermia include coagulopathy, thrombogenesis, cardiac arrhythmia, superficial cold injury, metabolic acidosis, hypo- and hyperkalemia, hypo- and hyperglycemia, pulmonary hypertension and increased support requirements for respiration and perfusion. Concern about increased frequency or severity of such adverse events or other problems in infants 33-35 wks GA subjected to

'therapeutic' hypothermia are real, but there are minimal human data to determine whether benefits will outweigh risks. Nonetheless, it seems probable that assessment of this intervention in later preterm infants will reveal fewer problems than might be seen in those ≤32 wks GA, since the frequency of such potentially confounding problems as intracranial hemorrhage (ICH: focus on intraventricular, intraparenchymal and intracerebellar), NEC and BPD will be much lower. Conversely, problems more commonly seen in infants ≥36 wks GA (e.g., PPHN treated with ECMO or iNO) may occur less frequently at <36 wks GA. Walsh et al recently compared outcome for 48 infants 32-35 weeks GA who underwent therapeutic hypothermia for NE to 1289 infants ≥36 weeks GA using the combined Pediatrix database and UK TOBY study cooling registry data (Walsh WF. PAS meeting 2011, Abstract 3675) and found no significant increase in mortality, but the small numbers and absence of a single rigorous protocol for cooling make it difficult to know how robust this conclusion is. A rigorous protocol driven-trial such as the current protocol is the only way to obtain appropriate safety and outcome data.

2.3 Neurologic exam for NE in infants 33-35 wk GA Challenges are posed by potential differences in the neurologic findings that may occur in preterm infants. Normal preterm infants may have systematic developmental differences in primitive reflexes, tone and posture that modify interpretation of Sarnat staging[22] (e.g., hypotonia is normal at <30 wks GA, "pupillary reaction to light begins to appear at 30 weeks GA, but is not present consistently until approximately 32 to 35 weeks"[23]), although a number of investigators have attempted to apply such staging to older preterm infants.[19,24,25] The Sarnat criteria, as well as the modified Sarnat criteria used for neurologic qualification in the original NRN hypothermia study[1], were based on findings in infants ≥36 wks GA at birth.[26] Primitive reflexes, tone, posture and autonomic function in infants <36 wks GA are less defined in otherwise well infants, not to mention those who sustain insults unrelated to NE. In this proposal, we include the requirement for an abnormal neurologic criterion that is unlikely to be influenced by GA, i.e., level of consciousness.

2.4 Why pursue this study in the NRN? Despite the potential problems posed by this proposal, it is clear that the NRN is uniquely positioned to address the safety and effectiveness of hypothermia in later preterm infants with moderate or severe NE. Experience with systemic cooling, ready access to appropriate hardware and expertise, large service and referral populations, growing expertise in neuroimaging following hypothermia, follow-up infrastructure and expertise in assessment of neurodevelopmental outcome are important contributory factors. Given the ongoing diffusion of 'brain cooling' for indications that are not evidence-based (including use at <36 wks GA) and the unknown risk-benefit ratios, it is important for systematic prospective data to be collected and analyzed in a rigorous manner.

3.0 Study design

This will be a staged prospective, randomized multicenter trial with qualifying infants 33 0/7 -35 6/7 wks GA to be randomized to whole body hypothermia (33.5 °C esophageal temperature [Tes]) for 72 hours or normothermia (with steps incorporated to avoid hyperthermia [>37.3°C Tes]). The treatment assignment will be unmasked.

3.1 Primary hypothesis

The risk of death or moderate to severe disability at 18-22 months will be decreased in infants 33 0/7 - 35 6/7 wks GA and ≥1500 grams birth weight with moderate or severe NE at <6 hrs age who undergo targeted temperature management with whole body hypothermia to 33.5°C (as monitored by esophageal temperature) for 72 hrs than in those with core temperature targeted at 37.0ºC (controls).

3.2 Primary objectives

1) To assess differences in death or moderate/severe disability at 18-22 months in enrolled infants with and without whole body hypothermia for 72 hrs.
2) To determine short-term safety (i.e., within 108 hours of baseline [time of insertion of esophageal probe]) of targeted temperature management with whole body hypothermia for 72 hrs in infants 33 0/7 -35 6/7 wks GA with moderate to severe NE at ≤6 hrs of age. Safety criteria will include intracranial hemorrhage, cardiac arrhythmia; persistent acidosis; thrombosis; bleeding; skin changes; necrotizing enterocolitis (NEC); perforation, ulceration or bleeding from the esophageal probe; hypo- and hyperglycemia; receipt of ECMO and death.

3.3 Secondary objectives

Differences between study groups will be assessed for:

1) Death up to 18-22 months of age only
2) Cause of death (withdrawal of support and reasons for such will be tracked, as will autopsy results)
3) Disability only
4) Death or profound disability (assignment of lowest score on Bayley III because untestable due to degree of impairment)
5) Severe disability and moderate disability only
6) Survival with no impairment
7) Each component of severe and moderate disability (as described below in Section 3.13)
8) Differences in MRI findings obtained at 10-17 days of postnatal age or at time of discharge or transfer to a non-NRN center (if <10-17 days of postnatal age); MRIs obtained at other times after cessation of cooling/control period will also be included and recorded
9) Evolution of encephalopathy
10) Other safety issues (including seizures after beginning of assigned treatment, BPD, NEC, culture-positive bloodstream or infection of other normally sterile site at >3 days, pulmonary hypertension, ICH, esophageal probe injuries, metabolic abnormalities, treatment with vasopressors and/or steroids, oliguria, anuria, liver dysfunction (AST>200 IU, ALT>100 IU and/or direct bilirubin >1.5 mg/dL),

thrombocytopenia, coagulopathy, days receiving oxygen/mechanical ventilation, receipt of ECMO, duration of NICU stay in survivors)

11) Neurological injury by cranial ultrasound within 24 hours of enrollment.
12) Incidence of seizures on EEG (see EEG Secondary Protocol)

The study population will also be used to determine features associated with NE in infants 33-35 weeks GA:

1) Demographic features
2) Frequency
3) Associated clinical factors (including maternal characteristics, pregnancy complications, acute perinatal/sentinel events, intrapartum complications, mode of delivery; GA, BW, head circumference, gender, delivery room resuscitation, cord gas results, blood gas within first hour of life, NICU course and complications)
4) Outcomes other than death or disability.

3.4 Inclusion criteria

Infants 33 0/7 to 35 6/7 wks GA (best obstetrical estimate) and ≥1500 grams birth weight (to minimize potential difficulties placing esophageal probe) who meet clinical, biochemical and neurologic criteria for moderate to severe NE in this GA range at ≤6 hrs age:

1) Biochemical:
   Cord gas or blood gas within first hour of life with pH ≤7.00 or base deficit (BD) ≥16 mEq/L, OR

2) Clinical:
   a) Acute perinatal event (e.g., abruptio placenta, cord prolapse, uterine rupture, maternal cardiac or respiratory arrest, severe FHR abnormality such as variable or late decelerations,) AND
   b) Requirement for positive pressure ventilation for apnea or poor respiratory effort since birth for at least 10 minutes OR 10 minute Apgar score ≤5

AND

3) Neurologic:
   Seizures OR modified Sarnat score with abnormalities in at least 3 of the 6 categories; at least one must be altered level of consciousness (lethargy or stupor/coma) as determined by a certified examiner

   (All infants who meet criteria for potential inclusion should undergo standard neurologic exam as for infants ≥36 wks GA being considered for hypothermia, with findings recorded)

3.5 Criteria for exclusion in hypothermia trial

1) Receipt of paralytic agent or sufficient sedative or analgesic agent that is considered by the examiner to confound the qualifying neurologic exam
2) Etiology of NE not likely to be hypoxic-ischemic in origin
3) Major congenital anomaly that may confound outcome
4) Considered to be moribund and will not be receiving full intensive care
5) Equipment and/or appropriate staff not available
6) Infant was cooled prior to arrival at the NRN center and had a core temperature <34.0º C for more than 1 hour at the time of screening (Cooling on transport is to be discouraged, in general)
7) Unable to randomize by 6 hours of age
8) Infant to receive or receiving ECMO
9) All blood gases (cord and neonatal at <1 hour of age) have a pH >7.15 and a base deficit <10 mEq/L
10) Consent not obtained from parent(s)
11) Concurrence not provided by responsible attending neonatologist

3.6 Randomization and stratification: After informed consent is obtained, qualifying infants will be randomized by 6 hours of age to either hypothermia or the non-cooled control group. Randomization will occur 24 hours a day, 7 days a week by the Data Coordinating Center at RTI International, Research Triangle Park, NC. Infants will be stratified by center and degree of NE (moderate versus severe). In the unlikely event of a multiple gestation qualifying for inclusion, all who qualify will be randomized independently.

3.7 Intervention: Caregivers will be unmasked to randomization. An esophageal temperature probe will be inserted into the distal third of the esophagus after randomization and will be considered to mark the temporal baseline for this study. For infants between 1500 and 1800 grams, placement of the esophageal probe by the oropharyngeal route may be preferred if resistance is encountered during attempts at nasopharyngeal placement. Only CSZ temperature probes will be used (outer diameter 3.0 mm). Although probes used with the Mona-therm temperature monitor system are slightly smaller (outer diameter 2.9 mm), this system is not available at all centers and would add potential variability that we prefer to avoid. Appropriate placement of the probe will be confirmed in all subjects by radiograph within 12 hours of placement.

Hypothermia will be implemented with whole body cooling to an esophageal temperature (Tes) of 33.5º C. using a Cincinnati Sub-Zero Hyper/Hypothermia Blanketrol® device (CSZ) for 72 hours. Previous NRN experience has suggested that smaller infants may have unexplained intermittent drops in Tes to <32º C with the CSZ device.[26]

Infants in the control group will have their core temperature (using Tes) maintained at 37.0º C. by servo-control of skin temperature and adjustment of the setpoint based on esophageal temperature. A range of 36.5-37.3º C. will be considered acceptable for the control group. As in the Late Hypothermia trial, steps will be incorporated to prevent hyperthermia and potential associated adverse effects in the control group[27, 28] For Tes >37.3º C., 1) steps will be undertaken to assure that standard thermoregulatory

management is in place, 2) if Tes >37.5º C., active cooling will be implemented first with a single tepid sponge bath and, if that is unsuccessful, with cooling blanket and CSZ until target temperature range is attained.

3.8 Re-warming After 72 hours of hypothermia, the set point of the control on the CZT will be increased by 0.5º C. per hour until Tes is ≥36.5°C. Once Tes is ≥36.5°C and <37.3°C for ≥4 consecutive hours, the esophageal probe will be removed, and skin temperature control changed to a radiant warmer or carefully regulated incubator. For this trial, 'normothermia' in the re-warmed, previously cooled group will be operationally defined as having attained ≥36.5-37.3°C. for ≥4 hours with removal of the esophageal probe. The control group will undergo thermoregulation by the radiant warmer servomechanism or carefully regulated incubator, except for the previously noted circumstance of NE-associated hyperthermia.

3.9 Safety monitoring

Routine clinical care will be provided to all subjects, including vital signs, surveillance for organ dysfunction, and provision of metabolic, nutritional, respiratory and hemodynamic support as needed. Cardiorespiratory, glucose, renal, metabolic, skin, hematologic and neurologic status will be monitored intensely during the 72 hours of intervention/control as clinically indicated except for monitoring of platelet counts.

1. Esophageal and skin temperatures will be monitored continuously for all infants in both groups (including those who are seizing or have recently seized and are receiving anticonvulsants) and recorded every 15 minutes for the first 4 hours after baseline, every hour for the next 8 hours and every 2 hours for the remainder of the hypothermia/control intervention period. Hypothermia in the targeted range is typically attained within 3 hours of initiation. Temperature monitoring for control infants will be similar except for hourly temperatures during the first 4 hours of the intervention. Temperature deviations more than ±1.5°C. from the setpoint (i.e., <32.0 or >35.0° C. in the hypothermia group will be recorded, as will circumstances and interventions to return to the targeted range.
2. Metabolic: Serum, plasma or whole blood glucose determinations will be determined per the clinical team for the first 24 hours of cooling/control, coordinated with other clinically indicated laboratory studies as possible, as part of standard care. Electrolytes will be obtained per clinical routine,although we will strongly encourage that they be obtained at least every 24-48 hours during the hypothermia/control intervention period..
3. Respiratory: Blood gases will be assessed per the clinical team and corrected for body temperature. Respiratory support will be adjusted as needed per standard care.
4. Cardiovascular: Heart rate, blood pressure and use of vasoactive and inotropic agents will be recorded at baseline and every 4 hours throughout the intervention period. Cardiac arrhythmias will be sought, necessary interventions implemented and both recorded. Receipt of ECMO will be monitored.

5. Renal: Body weight and urine output will be recorded daily. Serum BUN and creatinine will be assessed and recorded as per standard care. Since BUN and creatinine usually reflect maternal values in the first 24 hours of life, we will strongly encourage assessing and recording them at 24 hours and at 24-48 hours during the hypothermia/control intervention period.
6. Neurologic status: A cranial ultrasound will be obtained prior to initiation of interventions for the assigned study group if possible or within 24 hours of such initiation, to assess for structural abnormalities, intracranial hemorrhage (ICH: with particular focus on intraventricular, intraparenchymal, intracerebellar and extra-axial hemorrhage) and other pathology associated with HIE. **This is mandatory and is paid for by the study**. Mastoid views should be obtained whenever possible. Local readings will be employed for this study. Other CNS imaging during the intervention/control period will be obtained as clinically indicated. Follow-up cranial ultrasound studies are specifically strongly encouraged if any subject has a drop in hematocrit >10% (e.g., 40% to 30%).The presence of clinical seizures at baseline, during intervention and re-warming will be recorded. EEG evaluation will be sought for clinical seizure activity. Neurologic examination will be performed and recorded 36 hours after intervention begins, the day after re-warming and then in survivors at 38-40 weeks corrected GA or at discharge or transfer to a non-NRN facility, if those should occur before 38 weeks corrected GA.
7. Hematologic: Complete blood counts, including platelet count, if not routine practice (or usual care) will be obtained on all infants as close as possible to baseline, at 48 hours, at 96 hours, and recorded. PT/PTT and fibrinogen will also be assessed as clinically indicated for spontaneous bleeding or unexplained fall in hematocrit by >10%. Given the potential for increased viscosity with lower temperature, careful surveillance will be conducted for associated complications (including, but not limited to, thrombosis and NEC).
8. Infection: Culture results from normally sterile body sites will be recorded. Pneumonia will be considered to be present if a new chest infiltrate with associated requirement for increased respiratory support is noted.
9. Liver: Liver function tests including AST, ALT and fractionated bilirubin will be obtained as clinically indicated and recorded. Total or unconjugated bilirubin will also be assessed as clinically indicated and recorded.
10. Skin: Areas of necrosis, erosion, cyanosis, erythema and subcutaneous fat necrosis will be assessed throughout the intervention period.
11. Esophageal probe: Proper positioning and possible esophageal perforation will be assessed by review of a radiograph obtained within 12 hours of initial placement and all subsequent radiographs performed during the period the probe is being used in the infant. It is anticipated that such films will be obtained at least daily during the intervention/control period as part of standard care.
12. A brain MRI will be strongly recommended at 10-17 days of postnatal age or prior to discharge or transfer to a non-NRN facility (if <10-17 days of postnatal age) for assessment of intracranial abnormalities. Results of MRIs obtained at other times after cessation of cooling will be recorded and included. MRI should include sagittal T1, axial T1 (SE or SPGR at 1.5 T and FLAIR at 3T), axial

heavily T2 weighted fast spin echo, axial DWI (b=1000) with ADC maps, single voxel short TE MRS placed over left basal ganglia, and (if available) axial DTI (b=700; >21 directions; 2 mm^3 voxel). Local readings will be employed for this study. Based on experience from the SUPPORT Neuro secondary, it is anticipated that <10% of subjects will require sedation for this MRI and that a portion of those requiring sedation may require mechanical ventilation during the MRI. Other imaging studies may be obtained as required for clinical care as determined by the responsible clinicians. A secondary MRI study using centralized reader(s) is under development.

13. Nutrition: We strongly recommend that no infant in the hypothermia group receive enteral feedings before re-warming and removal of the esophageal probe, unless deemed to be medically necessary.

Following the cooling/control period, clinical care will be managed per the usual standards of the NICU and according to the judgment of the responsible attending physician and staff.

3.10 Interrupion of hypothermia. Infants may have hypothermia interrupted (stopped, then restarted) if the following occur:

1. Imaging
2. Equipment malfunction
3. Equipment safety shut off
4. Serious adverse event at the discretion of the attending physician following consultation with the study/site PI. (Examples include one or more of the following requiring intervention: cardiac arrhythmia, persistent acidosis, major thrombosis or bleeding)
5. Surgery
6. For other reasons

If cooling is interrupted at any time during the randomized treatment assignment, cooling time will not be extended. For example, rewarming will begin at 72 hours from baseline regardless of the number of interruptions. Any interruption of intervention greater than 15 minutes will be documented.

3.11 Discontinuation of hypothermia. Infants will exit the hypothermia arm if the infant receives placement on ECMO or if the parents or neonatologist withdraw consent. Discontinuation for a serious adverse event requiring therapy (arrhythmia, persistent acidosis, major thrombosis or bleeding, extensive skin necrosis, NEC) will be at the discretion of the attending neonatologist after consultation with the site/study PI. Re-warming will occur as outlined in section 3.8 above. Recording of temperature data as prescribed for use during the intervention will cease after discontinuation of the intervention, as done in the Late Hypothermia and Optimizing Hypothermia protocols. The infant will remain in the study as per intention-to-treat, unless permission to use any data is explicitly withdrawn by the parents.

3.12 Withdrawal of support or limitation of care
Decisions regarding withdrawal of life support during the period of hypothermia/control and/or limitation of clinical care following the intervention/control period will be made in consultation with an attending neonatologist other than the site NRN PI (as in the Late Hypothermia and Optimizing Cooling NRN protocols).

3.13 Follow-up
Appropriate contact information will be obtained from the parent(s) or guardian of each surviving infant prior to discharge from the NICU. Standard steps to optimize return for follow-up as currently practiced within the NRN will be employed. In the event of death prior to or following NICU discharge, efforts will be made to obtain an autopsy.

Survivors will be tracked following discharge and evaluated at 18-22 months of age for growth, neurologic exam, as well as formal psychometric assessment using the Bayley Scales of Infant Development III as currently specified by the NRN. Results of ophthalmologic and audiologic assessments will be obtained. Individuals performing psychometric testing and neurological evaluations will be masked to group assignment and will undergo training and annual certification per the NRN Follow-up protocol. If an infant is not evaluated at the 18-22 month clinic visit, appointments will be re-scheduled until evaluation is complete.

3.14 Primary outcome
Primary outcome will be death or moderate to severe disability at 18-22 months corrected age. The presence or absence of disability will be determined by the standard NRN interdisciplinary follow-up exam. *Severe* disability will be defined by the presence of any of the following: a Bayley III cognitive score <70, Gross Motor Functional (GMF) Level of 3-5, blindness (visual acuity <20/200 in the best eye as determined by an ophthalmologist) or profound hearing loss (inability to understand commands despite placement of a cochlear implant or amplification). *Moderate* disability will be defined by a Bayley III cognitive score between 70-84 and either a GMF level of 2, an active seizure disorder, or a hearing deficit requiring amplification or cochlear implant to understand commands. *Mild* disability will be defined by a cognitive score 70-84, or a cognitive score ≥85 and any of the following: GMF level I, seizure disorder or hearing loss not requiring amplification. *Normal* will be defined by a cognitive score ≥85 and the absence of any neurosensory deficits. Although the Bayley III cognitive score is thought to systematically give higher scores at 18-22 months than the Mental Developmental Index of the Bayley II used in the original NRN hypothermia trial for infants ≥36 weeks GA, this is likely to affect both study groups in this study similarly. Please note that the follow-up for this trial will be at 18-22 months corrected age in keeping with all previously performed RCTs of hypothermia for HIE in newborn infants 36 weeks gestation or beyond.

3.14 Secondary outcomes

These include death only, cause of death (withdrawal of support and reasons for such will be tracked), disability only, death or profound disability (assignment of lowest score on

Bayley III because untestable due to degree of impairment), severe disability and moderate disability only, survival with no impairment, each component of severe and moderate disability (as described above), differences in MRI findings after cessation of cooling/control , evolution of encephalopathy, and other safety issues (including seizures after beginning of assigned treatment, BPD, NEC, late onset bloodstream infection, pulmonary hypertension, ICH, great vessel or intracranial thrombosis, esophageal probe injuries, metabolic abnormalities, treatment with vasopressors and/or steroids, oliguria, anuria, liver dysfunction (AST>200 IU and/or ALT>100 IU), clinically diagnosed coagulopathy, days receiving oxygen/mechanical ventilation, receipt of ECMO, duration of NICU stay in survivors and neurological injury by cranial ultrasound within 24 hours of enrollment.

In addition, frequency and clinical factors associated with NE in infants 33-35 wks GA with NE (including maternal characteristics, pregnancy complications, acute perinatal/sentinel events, intrapartum complications, mode of delivery; GA, BW, head circumference, gender, delivery room resuscitation, cord gas results, blood gas within first hour of life, NICU course and complications) as well as outcomes other than death or disability.

4.0 STATISTICAL CONSIDERATIONS

4.1 Sample size and data analysis

As noted previously, the potential number of eligible subjects is uncertain due to limited but quite variable data such that traditional sample size determination is less likely to be helpful. The original NRN hypothermia trial in infants ≥36 weeks GA found a frequency of 62% death or moderate/severe disability in control infants; there are no data regarding the incidence of death or disability after NE at the targeted gestational age of this study. Given increased fragility associated with this degree of prematurity, it seems likely that the incidence of death or moderate/severe disability in the control arm is likely to be at least 70%.

A Bayesian approach is to be used for this trial. As quoted from other authors in the NRN Late Hypothermia protocol, (to argue) that trials should not be conducted “unless an arbitrarily defined level of statistical power can be assured, make(s) no sense if the alternative is acquiescence in ignorance of the effects of healthcare interventions… Unbiased trials with imprecise results trump no results at all.”

A Frequentist (i.e., conventional) study design is intended to determine the probability that accumulated data are consistent with the null hypothesis. It is dependent on a number of factors specified beforehand by the investigator(s): 1) the size of the difference between groups that is sought, 2) the frequency of the outcome of interest in the control group, 3) P value considered to be meaningful, and 4) size of types I and II error to be excluded. Necessary sample size is largely determined by these factors. It employs no prior trial results regarding similar issues. Meaningful assessment of uncommon problems or those of unknown frequency (e.g., moderate/severe NE in preterm infants 33-0/7 to 35-6/7 wks GA) is often not plausible. To achieve 80% power to detect a

decrease in death or moderate/severe disability from 70 to 50% would take ≈110 subjects per arm. A decrease from 70 to 55% would also be clinically significant but would require ≈190 subjects per arm.

A Bayesian design seeks to determine and update probabilities of alternative hypotheses being true, a conceptually more direct process than rejection of the null hypothesis within specified limits. The prior probability (i.e., prior to the conduct of the proposed trial) is established by thoughtful review and consideration of earlier work. The absence or paucity of prior data does not preclude Bayesian analysis, but still requires cautious consideration and transparent delineation of the basis for the prior probability. After data from the trial are accumulated, the new data are used to determine the posterior probability of the hypothesis using Bayes' theorem – an adjustment of the prior belief. The mathematics is not always simple and straightforward, but the availability of appropriate software has enhanced the practicality and utility of Bayesian analysis. This approach may yield illuminating and important results that influence subsequent practice, even with relatively rare events. Findings that eventually prove to be erroneous are still possible (as is also true of the Frequentist approach), but prospective rigorous design limits the potential for such an occurrence. This includes (but is not limited to) determination of prior probability, sensitivity analyses (optimistic/enthusiastic, neutral, skeptical), assessment of the design's operational characteristics via simulation and other considerations. Even if sample size is limited, Bayesian analysis will allow determination of the probability of the observed findings. Further overview and discussion of Bayesian study design and analyses can be found in the Late Hypothermia protocol at the NRN website.

For primary application of the Bayesian strategy to this proposal, we propose to apply a log-binomial regression model and a neutral prior that will be centered at RR=1.0 to final efficacy and safety data, where final data is defined as data contained in the study database after database lock. Database lock will occur either after 168 infants have completed the study or the study is terminated early for efficacy, futility, or safety.. In addition to using a neutral prior, final analyses on locked data and some interim analyses will develop posterior distributions using skeptical (centered at RR = 1.1) priors and/or enthusiastic (centered at RR = 0.75) priors as well. Guidelines for interim determinations of safety, effectiveness, and futility using a Bayesian approach, including necessary information to fully define these priors, are proposed in sections 4.2, 4.3 4.5, and 4.6 below. Section 4.7 covers guidelines related to the final analysis. In all analyses, MCMC methods will be used to sample from the Posterior distribution.

A potential benefit for this trial is that Bayesian analysis provides estimates of the probability of any benefit (which frequentist analyses cannot supply) as well as estimates of specific benefit (e.g., >0, >10 or >20%).[29]

4.2 Bayesian Model and Prior Specification

As noted above, Bayesian analyses for this trial will utilize skeptical, neutral and enthusiastic priors for estimation of posterior distributions for relative risk. Skeptical priors will be used during interim efficacy analyses. Neutral priors will be used during interim safety analyses, and enthusiastic priors will be used during interim futility analyses. For descriptive purposes, all three types of priors will be used during the final analysis, but the primary focus will be on results based on neutral priors.

All analyses will involve estimating a Posterior distribution based on a log-binomial regression model with covariates and MCMC methods. For frequentist analyses, the log-binomial regression model is equivalent to robust Poisson regression. The log-binomial regression model has the benefit of being fit easily using MCMC techniques, thus allowing for direct estimation of the log relative risk due to treatment, correct estimation of its uncertainty, and correct estimation of its posterior distribution. In addition to estimating the treatment effect on the log relative risk scale, the log-binomial regression will adjust for level of encephalopathy and center. The planned number of enrolling centers is 18, and center will be included in the linear component of the model as an additive random effect. For the intercept and the encephalopathy terms, Normal(mean = 0, sd=100) priors will be used. For the random center effect, we will assume a Normal distribution centered at 0, and the standard deviation parameter will be assumed to follow a uniform hyperprior with limits based on observed variability from a previous neonatal network trial. For the term representing the log relative risk, skeptical, neutral, and enthusiastic priors will be used to estimate posterior distributions for the relative risk.[30] Because the log-binomial model will estimate the log relative risk as the treatment parameter, the priors for the treatment effect will be normal priors. These normal priors for the log relative risk are be defined below so that the resulting priors in the relative risk scale have specific properties.

In the relative risk scale, the skeptical prior for relative risk will be centered on 1.1 and have a 95% credible interval with an approximate range of 0.37 to 3.3. This skeptical prior will be defined by a normal prior on the log relative risk scale centered at 0.0953 with standard deviation (SD) of 0.5605. In the relative risk scale, the neutral prior for the relative risk will be centered on 1.0 and have a 95% credible interval with an approximate range of 0.33 to 3.0. This neutral prior will be defined by a normal prior on the log relative risk scale centered at 0 with standard deviation (SD) of 0.5605. In the relative risk scale, the enthusiastic prior for the relative risk will be centered on 0.75 and have a 95% credible interval with an approximate range of 0.25 to 2.25. This enthusiastic prior will be defined by a normal prior on the log relative risk scale centered at -0.2877 with standard deviation (SD) of 0.5605.

Table 1: Interim Analysis Schedule

Interim Analysis	Sample Size*	Purpose	Primary Prior for RR	Endpoint**	Stoppage Criteria
1	20	Safety	Neutral	Interim Safety	$Pr(\theta>1\|X) > 0.999$
2	40	Safety	Neutral	Interim Safety	$Pr(\theta>1\|X) > 0.99$
3	60	Safety	Neutral	Interim Safety	$Pr(\theta>1\|X) > 0.98$

4	80	Safety	Neutral	Interim Safety	$Pr(\theta>1\|X) > 0.95$
		Efficacy***	Skeptical	Primary Efficacy	$Pr(\theta<1\|X) > 0.975$
5	100	Futility***	Enthusiastic	Primary Efficacy	$Pr(\theta<1\|X) < 0.10$
		Safety	Neutral	Interim Safety	$Pr(\theta>1\|X) > 0.925$
		Efficacy***	Skeptical	Primary Efficacy	$Pr(\theta<1\|X) > 0.975$
6	130	Futility***	Enthusiastic	Primary Efficacy	$Pr(\theta<1\|X) < 0.10$
		Safety	Neutral	Interim Safety	$Pr(\theta>1\|X) > 0.90$

* All interim analyses, analyses 1 through 6, will be completed prior to database lock. For these analyses, the sample size will include infants who have reached NICU discharge, alive at 60 days of age, or have died by 60 days of age.
** The interim safety endpoint is defined in Section 4.3. The primary efficacy endpoint is defined in Section 3.14.
*** It is expected that at the time of the 5th and 6th interim analysis, the number of infants, 100 and 130 respectively, available for interim analysis of safety will be greater than the number of infants available for interim analysis of efficacy and futility.

4.3 Staged analysis and safety monitoring

Formal interim analyses of safety will occur when 20, 40, 60, 80, 100, and 130 infants reach NICU discharge, alive in the NICU at 60 days or death by 60 days of age, Table 1. For the first interim analysis of safety, enrollment will be temporarily paused once the first 20 infants reach NICU discharge, alive in the NICU at 60 days or death by 60 days of age. At that point, the NRN Data Safety Monitoring Committee (DSMC) will review safety data to determine if resumption of the trial is indicated. Safety will be assessed by the incidence of the interim safety endpoint which will involve death within the first 60 days, severe intracranial hemorrhage, or intracranial or great vessel thrombosis within 108 hours of baseline (insertion of esophageal probe). The full definition of the interim safety analysis endpoint will include large intraventricular, large intraparenchymal, large cerebellar hemorrhages or extra-axial hemorrhages which causes a midline shift of cerebral structures or deemed 'large' by the local image reader, or major thrombosis of a great vessel (aorta or vena cava) or cerebral artery, vein or sinus (see study Manual of Operations [MOP] for details). Additional descriptive summaries of the following secondary safety endpoints may be found in the MOP:

a) Intracranial hemorrhage
b) Cardiac arrythmia
c) Persistent acidosis
d) Major vessel thrombosis
e) Bleeding
f) Major skin changes
g) Necrotizing enterocolitis (Bell stage II or greater)
h) Hyperglycemia or hypoglycemia
i) Perforations or major bleeding from the esophageal probe
j) PPHN developing after randomization
k) ECMO

l) Death or any of the preceding within 108 hours of baseline (insertion of esophageal probe)

Bayesian interim monitoring will be used to assess whether systematic differences are evident among the treatment groups for the occurrence of the interim safety analysis endpoint. The posterior probability that the hypothermia group has a higher incidence of the interim safety analysis endpoint will be determined. These posterior probabilities for safety will be assessed using a likelihood that incorporates a log-binomial regression model with covariates, described more completely in section 4.2. The treatment will be considered harmful (i.e., the DSMC may consider termination of the trial) if for a pre-specified threshold η, the posterior probability of treatment harm exceeds η. In statistical notation, if the posterior probability $Pr(\theta>1|X) > \eta$ based on the neutral prior, where θ is the relative risk favoring the treatment group and X is the available data, then the hypothermia treatment will be considered harmful. We propose a conservative value of η = 99.9% for the first interim safety analysis, requiring very strong evidence of harm from hypothermia, Table 1.

In addition to reporting the above posterior probability during the first interim safety analysis, the 95% credible interval for θ, and the entire posterior distribution of θ will be presented graphically to the DSMC in order to assure a full appreciation of the range of possible values of θ. Since no data are currently available for infants of this gestational age re: these adverse events, we will use the neutral prior distribution defined in section 4.2 for formal interim safety monitoring. In addition, we will also report posterior results based on the skeptical and enthusiastic priors defined in section 4.2 to facilitate decision-making and limit the possibility of inconclusive results upon stopping. Because of the small number of infants and the likelihood that important factors (e.g., moderate versus severe encephalopathy) may not be equally distributed at the time of the first safety review, we propose that a conservative approach to decisions about study termination be employed. Using a stoppage criterion of $Pr(\theta>1|X) > 0.999$ for this first safety interim analysis reinforces the need for conservative interpretation of the data.

After each of the first five interim analyses of safety, if continuation is approved, additional infants will be enrolled until the next pre-specified number of infants reaches NICU discharge, alive in the NICU at 60 days, or death by 60 days of age, Table 1. At that time, the DSMC will review the study data in a similar manner as reviewed after the first interim safety analysis except the study will not be temporarily paused. For the second through sixth interim analyses of safety, the following values of η will be used:

a) η = 99% will be used for the second (at 40 infants),
b) η = 98% will be used for the third (at 60 infants),
c) η = 95% will be used for the fourth (at 80 infants),
d) η = 92.5% will be used for the fifth (at 100 infants),
e) η = 90% will be used for the sixth (at 130 infants).

After the sixth interim analysis of safety at 130 infants, additional infants will be enrolled until 168 infants have been enrolled and reach the primary outcome. Once 168 infants have reached primary outcome, safety, efficacy, futility analyses will be completed

according to the final analysis plan specified for all post database lock analyses in Section 4.7.

In addition to the above formal interim monitoring of safety, severe adverse events will be reported within 24 hours of discovery to the DCC and the NICHD, who will report to the DSMC if needed. These events will be reported to local IRBs per the policy of each institution. Finally, the Preemie Hypothermia Study Subcommittee will review study status, all protocol violations, all severe adverse events and temperature profile of study infants on every conference call and face-to-face meeting (every 6-8 weeks). For this review, the subcommittee will only have access to overall data and not treatment group information.

The DCC will create a system to monitor accruing data and events and will communicate these to the DSMC as needed. The DSMC currently meets annually in person and quarterly as needed by conference call.

4.4 Study Continuation Review after Two Years

If safety reviews have permitted the study to continue for two years after 100% of NRN centers have been IRB-approved and screening, subject accrual will be reviewed at that time by the Sub-committee with recommendations to the Steering Committee and the NRN DSMC. That review will permit more precise determination of attainable sample size by extension of enrollment to 3-5 years. The primary outcome will not be uniformly available at two years, so decisions about continuation of the trial will be based on recruitment and safety data. We will use the accrual of subjects in the Late Hypothermia trial as a benchmark (i.e., enrollment of approximately 50 subjects by the two year cumulative review) to help determine if further continuation is indicated. Extension or termination of the trial would be evaluated and approved or rejected at this two year cumulative review. The recommendations made in the two year review will be given to the DSMC for consideration in conjunction with the formal safety, efficacy, and futility analyses. An informal discussion concerning actual enrollment and formal interim efficacy, safety and futility analyses will suffice to allow the DSMC to advise on matters regarding early trial stoppage.

4.5 Interim Monitoring and Final Determination of Effectiveness Outcome

Formal interim analyses of efficacy will be completed after 100 and 130 infants have reached the interim safety endpoint, so the interim analyses of efficacy will occur simultaneously with the 5^{th} and 6^{th} interim analysis of safety, Table 1. It is expected that at the time of the 5^{th} and 6^{th} interim analysis of safety, the number of infants available for an efficacy assessment due to the availability of primary endpoint data will be less than the number of infants available for the safety assessment. These interim analyses of efficacy will assess whether hypothermia is better, equivalent, or adverse relative to normothermia with respect to the primary outcome of death or moderate to severe disability at 18-22 months corrected age.

During the interim analyses of efficacy, posterior probabilities will be assessed using a likelihood that incorporates a log-binomial regression model with covariates, described more completely in section 4.2. For interim efficacy analyses, the skeptical prior defined in section 4.2 will be used to calculate the posterior distribution for the relative risk as well as estimate the probability of hypothermia being better than control and the corresponding 95% credible intervals.

During the interim analyses of efficacy, the DSMC may consider termination if the upper 95% credible limit for the posterior RR of treatment benefit using a skeptical prior is <1.0, Table 1. Use of a skeptical prior assures a more conservative basis for termination. It also reduces the likelihood of biased and sometimes misleading estimates of treatment effect associated with early stopping of a trial.[31,32] Justification for this process of interim efficacy monitoring is given in the Appendix, sections 7.1, 7.2 and 7.4.

4.6 Interim Determination of Treatment Futility

Formal interim analyses of efficacy will be completed after 100 and 130 infants have reached the interim safety endpoint, so the interim analyses of futility will occur simultaneously with the 5th and 6th interim analyses of safety and the 1st and 2nd interim analyses of efficacy, Table 1. For the interim assessments of futility available primary outcome data will be used to determine the posterior probability that the risk of death or disability in the hypothermia group will be less than that in the control group thus clearly demonstrating the futility of hypothermia. Similar to efficacy, futility will be assessed using a likelihood that incorporates a log-binomial regression model with covariates as defined in Section 4.2. To provide a more conservative estimate for this purpose, we will principally use an optimistic prior for this determination with the prior belief set at a treatment benefit similar to that in the original NRN hypothermia trial with steps to avoid hyperthermia in both groups (RR=0.75). To provide a broad perspective and assess the robustness of this estimate with respect to prior assumptions, posterior probability and 95% credible intervals will also be determined and presented for the skeptical and neutral prior perspectives. The DSMC may consider termination if the posterior probability of observing any treatment benefit (RR≤1.0) is less than 10%, Table 1. Justification for this process of interim futility monitoring is given in the Appendix in sections 7.1, 7.2 and 7.4, As for the Late Hypothermia trial, early termination for futility should be tempered by other considerations: 1) a major goal is to obtain the most unbiased estimate of effectiveness for hypothermia in premature infants 33-35 weeks GA with NE, 2) absence of effectiveness may also influence future clinical practice, if the study population is suitably large, and 3) evolving bias in clinical practice makes it probable that many late preterm infants will be cooled in the future without a reasonable base of evidence.

4.7 Final (Post Database Lock) Analyses

The final analysis will occur after 168 infants have completed the trial and contributed primary outcome data, or the trial is stopped early for efficacy, futility, or safety. The final analysis will involve Posterior distribution estimation for relative risk using the log-binomial regression model defined in Section 4.2 where the outcomes are the primary

efficacy endpoint and safety endpoints. Since database lock will not occur until all enrolled infants have reached primary endpoint, if the study is stopped early there will be a period of time between the stoppage of infant enrollment, data base lock, and final analysis. If the study is stopped early for safety, during the period when follow-up outcomes are being accrued, we may write and submit a paper to a peer-reviewed journal disseminating the safety analysis results found. This paper, if it is written, will be limited to hospital outcomes for the enrolled infants and will not include any follow-up data. If the study is stopped early for efficacy or futility, no attempt will be made to disseminate information about safety until the study database is locked.

With respect to the primary efficacy endpoint, the estimated Posterior distribution of relative risk based on the data and the neutral prior will be used to determine posterior probabilities of >0, >10 and >20% decrease in death or moderate/severe disability, as well as graphical displays of the Posterior distribution and appropriate 95% credible intervals for the relative risk. In addition to adjusting for level of encephalopathy and center in the log-binomial model, the final analysis of efficacy will also perform sensitivity analyses concerning possible treatment effect confounders present at randomization. All additional covariates added to the model will utilize Normal(mean=0, sd=100) priors.

The lack of preexisting data suggests that the neutral prior distribution may be most appropriate for presentation of final results; however, generation of final results using the skeptical (RR of 1.10) and enthusiastic priors (RR of 0.75) will also be produced to give a broad overview of the implications of the trial's efficacy results.

With respect to the safety endpoints, posterior distributions of safety outcomes will be estimated and the posterior probability of the normothermia group having a higher prevalence relative to the hypothermia group, $Pr(\theta>1|X)$, will be calculated along with 95% credible intervals for the relative risk, θ. In a similar process as used in the interim safety analysis, the final safety analysis will also make note if $Pr(\theta>1|X) > 0.82$, which was used as the final cutoff criteria for identifying a harmful effect in the simulation study used to design the trial..

In addition to reporting the above, the entire posterior distribution of the relative risk for safety endpoints will be graphically presented. Since no data are currently available for infants of this gestational age with respect to the safety outcomes we will use the neutral prior distribution defined in section 4.2 for the primary analysis of final safety data. Secondarily, we will also report posterior results based on the skeptical and enthusiastic priors defined in section 4.2 to facilitate final safety data interpretation.

5.0 TIME FOR COMPLETION

If all feasibility and safety thresholds are met, we anticipate that recruitment will take 3-5 years, with another 18-22 months (potentially a few more for obtaining maximal compliance with follow-up) required for follow-up examinations. If safety and recruitment thresholds are not attained, discontinuation may occur at 2 years or as early as assessment after short-term safety in the first 20 subjects.

6.0 CONFLICTS WITH ONGOING RESEARCH

To the best of our knowledge, no other NRN study will include infants in this GA group with NE. Although there is no overlap in patient group recruitment, the ongoing Late Hypothermia and Optimizing Hypothermia trials may result in limited availability of Cincinnati Sub-Zero Blanketrol® units. Additional units may be necessary for conduct of all 3 studies, if this protocol is approved and implemented.

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