OPTIMIZING COOLING STRATEGIES AT < 6 HOURS OF AGE FOR NEONATAL HYPOXIC-ISCHEMIC ENCEPHALOPATHY (HIE)

Short Title: Optimizing Cooling for HIE

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Optimizing Hypothermia as Neuroprotection at < 6 Hours of Age for Neonatal Hypoxic Ischemic Encephalopathy

Objective: Evaluate whether whole body cooling initiated at < 6 hours of age and continued for a duration of 120 hours or a depth at 32.0°C in infants ≥ 36 weeks gestation with hypoxic ischemic encephalopathy will reduce death and disability at 18 months of age

Study Design: A prospective, randomized, 2x2 factorial design multicenter trial. All study infants will receive whole body hypothermia. The intervention will be unmasked. The individual factors tested will be (a) a comparison of two cooling durations (72 versus 120 hours) and (b) a comparison of two different depths of cooling (33.5°C versus 32.0°C)

Eligibility criteria: Infants ≥ 36 weeks gestation with a pH (cord or < 1 hour neonatal) ≤ 7.0 or a 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 infants must have signs of moderate or severe encephalopathy at ≤ 6 hours of age at the time of enrollment.

Study Intervention: Infants will be randomized to either usual depth of cooling (33.5°C) or deeper cooling (32.0°C) and then to usual length of cooling (72 hours) or longer cooling (120 hours). Hypothermia will be achieved with whole body cooling using the Cincinnati Sub-Zero Hyper/Hypothermia Device. Safety measures will be monitored and adverse events will be compared between groups using sequential analyses methods. The first interim analysis for safety will occur after the first 40 infants are accrued into the study (10 in each arm of the factorial design). The study will proceed after DSMC review.

Primary outcome: The primary outcome will be death or moderate/severe disability at 18-22 months of age.

Sample size estimates: Estimated event rates are 37.5% for usual duration vs. 27.5% for longer duration or 37.5% for usual depth of cooling vs. 27.5% for deeper cooling. With a two-tailed, type 1 error of 5%, power set at 80%, with 5% lost to follow-up, 363 infants per group (longer cooling, deeper cooling) or a total of 726 subjects will be enrolled. A Bayesian analysis will be used for examining the results if the treatment effect is smaller than hypothesized.

Duration of study: Based on the current NICHD Neonatal Research Network Centers survey of infants receiving whole body cooling at < 6 hours of age as part of usual care, 5 years will be adequate for enrollment and an additional 1.5 years for follow-up.

1.0 STATEMENT OF THE PROBLEM

The NICHD Workshop on Hypothermia and Perinatal Asphyxia (Higgins 06) and the Committee of the Fetus and Newborn of the American Academy of Pediatrics (Blackmon 06) have recommended that therapeutic hypothermia, if offered, should be used only under published protocols (Shankaran 05, Gluckman 05). Although it is postulated that deeper, longer and earlier therapy with hypothermia is preferred, the optimal degree and duration of cooling is unknown (Gunn 98, Higgins 06 and Barks 08). It is also unclear whether the degree and duration of therapy should be based on the cause, severity, and stage of brain injury (Higgins 06). Since these statements by NICHD and COFN were published, several meta-analyses evaluating the safety and efficacy of hypothermia in term infants with encephalopathy have become available; all the published meta-analyses have concluded that in term infants < 6 hours of age with moderate or severe encephalopathy, hypothermia to 33.5 to 35.0°C for 72 hours decreases mortality and disability at 18 months of age (Azzopardi and Edwards 07, Shah 07, Jacobs 07 and Schulzke 07).

Currently the NICHD NRN sites are offering cooling to term infants (defined as $\geq$ 36 weeks gestation) who are < 6 hours of age with encephalopathy presumably due to hypoxia-ischemia (HIE). We now have the opportunity to examine whether greater depth of cooling or longer duration of cooling could safely offer more neuroprotection than the depth and duration of cooling currently offered as usual care in the NRN sites. No other neuroprotective approach with pharmacological therapy (antioxidant, anti-inflammatory and immunomodulatory, growth factors, erythropoietin or stem cells) is ready for clinical use (Gressens 07).

We propose to evaluate both deeper cooling and longer cooling in a randomized trial using a 2 by 2 factorial design. Given the sample size challenges for evaluating two modifications of cooling therapies, we propose this factorial design which will be testing two approaches to optimize cooling within one trial. The NICHD NRN is the only multi-center network positioned to perform this trial with efficiency. Our hypothesis is: Whole body cooling initiated within 6 hours of age can be optimized to further decrease the outcome of death and disability at 18 months of age, by a greater depth of cooling or a longer duration of cooling among infants with moderate and severe encephalopathy.

2.0 BACKGROUND AND SIGNIFICANCE

The NICHD trial of whole body cooling for 72 hours at 33.5°C demonstrated that death or moderate or severe disability occurred in 45 of 102 (44%) in the hypothermia group and 64 of 103 (62%) in the control group, risk ratio (95% confidence interval) RR (95%CI) 0.72 (0.54-0.95), P=0.01 (Shankaran 05). Three infants had moderate disabilities; 2 hypothermia and 1 control group infant (Shankaran 08). Among infants with moderate encephalopathy at randomization, the rate of death or disability was reduced from 30/63 (48%) in the control group to 22/69 (32%) in the hypothermia group,

RR 0.69 (0.44-1.07), P = 0.09. Among infants with severe encephalopathy at randomization, the rate was reduced from 34/40 (85%) to 23/32 (72%), RR 0.85 (0.64-1.13), P = 0.24. In the Cool Cap trial, using both clinical and aEEG entry criteria for enrollment with cooling at 34 to 35°C for 72 hours, death or severe disability occurred in 73 of 110 (66%) of conventional care and 59 of 108 (55%) assigned to head cooling, OR 0.61 (0.34 to 1.09), P=0.10. After adjustment for severity of aEEG changes, OR for hypothermia was 0.57 (0.32 to 1.01), P=0.05 (Gluckman 05). Recently, the Cool Cap study investigators published data on outcome based on severity of encephalopathy at randomization (Wyatt 07). Among infants with moderate encephalopathy at enrollment, death or disability at 18 months was 39/69 (57%) in controls and 28/62 (45%) in the cooled group. Among infants with severe encephalopathy at enrollment, the control group rate of death or disability was 32/35 (91%) and in the cooled group it was 28/40 (70%). In both trials, approximately 66% of neonates had moderate encephalopathy at randomization. Table 1 summarizes primary outcome data by severity of encephalopathy from the two large trials.

Table 1: Proportion of Infants with Moderate and Severe Encephalopathy with Primary Outcome of Death and Disability in the NICHD and Cool Cap Trials

	Cooled Death/disability	Control Death/disability
MODERATE HIE		
Whole body Hypothermia NICHD trial (Shankaran 05)	32%	48%
Cool Cap trial (Wyatt 07)	45%	57%
SEVERE HIE		
Whole body Hypothermia NICHD trial (Shankaran 05)	72%	85%
Cool Cap trial (Wyatt 07)	70%	91%

In summary, cooling for 72 hours at a core temperature of ≥ 33.5°C resulted in a death or disability rate of 32 to 45% with moderate HIE and 70 to 72% with severe HIE. Therefore the rate of death or disability continues to be high.

The NICHD trial, the Cool Cap trial, the TOBY trial and other ongoing trials (ICE trial, European trial) have used a target temperature ≥ 33.5°C for 72 hours. Debate is now

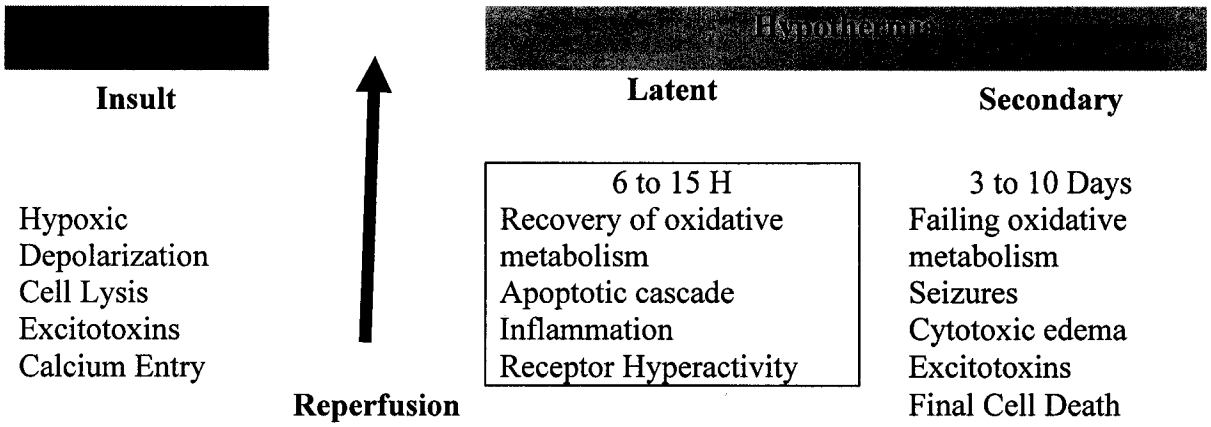
occurring whether: a) cooling initiated earlier than the current RCT will be more beneficial, b) infants with moderate HIE should be treated differently than those with severe HIE, c) a greater depth of cooling may be more beneficial and d) a longer duration of cooling may offer greater neuroprotection?

2.1 Initiation of cooling earlier than published trials: Animal data supports the concept that earlier initiation of therapeutic hypothermia is associated with greater neuroprotection compared to later initiation (Gunn 97, 98). However in clinical practice, four to five hours of age appears to be the earliest time period for cooling to be initiated after the following procedures occur: screening, stabilization, evaluation and diagnosis of encephalopathy, consent and randomization. (Gluckman 05, Shankaran 05). Furthermore, the neurological examination often changes during the early hours following birth and initiation of therapeutic hypothermia at < 2 hours may lead to treatment of infants who would not merit the intervention at 4-6 hours.

2.2 Should infants with moderate HIE be treated differently than infants with severe HIE? Performing an RCT with different cooling regimens for moderate and severe HIE may be prohibitive regarding sample size requirements. *In the NICHD trial, infants with moderate and severe HIE had the same direction of benefit with cooling (Shankaran 08). We speculate, therefore, that a greater depth and duration of cooling for both moderate and severe HIE may further improve outcome.*

2.3 Greater depth or longer duration of cooling: At the present time, the optimum depth or duration of cooling for neonatal encephalopathy is unknown. Infants with severe HIE may have brain injury before birth and be in secondary energy failure or may rapidly progress into secondary energy failure (Rutherford 06, Westgate 99). Therefore there may be a reduction in the benefit of hypothermia as currently applied (Gunn and Thoresen 06). It has been noted in studies performed prior to the introduction of hypothermia that term infants with severe HIE do not show any recovery of cerebral oxidative metabolism (Azzopardi 89) and childhood or school age outcome is associated with higher rates of death and disability among infants with severe as compared to moderate encephalopathy (Robertson 89, Shankaran 91). The NIH Neurology Group on HIE has suggested that treatment for moderate encephalopathy should start with modest hypothermia, while treatment for severe encephalopathy could include deeper hypothermia, more prolonged cooling, or modest hypothermia plus other strategies (Perlman 06). Therefore, cooling of 5°C to a depth of 32°C may be better than cooling of 3°C to 34°C; more cooling might show greater cerebral protection (Gunn and Thoresen 06). Longer cooling may protect against the continued cascade of injury, especially apoptosis and inflammation that has been shown in the animal model to extend over several days (Lorek 94, Bennet 06, and Gunn 97). Figure 1 shows the phases of cerebral injury after a severe but reversible period of hypoxia-ischemia (Gunn and Gluckman 07).

Figure 1: Phases of Cerebral Injury



During **reperfusion** after the insult, there is a period of approximately 30 to 60 minutes during which cellular energy metabolism is restored, with progressive resolution of the acute cell swelling secondary to hypoxic-depolarization. This is followed by a **latent** phase, during which oxidative metabolism has normalized (Thoresen 95), but there is hyperactivity of glutaminergic receptors, the intracytoplasmic components of the apoptotic pathway are activated and secondary inflammatory reaction is initiated. This may be followed by **secondary** deterioration leading to delayed neuronal death after 3 days. As indicated in the figure, treatment with cerebral hypothermia needs to be initiated as early as clinically feasible in the latent phase before the onset of secondary deterioration, and then continued for long lasting neuroprotection. The duration of the therapeutic time window depends on the severity of cerebral hypoxia-ischemia under normothermia and delayed hypothermia in newborn piglets (Iwata 07).

2.4 Cooling to a depth of 4 to 6°C vs. control in the animal model of hypoxia-ischemia: The detrimental effects of 24 to 48 hours of cooling to a depth of **8°C (37 to 29°C)** in the primate stroke model (Michenfelder 77), cats and monkeys (Steen 79) and dogs (Steen 80) have been reported. There is however, established evidence in fetal and neonatal models, and across species, that cooling by 4-6°C vs. controls has been neuroprotective while being well tolerated in animal models (Bona 98, Busto 87, Carroll 92, Colbourne 94, Gunn 97, Gunn 98, Haaland 97, O'Brien 06, Sirimanne 96, Thoresen 96, Thoresen 01, Tooley 02, Tooley 03, Tooley 05, Yager 96). The duration of cooling in these studies varied from 3 to 72 hours, and each study compared **a** specific depth of cooling to controls. The depth of cooling achieved in each of these studies was a rectal temperature of 28°C (Carroll 92), 32°C (Bona 98), 32.5°C (Thoresen 96) or 33°C (O'Brien 06). Scalp temperatures achieved in other studies were 21.0-23.9°C (Tooley 03). Extradural brain temperatures in studies by Gunn are reported as low as 30°C (Gunn 98, 99). Actual brain temperature studies document temperatures of 30-32.2°C (Tooley

02), 31.1°C (Tooley 05) and 32°C (Colbourne 94). None of these studies comparing a specific depth of hypothermia to controls report any adverse effects except one report of a piglet shivering during the cooling (Tooley 05). There are no studies where a temperature of 32.0°C has been maintained for longer than 72 hours.

2.5 Temperature-specific neuroprotective pattern of hypothermia: The neuroprotective pattern of therapy with hypothermia is temperature specific. There is suggestive data that optimal neuroprotection appears to occur at different temperatures in the cortical and deep gray matter. Neuroprotection with hypothermia by 4 to 6°C has been documented by many modalities, including a decrease in brain energy utilization measured by magnet resonance spectroscopy (Laptook 95), reduction of infarct size (Taylor 02), decrease in neuronal cell loss (Gunn 98), retention of sensory motor function (Bona 98), preservation of hippocampal structure (Carroll 92, Colbourne 94) and recovery of electroencephalographic activity (Gunn 98). Hypothermia initiated immediately post reperfusion was protective, with progressively increased protection with increasing depth of temperature, noted in studies that compared different depths of hypothermia vs. control. Hypothermia ranging from 34 to 31°C compared to 37°C has been found to preserve cerebral energy metabolism and suppress oxidative metabolism (Williams 97). Multiple other processes are involved in neuroprotection with hypothermia including decreasing apoptosis, limiting free radical injury, suppression of the inflammatory response, and a decrease in the inhibition of protein synthesis (Gunn and Thoresen 06). There is a significant correlation of both phospho creatinine/inorganic phosphorus and ATP levels with brain swelling. Tissue swelling was minimized at 31°C as compared to higher temperatures. Thoresen has also noted that seven day old rats treated with hypothermia to a temperature of 32.5°C had significantly less damage based on histological sections of the brain than normal control animals maintained at 38.3°C (Thoresen 95). Although intraischemic variations of brain temperature had no significant influence on energy metabolite levels measured at the conclusion of ischemia in the rat model, the histopathological consequences were markedly influenced, however, with preservation of cell counts at 31 and 34°C (Busto 87). The mechanisms of neuroprotection at a greater depth (32-33°C) of hypothermia may or may not be different from those known to be neuroprotective at 33-34°C.

2.6 Safety of cooling to < 33.0°C in the clinical setting: The pilot trial by Eicher and colleagues was performed with whole body cooling to a temperature of 33°C for 48 hours (Eicher 05). This is a lower temperature and shorter duration than that used in the NICHD or the Cool Cap trial (Shankaran 05, Gluckman 05). The average temperature in this pilot RCT was 32.8 $\pm$ 1.4°C at two hours of age. In this trial, 77% of the neonates had severe encephalopathy at randomization (unlike the NICHD and Cool Cap trial where 33% of infants had severe encephalopathy). Death or severe motor scores were significantly lower at 12 months of age in 52% of cooled infants compared to 84% of control infants (Eicher 05). The safety concerns of a target temperature of 33°C raised by Eicher included a higher incidence of bradycardia and a greater use of inotropic agents during cooling in the hypothermia group as compared to the control group. A longer use of pressor medication, longer prothrombin times and lower platelet counts were noted in the

hypothermia group as compared to infants in the control group. In addition, clinical seizures after enrollment were noted more commonly among the infants that underwent cooling as compared to the control infants.

In the NICHD Neonatal Research Network randomized controlled trial, hypothermia to a target esophageal temperature of 33.5°C for 72 hours was achieved using the Blanketrol II Hyper-Hypothermia Cincinnati Sub-Zero cooling system. On the servo mechanism, an expected overshoot occurs with initiation of cooling. This is followed by establishment of an equilibration near the target set point within 0.1°C (Shankaran 05). While evaluating safety outcomes of this trial (Shankaran 08), we noted unexplained intermittent drops of temperature remote from the initial overshoot. We found that the maximum overshoot below the target temperature was minus 1.4 ± 0.6°C (range was 0.0 to 4.1°C). The duration of time spent below the target of 33.5°C was 1.25 to 75.5 hours among all infants; one infant never achieved target temperature. There were 40 temperatures recorded < **32.0°C** after the initial overshoot among infants in the hypothermia group. There were 17 infants with temperatures < **32.0°**C after the initial overshoot and 10 infants who had temperatures < 32.0°C after equilibration. In spite of these decreases in temperature to < 32.0°C, no adverse events were temporally related during the 72 hour intervention period between infants with these temperature decreases and those who did not have the temperature decreases. Among infants who were cooled, there were no significant differences in esophageal temperatures among infants who received anticonvulsants and those who did not receive these medications. Similarly, there were no significant differences in esophageal temperatures among infants who received sedatives or analgesics and those who did not receive these medications. The use of inotropic agents to support blood pressure during study intervention was comparable among all the infants in the hypothermia and control groups. The two groups were also comparable in the number of infants receiving volume expanders, blood transfusions and platelet transfusions during the study intervention period. The number of infants with clinical seizures at baseline, 48 and 72 hours of study intervention were similar in the hypothermia group as compared to the control group. At 24 hours of study intervention, fewer infants in the hypothermia group had seizures as compared to the control group (Shankaran 08).

The first study evaluating safety of whole body hypothermia to a depth of 30 to 33°C in a small group of term infants with HIE was recently published (Compagnoni 08). Three groups of infants were studied; the control group (n=11) was treated with routine standard methods. A second group of infants (n=10, categorized as mild hypothermia) was treated with cooling to a temperature of 32 to 34°C and a third group (n=18, categorized as deep hypothermia) was treated with target temperature maintained at 30 to 33°C for 72 hours. Cerebral magnetic resonance imaging was performed after the second week of life and neurological examinations recorded in all survivors at 12 months of age. During the study intervention of cooling for 72 hours, disseminated intravascular coagulation was noted in two cases in the control group, pulmonary hypertension in two infants in the group with mild hypothermia and pneumonia was noted in three infants in the group with deep hypothermia. There were 5 deaths; two in the control, 1 in the mild and 2 in the deep hypothermia group, respectively.

2.7 Efficacy of cooling to 30.0 to 33.0°C in neonates: In the study of Compagnoni et al, severe cerebral lesions on magnetic resonance imaging and poor neurologic outcome was observed in four of nine cases in the control group (44.5%) compared to one of nine cases in the mild hypothermia group (11.2%) and one of 16 cases (6.3%) in the group with deep hypothermia, (control vs. mild or deep hypothermia groups $P < 0.05$) (Compagnoni 08). This study was not adequately powered to evaluate efficacy of cooling to 30.0 to 33.0°C.

2.8 Justification for a longer duration of cooling: Cooling of the brain for a few hours can be modestly protective, but is exquisitely dependent on the timing at the end of hypoxia-ischemia (Gunn and Thoresen 06). Neuroprotection with cooling that was initiated within 6 hours has required relatively prolonged periods of cooling, typically longer than 12 hours. Cooling was continued for three days in the fetal sheep studies because pilot studies demonstrated intense rebound of seizure activity and increased cell loss if cooling was stopped after less than 24 to 48 hours. In contrast, spontaneous re-warming after three days of cooling was associated with only minor transient epileptiform activity (Gunn 06). Since rebound seizure activity after re-warming from 72 hours of cooling has been reported in animal models (fetal sheep, Gerrits 05 and newborn piglets, Iwata 05), it is possible that cooling for four or five days may provide further benefit. Rebound seizure activity during re-warming after a cooling period of 72 hours has been noted in clinical practice in human neonates (Battin 04); seizure activity during rewarming was not seen in either of the 2 large RCT (Gluckman 05, Shankaran 05).

The need for prolonged cooling is also justified based on experimental evidence that biphasic edema after hypoxic ischemic brain injury in the neonatal rat reflects early neuronal damage and late glial injury (Nedelcu 99). Brain injury is an evolving process with necrosis (predominant cell death during the acute phase) and apoptosis (predominant cell death with less severe insults) occurring over days and months (Robertson 07). In the human neonate, despite adequate oxygenation and circulation following resuscitation for HIE, phosphocreatine (PCr) and nucleotide triphosphate (NTP mainly ATP) decreased and inorganic phosphate (Pi) increased (Azzopardi 89). These findings, along with increased brain lactate levels (Robertson 99) and an alkaline intracellular pH (Robertson 02) in the first few *days* after birth were associated with neurodevelopmental impairment and increasing mortality. These changes have been termed secondary energy failure on the basis that cerebral metabolism recovered on resuscitation but deteriorated again following a variable period (the latent phase). Adverse biological processes contributing to secondary energy failure after intrapartum hypoxia-ischemia include the inflammatory cascade, accumulation of excitatory neurotransmitters, intracellular calcium accumulation, and generation of oxygen free radicals, mitochondrial dysfunction and increased apoptosis (Northington 01, Taylor 99, Johnston 01, Orrenius 03, and Brown 03). *The inflammatory changes and histological changes following acute perinatal asphyxia can occur for a prolonged period of time, from 3 to 10 days in pre-clinical models (Figure 1). This is additional justification for prolonging the duration of cooling, while the latent phase and secondary injury continues to be recognized as occurring remote from the primary insult.* In the proposed study we have selected the duration of

120 hours which will be 48 hours longer than the current duration of clinical cooling of 72 hours. We wish to examine a duration that is both longer than and as safe as current practice.

2.9 Justification for deeper cooling: Covey noted hypothermia of 5°C administered post insult for 6 hours in 7 day old rat pups offered better neuroprotection for striatal neurons than 2°C (Covey 07). Iwata and colleagues have demonstrated that cooling at 2 different regimens (rectal temperatures of 35 and 33°C compared to normothermia of 38.5 to 39.0°C) for 48 hours in newborn piglets demonstrated progressive increase in neuronal viability in gray matter (Iwata 05). Laptook has demonstrated a linear relationship between brain energy utilization rate and brain temperature over the range of temperatures between 27.6 to 41°C, with a 1°C reduction in brain temperature leading to a 5.3% reduction in brain energy utilization rate in 8-9 and 15-16 day piglets (Laptook 95). Taylor looked at infarct size in 14 day old rats with cooling to 33.0 and 30.0°C compared to normothermia, and found smaller infarct size at both depths compared to normothermia (Taylor 02). Williams has evaluated cerebral energy metabolism during hypoxia-ischemia, and demonstrated that when compared to controls, NMR metabolites were preserved at 31.0 and 34.0°C in 7 day postnatal rats (Williams 97). None of these studies comparing differing depths of temperature to controls documents adverse effects. In addition, adjusting brain temperatures from 28.0°C and 41.0°C did not alter any systemic variable in the piglet model except for heart rate, which directly correlated with brain temperature (Laptook 95). In the proposed study we have selected a depth of 32.0°C which will be 1.5°C lower than the current depth of clinical cooling of 33.5°C since we wish to examine a depth that is greater than and as safe as current practice.

2.10 Hyperthermia in infants with hypoxic ischemic encephalopathy: The NICHD trial of whole body hypothermia demonstrated occurrence of **elevated core body temperature** in the control group infants when temperatures were measured in a consistent manner in the 76 hours of study intervention and re-warming phase (Shankaran 05). Of the 102 infants randomized to the usual care group, 50 infants had a maximum esophageal temperature $\geq$ 38.0°C. Higher core temperatures were associated with significant increases in risk of death or impairment in the control group (Laptook 08). In a secondary analysis of the Cool Cap trial, investigators also noted an association between elevated temperatures in the control group and increased risk of death or disability (Wyatt 07). Hyperthermia after brain injury adds to the risk of more severe neurologic damage and studies in adults and pediatric subjects consistently support association between higher core temperatures and worse outcome (Dietrich 07, Bramlett 07). In the animal model, seizures associated with a hypoxic ischemic insult result in aggravation of neuronal cell death, specifically within the hippocampus (Yager 04). The damage to the hippocampus occurs in the setting of spontaneously occurring hyperthermia of 1.5°C; rat pups in whom hyperthermia was prevented during seizures displayed significant reduction in brain damage compared to controls. In another study, neonatal rats subjected to hypoxic ischemic injury were noted to have selective and long lasting learning and memory impairments during behavioral tasks, and hypothermia to 27.0°C significantly reduced the attentional deficit in behavioral tasks, whereas hyperthermia aggravated the behavioral deficit and the brain injury (Mishima 04). *These*

studies indicate that preventing spontaneous hyperthermia in the model of hypoxic ischemic seizures in the newborn is neuro-protective. Therefore, it is imperative that breakthrough hyperthermia should be prevented in neonates after the cooling period to optimize neuroprotection.

3.0 PRELIMINARY DATA ANALYSIS OF DEEPER COOLING

3.1 Preliminary data analysis performed for this protocol: Preliminary analysis of data from the randomized control trial (Shankaran 05) was carried out in an attempt to optimize cooling strategies for the current protocol. Neonates being cooled on the servo controlled mechanism of the Blanketrol Hyper-Hypothermia cooling system do drop their core esophageal temperature initially below target temperature (overshoot of temperature) for varying periods of time before they reach equilibrium defined as within 0.1-0.2 of target (Shankaran 08). We examined details of recorded temperatures of all cooled infants and performed three analyses of the whole body hypothermia trial.

1) The first analysis was to evaluate whether there was an association between time spent below target temperature (< 33.5°C) and primary outcome. As noted in Table 2 below, there was no significant association between times spent below 33.5°C and primary outcome, or components of the primary outcome, among infants in the hypothermia group; however there is a trend for a lower frequency of primary outcome with greater time spent < 33.5°C. One explanation for lack of a significant association could be that time spent below 33.5°C in this study was not adequate enough, hence there is a need to examine whether deeper and longer cooling is neuroprotective.

Table 2: Hypothermia infants – Hours spent with esophageal temperature < 33.5

	N	Mean	SD	Q1	Median	Q3	p-value†
All infants	101*	48.72	18.71	36	52	63.25	-
Death or mod-severe disability	44	45.44	21.27	31.25	48.25	60.5	0.22
Infants without primary outcome	57	51.25	16.21	39.75	52.25	65	
Death	23	42.26	23.78	15.25	45.75	59	0.18
Survival	78	50.62	16.64	37.5	52.875	64.5	
Among survivors							
Mod-severe disability	21	48.92	18.06	33.5	54	61	0.54
No mod-severe disability	57	51.25	16.21	39.75	52.25	65	

*One infant had missing esophageal temperatures. Q represents quartiles. N = number of infants. Mean and Median is time in hours.
† P-values are from Wilcoxon Two-Sample Test, t approximation.

2. A second analysis was performed to understand why some infants in the hypothermia group had excessive decreases in temperature (either following the initial overshoot or after achieving equilibration) because this information may enable us to target specific approaches among those infants who we have predicted would tolerate deeper temperatures less well. We compared the perinatal characteristics (age of randomization, 10 minute Apgar score, cord pH, and base deficit) and neonatal characteristics (birth weight, seizures at randomization, level of encephalopathy and inotropic support at randomization) between infants who had no temperatures recorded below 32°C after the initial overshoot compared to those infants who had a temperature of less than 32°C after the initial over shoot and those who had a temperature of < 32°C after achieving equilibration. As noted in Table 3, there were no significant differences between the infants who did not have an overshoot below 32°C and those who dropped their temperatures < 32°C after the overshoot or after equilibration. *There is however a trend for a greater need for inotropic support at randomization between those infants who dropped their temperature below 32°C after initial overshoot (7/17 or 41%) and those who dropped their temperatures after equilibration (6/10 or 60%) compared to those who had no drops below 32°C (20/74 or 27%).*

Table 3: Hypothermia infants—comparison of infants with esophageal temperatures < 32°C after initial overshoot or equilibration.

	No drops below 32°C after initial overshoot (N=74)		Esop. temp. < 32°C after initial overshoot (N=17)		Esop. temp. < 32°C after equilibration (N=10)		p-value (Fisher's Exact Test)
	N	%	N	%	N	%	
Outborn	34	46%	9	53%	4	40%	0.85
Male gender	36	49%	12	71%	3	30%	0.11
10 minute apgar ≤ 5 *	59	86%	11	73%	9	90%	0.52
Seizures at randomization	30	41%	10	59%	4	40%	0.38
Severe level of initial HIE † Moderate level of initial HIE	22 51	30% 70%	6 11	35% 65%	3 7	30% 70%	0.94
Inotropic support at randomization	20	27%	7	41%	6	60%	0.08

* 7 infants are missing this data, N=94. Five are in the first group (N=69), and two are in the middle group (N=15). † 1 infant is missing initial HIE in the first group (N=73).

3. Lastly, variables were examined between infants who had no decreases in esophageal temperature below 32°C with those who had decreases after either overshoot or after equilibration (Table 4). *As noted, infants with a lower birth weight (when weight is evaluated as a continuous measure) had more frequent decreases in temperature below 32°C.*

Table 4: Hypothermia infants: Comparison of infant with esophageal temperatures < 32°C after initial overshoot or equilibration.

Age at randomization	N	Mean	SD	Min	Q1	Median	Q3	Max	p-value
No < 32°C after overshoot	74	4.29	1.27	0.77	3.5	4.48	5.1	7.33	0.87
< 32°C after overshoot	17	4.27	1.34	2.08	3.5	4.25	5.53	6.42	
< 32°C after equilibration	10	4.06	1.26	2.2	3.25	3.92	5.23	5.58	

Birth weight	N	Mean	SD	Min	Q1	Median	Q3	Max	p-value
No < 32°C after overshoot	74	3475.9	642.8	2050	3020	3322	3860	5432	0.04
< 32°C after overshoot	17	3153.8	484.8	2570	2761	3100	3461	4110	
< 32°C after equilibration	10	3108.6	499.9	2430	2755	2960.5	3510	3960	

Cord pH	N	Mean	SD	Min	Q1	Median	Q3	Max	p-value
No < 32°C after overshoot	54	6.87	0.20	6.55	6.71	6.89	6.99	7.27	1.0
< 32°C after overshoot	10	6.84	0.17	6.47	6.78	6.89	6.98	7.02	
< 32°C after equilibration	8	6.87	0.15	6.69	6.78	6.88	6.91	7.19	

Cord base deficit	N	Mean	SD	Min	Q1	Median	Q3	Max	p-value
No < 32°C after overshoot	45	18.38	7.36	3	14	18	23	34	0.95
< 32°C after overshoot	9	18.33	4.5	10	16	20	21	24	
< 32°C after equilibration	8	19.25	4.80	12	16	18.5	24	25	

* P-values are from Kruskal-Wallis Test (one-way ANOVA). Variables tested are continuous measures

Therefore, in the current protocol it will be necessary to identify those infants at higher risk for temperature decreases below target (infants requiring blood pressure support and those <25th percentile for weight) and an algorithm will be developed to control target set point temperatures in these infants.

3.2 Current status of trials evaluating cooling for HIE initiated < 6 hours of age: The current management of encephalopathy in the NICHD Neonatal Network Centers is to provide whole body cooling to 33.5°C for 72 hours. Centers in the Cool Cap study currently offer selective head cooling at all centers. The Total Body Cooling (TOBY) trial recruited 325 infants and demonstrated that among survivors, cooling resulted in reduced risks of CP, and improved scores on the BSID MDI and PDI and the GMFCS. The Infant Cooling Evaluation (ICE) trial terminated enrollment at 218 infants (total sample size was 276) due to "lack of equipoise among the investigators". The European trial (Neo. network website) was terminated because "current evidence of the benefit of therapeutic hypothermia did not justify further randomization". The ICE and the European trial results are pending publication. The primary outcome of both the completed and ongoing hypothermia trials are outcome at 18-22 months of age; hence 3-5 years must elapse following enrollment of the first study subject to examine the endpoint of death and disability.

3.3 Current Status of Pediatric Trials: The NICHD Pediatric Critical Care Network has initiated a protocol titled: Whole Body Cooling to 32.0°C to 34.0°C for Cardiac Arrest (subjects 48 hours of age to 18 years). The Wayne State University NRN PI (Seetha Shankaran MD) is a member of the Steering Committee of this trial.

4.0 STUDY DESIGN

This will be a prospective, randomized, 2 X 2 factorial design multi-center trial conducted by the NICHD Neonatal Research Network. The individual factors to be tested will be:

1) A prospective comparison of 2 cooling durations (72 vs.120 hours)
2) A prospective comparison of 2 different depths of cooling (esophageal temperatures of 33.5°C vs. 32.0°C)

4.1 Primary Hypothesis:

1) Relative to infants receiving whole body cooling for 72 hours, cooling for 120 hours will reduce death or disability
2) Relative to infants receiving whole body cooling at an esophageal temperature of 33.5°C, cooling to an esophageal temperature of 32.0°C will decrease death or disability

4.2 Secondary Hypotheses:

1) There will be no statistical interaction between the two factors tested in this trial
2) Cooling to a greater depth and/or longer duration will result in the following:
   a. No increase in acute adverse events among infants cooled for 120 hours
   b. No increase in acute adverse events among infants cooled to 32.0°C
   c. Mean Cognitive score at 18-22 months of age will be higher among infants cooled for 120 hours
   d. Mean Cognitive score at 18-22 months will be higher among infants cooled to 32.0°C

A factorial design has been selected because the conditions are ideal for such an approach (Piantadosi 05). Namely, the interventions can be administered together without significantly changing the intensity/magnitude of each in the presence of the other. In addition, there is interest in learning about the effect of the two combined interventions on outcome, and large statistical interactions between the 2 treatments (longer cooling, deeper cooling) are not anticipated. The mechanisms of neuroprotection associated with longer or deeper cooling are likely to be similar to mechanisms associated with neuroprotection using shorter or lesser degrees of hypothermia but may differ in the extent of mechanisms involved.

Since safety and feasibility of cooling infants with HIE to the duration and depth proposed in this study has not been performed to date, the Data Safety Monitoring Committee (DSMC) of the NRN will examine data after the first 40 subjects (10 in each arm of the factorial design) are randomized into the proposed study. Enrolment will be temporarily halted while the DSMC reviews the first interim analysis for safety and will be resumed after the DSMC, on review of this data, decides that recruitment may commence. This approach is similar to the first RCT of whole body hypothermia performed by the NRN in 1998 where 20 infants were cooled to 34.5°C vs. control with no adverse events (Shankaran 02) before the whole body hypothermia trial was initiated with cooling to 33.5°C.

4.3 Inclusion Criteria: The inclusion criteria are similar to the first whole body hypothermia for HIE trial (Shankaran 05). All infants with a gestational age $\geq$ 36 weeks will be screened for study entry if they are admitted to the NICU with an admitting diagnosis of fetal acidosis, perinatal asphyxia, neonatal depression or encephalopathy. Infants will be evaluated by physiological criteria, followed by a neurological examination. Eligibility criteria will include a pH $\leq$ 7.0 or a base deficit $\geq$ 16m mEq/ L on umbilical cord or any postnatal sample within 1 hour of age. If, during this interval, a pH is between 7.01 and 7.15, a base deficit is between 10 and 15.9 mEq/L, or a blood gas is not available, additional criteria will be required. These include an acute perinatal event and either a 10-minute Apgar score $\leq$ 5 or assisted ventilation initiated at birth and continued for at least 10 minutes. Once these criteria are met, all infants will have a standardized neurological examination performed by a certified physician examiner. Infants will be candidates for the study when encephalopathy or seizures are present. Encephalopathy will be defined as the presence of 1 or more signs in 3 of the following 6 categories: 1) level of consciousness: lethargy, stupor or coma; 2) spontaneous activity:

decreased, absent; 3) posture: distal flexion, decerebrate; 4) tone: hypotonia, flaccid or hypertonia, rigid; 5) primitive reflexes: a) suck, weak, absent; b) Moro, incomplete, flaccid; and 6) autonomic nervous system: a) pupils: constricted, unequal, skew deviation or non reactive to light; b) heart rate: bradycardia, variable heart rate or c) respiration: periodic breathing, apnea. Infants will be classified as moderate or severe encephalopathy based on a predefined algorithm. Determination of the stage of encephalopathy will be based on a modified Sarnat stage by scoring the presence of moderate or severe abnormalities in 6 categories. The number of moderate or severe signs determines the extent of encephalopathy and if signs are equally distributed the designation of moderate or severe encephalopathy will be based on the level of consciousness. Multiple births will be enrolled in the same arm of the study.

4.4 Exclusion Criteria: Exclusion criteria will include the following: a) inability to randomize by 6 hours of age, b) major congenital abnormality, c) major chromosomal abnormality (including Trisomy 21), d) severe growth restriction (≤ 1800gm birth weight), e) infant is moribund and will not receive any further aggressive treatment, f) refusal of consent by parent or g) refusal of consent by attending neonatologist. In addition, infants with a core temperature < 32.5°C for ≥ 2 hours at the time of randomization by the research team would not be eligible for the study.

4.5 Randomization and Stratification: After informed consent is obtained, infants with moderate /severe encephalopathy will be randomized to either usual depth of cooling (at 33.5°C) or deeper cooling (at 32.0°C), and then to usual length of cooling (for 72 hours) or longer cooling (for 120 hours). This double randomization will create the four groups shown in **Figure 2** below.

Figure 2: Design outline of proposed trial

		Depth of Cooling		**Margin**
		33.5°C (Group A)	*32.0°C (Group B)*	
Duration of Cooling	*72 hours (Group X)*	*AX*	*BX*	**X**
	120 hours (Group Y)	*AY*	*BY*	**Y**
Margin		**A**	**B**	

Randomization will be conducted using permuted block design and stratified by clinical site and stage of encephalopathy. Telephone randomization will occur 24 hours a day, 7 days a week, by the Data Coordinating Center at RTI International, Research Triangle Park, NC. Randomization should occur within 6 hours of age. Cooling will occur at < 6 hours for all eligible infants since this is usual care at all NRN sites.

4.6 Intervention: Care-givers will not be masked to therapy. All infants will be cooled using the Cincinnati Sub-Zero Hyper-Hypothermia Blanketrol System. An esophageal temperature probe will be placed in the lower third of the esophagus and the probe will be

interfaced with the Blanketrol System. The esophageal temperature will be controlled in the automatic control mode ("servo") at the target temperature for the duration of cooling. At the completion of cooling, the control set point will be increased 0.5°C per hour until the esophageal temperature is $\geq$ 36.5 °C for four hours. Once achieved, the esophageal probe will be removed, the infant will be taken off the cooling/heating blanket, and continued temperature control will be adjusted per skin temperature if servo- controlled, or environmental temperature if in an incubator (not on servo) to maintain temperature (axillary) between 36.5°C and 37.0°C.

The esophageal temperatures of all infants will be monitored closely on an ongoing basis to evaluate overshoot, depth of overshoot and time to equilibration. In addition, decreases of temperature following the initial overshoot and following equilibration will be monitored on an on-going basis. The type and timing of sedatives, analgesics and anticonvulsants will be recorded; use of these medications will be based on site practices.

4.7 Algorithm to prevent decreases of temperature to less than 31.0°C: Infants assigned to 32.0°C arms of the study will have the target temperature set at 33.5°C initially. Once equilibration with 33.5°C is achieved (after overshoot) then the target will be reset to 32.0°C. All temperatures recorded < 32.0°C will be reviewed on an on-going basis.

4.8 Discontinuation of Hypothermia: Infants will exit the assigned hypothermia intervention arm of the study if any of the following occur: parents withdraw consent, neonatologist withdraws consent or infant requires ECMO. Discontinuation of hypothermia for a serious adverse event requiring therapy (one or more of the following: cardiac arrhythmia, persistent acidosis, major thrombosis or bleeding or extensive skin breakdown) will be at the discretion of the attending physician after consultation with the study/site PI. If hypothermia is discontinued, rewarming will occur at 0.5°C per hour with further management per usual care at the site. The infant will continue to be part of the study as per intent-to-treat study protocol (unless parents explicitly withdraw permission to use any data).

4.9 Withdrawal of Support or Limitation of Care: Decisions made with the family to limit or withdraw care will be documented. If the Study PI is the attending physician, a neonatologist other than the Study PI is encouraged to participate in these discussions. A neurological examination will be performed on the day support is withdrawn.

4.10 Post Randomization Exclusion of Infants: The study is designed as intent-to-treat, and therefore infants will not be excluded after randomization.

4.11 Safety Monitoring of Control and Experimental Infants:

a. Skin, esophageal, axillary, and servo set point temperature will be monitored every 15 minutes for the first 4 hours, every hour up to 12 hours, followed by every 4 hours during the maintenance phase of cooling and every 2 hours during the rewarming phase until normothermia is achieved.
b. Metabolic status: serum electrolytes will be monitored as per clinical routine.

c. Respiratory status: blood gases will be monitored every 4 to 6 hours. Since lower target temperatures (< 33.5°C) may be associated with risk of pulmonary hypertension, more frequent gas measurements may be required.

d. Cardiovascular: heart rate, blood pressure and use of inotropic agents will be recorded at baseline and every 4 hours throughout the study period. The risk of cardiac arrhythmia may be increased at < 33.5°C hence risk will be monitored along with treatment for arrhythmia. Echocardiograms will be performed as per site practice.

e. Renal status: urine output and body weight will be recorded daily during the intervention interval. Serum BUN and creatinine will be obtained at baseline and daily as per clinical routine.

f. Neurological status: To monitor for possible sagittal sinus thrombosis, a subset of infants will require a cranial sonogram performed within 48-72 hours following the end of the intervention period. Neurological examinations will be performed at baseline, following study intervention, pre-discharge and at time of withdrawal of support. The presence of seizures at baseline, during intervention and during rewarming will be recorded. All infants with clinical seizures will have EEG evaluations performed.

g. Hematological: Platelet counts will be obtained daily. PT/PTT will be obtained per clinical routine or if bleeding is suspected based upon clinical symptoms or an unexplained fall in hematocrit by more than 10%. Complete blood counts will be monitored as per clinical routine, including white blood counts and absolute neutrophil counts because of potential risk of infection. Since increased viscosity is also a potential problem at lower temperatures, a high index of suspicion will be maintained for complications associated with increased viscosity (such as thrombotic events, NEC).

h. Infectious Disease: Results of blood and CSF cultures will be recorded. In addition, the incidence of pneumonia (defined as infiltration on chest radiograph accompanied by increase in ventilatory support) and blood stream infections during intervention and during entire hospitalization will be noted.

i. All infants will have neonatal cranial MRI between 7 and 14 days, to evaluate the impact of lower target temperature and longer duration of cooling on cortical vs. deep gray matter. If clinically indicated, the MRI maybe obtained outside this window. Central reading of MRI will proceed following approval of MRI secondary study. Classification of MRI abnormalities will be based on the current NICHD NRN study evaluating the association of MRI abnormalities in the neonatal period and neuroprotection with hypothermia.

j. Liver function tests (including AST, ALT and bilirubin) will be obtained at baseline and at end of study intervention.

k. Evaluate for presence of aseptic subcutaneous fat necroses during the entire study period.

4.12 Treatment of Hyperthermia: Infants will be monitored for hyperthermia during the first 10 days of life. Hyperthermia will be treated as per usual care at the site.

4.13 Follow-up: All surviving infants will be followed to 18-22 months of age in the Neonatal Research Network Follow-Up Program with a compliance rate maintained at 90%. Tracking information will be recorded at the time of discharge from the NICU. An attempt will be made to obtain an autopsy in case of death occurring prior to and following NICU discharge. Growth parameters, a neurological examination and psychometric testing will be performed and vision and audiometric assessments will be recorded. Individuals performing the psychometric testing and the neurological evaluations will be masked to intervention status and they will undergo training and annual certification as per NICHD NRN Follow-Up protocol. In addition, the family's socio-economic and educational status will be assessed. If an infant is not evaluated at the 18-22 month clinic visit because of acute illness, behavior problems, or "other" reasons, appointments will be re-scheduled until the evaluation is complete.

4.14 Primary Outcome: The primary outcome will be death or disability (either moderate or severe in extent) at 18-22 months of age. *Severe disability* will be defined by any of the following: a Bayley III Cognitive score < 70, Gross Motor Functional (GMF) Level of III-V, blindness or profound hearing loss (inability to understand commands despite amplification). *Moderate disability* will be defined as a Bayley Cognitive score 70-84 and either a GMF level of II, a currently active seizure disorder, or a hearing deficit requiring amplification to understand commands. Infants without the primary outcome will be categorized as normal or mildly impaired. *Normal* will be defined by a cognitive score $\geq$ 85 and absence of any neurosensory deficits. *Mild impairment* will be defined by a cognitive score 70-84, or a cognitive score $\geq$ 85 and any of the following: presence of a GMF level 1-II, seizure disorder or hearing loss not requiring amplification.

4.15 Secondary Outcomes: These include number of deaths in the NICU and following discharge, number of infants with mild, moderate and severe disability, number of infants for whom aggressive care is withdrawn, adverse events (severe bradycardia, acidosis, bleeding, thrombotic or ischemic CNS abnormalities), clinical neonatal seizures and severe neonatal MRI abnormalities (defined by the NRN study evaluating MRI abnormalities). The treatment effect on the primary outcome by level of encephalopathy (with the understanding that the study is not powered for this analysis) will be evaluated. The MRI will be obtained between 7-14 days of age because of ongoing changes in brain injury; this timing is later than recommended by the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society (Ment 02). If it is found that clinical MRI studies are performed outside this window as part of usual care at participating centers, the studies performed closest to day 7 may need to be evaluated separately from those performed after 8 days of age since cooling may delay the evolution of brain lesions and imaging performed too soon after hypothermia may not reflect the ultimate appearance of lesions. Once the MRI secondary study is approved, two central readers (Drs Patrick Barnes and Nancy Rollins) will evaluate the clinical MRI on a rolling basis, within 1 month of MRI being shipped to the central readers. The intra-observer reliability of the central readers will be established prior to initiation of the readings.

Primary outcome---death or disability (moderate or severe) at 18-22 months of age
Secondary outcomes

- Normal infants
- Mildly disabled infants
- Mortality (including support withdrawn)
- Cognitive outcome
- Cerebral palsy
- Disability by stage of HIE
- Visual impairment
- Hearing impairment
- Multiple disabilities
- Acute adverse events
- Multiorgan dysfunction
- Neonatal seizures
- MRI findings (based on NICHD summary classification)
- Length of hospital stay
- Rehospitalizations after discharge
- Post neonatal deaths
- Growth parameters at follow up
- Bayley III Motor score

5. STATISTICAL CONSIDERATIONS

5.1 Sample Size: Sample size calculations for this 2x 2 factorial design assume that there are no large statistical interactions between the 2 factors being tested --- longer and deeper cooling. Note that this does not preclude us from testing for the presence of such an interaction (indeed, it figures as the first secondary hypotheses presented earlier), but that we are not powering the trial to detect such an interaction.

In a 2 by 2 factorial design we are essentially superimposing one trial on another -- in this case, a trial of longer vs. usual duration of cooling, and a trial of deeper vs. usual depth of cooling. So, unless we want to power for a statistical interaction (which we are not doing here), we power such a trial for a comparison between the 2 groups ("outside the table", or marginal analysis) -- longer vs. usual duration of cooling (regardless of depth of cooling), i.e., groups X vs. Y in Fig. 2 or deeper vs. usual depth of cooling (regardless of duration of cooling), i.e., groups A vs. B in Fig. 2.

The following event rates in the 4 cells of the factorial trial design are assumed: AX=45%, AY=BX=30%, BY=25%. An event rate of 45% for the control group (standard duration of cooling for 72 hours and standard depth of cooling to 33.5°C) is assumed based on the primary outcome of the NICHD RCT of whole body hypothermia for HIE (Shankaran 05). An event rate of 30% in AY and BX is an estimate. We acknowledge the event rates will depend on proportion of infants with moderate and severe HIE. We assume that BY (with the longer and deeper cooling combined) would have the lowest event rate of 25%. However, we are aware that this group may also be at highest risk for

complications of longer and deeper cooling. Assuming that we are principally interested only in testing the marginal effects of A vs. B or X vs. Y (which translates into a comparison of event rates of 37.5% vs. 27.5%), a sample size of 363 per group (A or B, or X or Y), for a total of 726, is needed with a two-tailed test, with Type I error set at 5%, power set at 80%, and allowing for 5% loss to follow up.

Two approaches will be used to monitor duration of the trial. Sites will be encouraged to increase the number of certified examiners, so that no eligible infant is missed. Secondly, the total duration of enrollment will be limited to 5 years.

It is possible that randomization would be discontinued for one group because of an unacceptable rate of adverse events in the neonatal period. In this scenario, more infants would then be randomized to the other three groups during the remainder of the trial. Assuming that the overall enrollment rate was unaffected, the number of patients would be increased for each of the three remaining groups over the number had infants been randomized to four groups. The power to conduct analyses at the margins as conventionally performed for a factorial trial would be decreased. However, there would no longer be interest in whether to use a treatment which has an unacceptable neonatal adverse event rate. With greater enrollment in the 3 remaining groups, the power to compare these specific 3 groups would not be compromised and in actuality would be somewhat higher than had the study been conducted as originally planned. (The same thinking would apply in the unlikely event that randomization was discontinued in two groups.)

5.2 Data Analyses. All data analyses will be performed according to the intention-to-treat principle. There are 2 main outcomes of this factorial design –effect of 120 hours cooling vs. 72 hours cooling and effect of 32.0°C vs. effect of 33.5°C, hence "at the margins" analyses will be carried out, testing for differences between groups X and Y, and groups A and B, from Fig. 2 (McAlister 03). We are aware that such "at the margins" analysis underestimates the efficacy of the new therapies when the interaction is antagonistic, while it overestimates efficacy when the interaction is synergistic (McAlister 03). On the other hand, "inside the table" analyses (pair-wise comparisons of groups BX, AY and BY, with group AX) use only half as many patients; the confidence intervals around treatment estimates are much wider for "inside the table" than for "at the margin" analysis and the use of the same control group (33.5 °C for 72 hours) creates a problem with multiple comparisons. Since our trial is not expressly powered for this purpose, "inside the table" analyses will only be pursued in this protocol as a secondary objective to test for statistical interactions.

The data in the two groups in the factorial design will be analyzed for treatment group differences with Chi square or Fisher's exact tests for the categorical variables and with t-tests for the continuous variables. The primary and secondary outcomes will be analyzed using robust Poisson regression models (for binary outcomes) to generate risk ratios adjusting for the stratification variables (level of HIE and site). The NICHD NRN DSMC will monitor progress of the study for safety at pre-specified time points. The DSMC will

be required to evaluate safety of greater depth and longer duration of cooling after the every 25 infants have been enrolled in the trial.

The Bayesian analyses for different scenarios are note below:

Factorial design with total of 726 subjects (363 per group, A or B, or X or Y) comparing event rates of 37.5% vs. 27.5%

	Posterior Probability of Benefit					
	>0% reduction in death or impairment		**>10% reduction in death or impairment**		**>20% reduction in death or impairment**	
Perspective	Prior	Posterior	Prior	Posterior	Prior	Posterior
Neutral	**.50**	**.997**	**.37**	**.96**	**.25**	**.72**
Skeptical	**.30**	**.99**	**.21**	**.94**	**.13**	**.67**

Factorial design with a total of 726 subjects (363 per group) comparing event rates of 35% vs. 30%

	Posterior Probability of Benefit					
	>0% reduction in death or impairment		**>10% reduction in death or impairment**		**>20% reduction in death or impairment**	
Perspective	Prior	Posterior	Prior	Posterior	Prior	Posterior
Neutral	**.50**	**.92**	**.37**	**.64**	**.26**	**.21**
Skeptical	**.30**	**.89**	**.21**	**.58**	**.13**	**.17**

Factorial design with total of 516 subjects (258 per group, A or B or X or Y) comparing event rates of 37.5% vs. 27.5%

	Posterior Probability of Benefit					
	>0% reduction in death or impairment		**>10% reduction in death or impairment**		**>20% reduction in death or impairment**	
Perspective	Prior	Posterior	Prior	Posterior	Prior	Posterior
Neutral	**.50**	**.99**	**.37**	**.92**	**.26**	**.66**
Skeptical	**.30**	**.98**	**.21**	**.89**	**.13**	**.60**

Factorial design with total of 516 subjects (258 per group, A or B or X or Y) comparing event rates of 35% vs. 30%

	Posterior Probability of Benefit					
	>0% reduction in death or impairment		>10% reduction in death or impairment		>20% reduction in death or impairment	
Perspective	Prior	Posterior	Prior	Posterior	Prior	Posterior
Neutral	**.50**	**.87**	**.37**	**.60**	**.26**	**.24**
Skeptical	**.30**	**.83**	**.21**	**.53**	**.13**	**.18**

5.3 Monitoring of Safety for the Trial:

- The protocol will be reviewed by the Institutional Review Board of each participating institution.
- The first interim analysis for safety will be conducted after 40 infants are enrolled (10 in each arm of the factorial design). Enrolment will be temporarily halted while the DSMC reviews the first interim analysis for safety and will be resumed only after the DSMC, on review of this data, is convinced that recruitment may commence.
- Following approval of the DSMC, the temperature data on the infants in the intervention arm of the study will be monitored by the PI and Subcommittee on an ongoing basis to document drops of temperature below target and plans to minimize this complication will be developed. There will be increased vigilance looking for potential complications of a greater depth of hypothermia on cardiac function, increased infection rates, increased bleeding or effects of increased viscosity.
- Serious adverse events will be reported on the MedWatch form to RTI. After the initial interim analysis for safety, serious adverse events will be compared between the treatment groups using sequential analysis methods after every 25 infants have been accrued into the trial. The 2 events that will be monitored include *arrhythmia* requiring therapy (excluding sinus rhythm or mechanical line-placement as a cause) and *major bleeding or thrombosis*. Neonates will be monitored with daily platelet counts and coagulation profile/CBC as clinically indicated. On-going masked central reading of cranial MRI will be undertaken once the MRI secondary study is approved, for evidence of CNS infarct/hemorrhage that is higher than the frequency noted in the NRN study evaluating cranial MRI among infants in the whole body hypothermia for neonatal HIE. The computed statistic will be compared to Pocock boundaries that are constructed beforehand so that an overall alpha level of 5% is maintained. RTI will be responsible for reporting adverse events to the DSMC of the Network.
- All protocol deviations/violations will be monitored by RTI.
- RTI will prepare reports for presentation to the DSMC at periodic intervals.
- DSMC will be responsible for monitoring the safety of the trial. Pre-specified looks will occur at 25%, 50%, 75% and 100% of data accrual at the conclusion of the study intervention.

- Efficacy of the trial with respect to the primary outcome will be monitored during the above specified looks at the data, as feasible, based on recruitment and follow up data accrual.

6.0 DURATION OF THE STUDY

The duration of the study is estimated to be 5 years for enrollment and 1.5 years for follow up, with a total of 6.5 years. This projection is based on a survey conducted in June 2009 of all the current NICHD NRN sites to examine study feasibility (see enclosed survey results). To summarize, the number of infants who have undergone whole body hypothermia at < 6 hours of age with eligibility criteria similar to the current proposal is as follows: In 2006, with 11 sites performing cooling for HIE, 108 infants were cooled. In 2007, with 12 sites, this number was 116. In 2008 200 infants were cooled while in 2009 with data from satellite sites (Duke University, Yale University and Wayne State University), 90 infants were cooled between January1 and May 31 2009.

The consent rate for the first NICHD trial of hypothermia for HIE was 87% (208 of 239 eligible, Shankaran 05). We anticipate a similar consent rate for the proposed study. The proposed study has estimated a 5% loss to follow up rate; however, it should be noted that the first NICHD NRN trial of hypothermia for HIE had primary outcome data available for 205/208 infants (Shankaran 05). Therefore, with a high consent rate, we are confident we can enroll 726 subjects prior to 5 years and complete follow up with a high compliance rate in an additional 1.5 years.

7.0 CONCLUSIONS

The goal of this protocol is to refine the intervention of whole body hypothermia for neonatal hypoxic-ischemic encephalopathy among term infants by testing both a longer duration of cooling and a greater depth of cooling. We anticipate recruitment into this study will be completed before newer pharmacological therapies (i.e. Erythropoietin) are ready to be in tested in randomized controlled trials following completion of pharmacokinetic, safety and efficacy studies with these agents (Juul 08, Fauchere 08).

The NICHD NRN is uniquely positioned to perform this trial; term infants with neonatal HIE are a non-competing population for research in the Network. A short study start-up time is expected as NRN sites are already trained, and have the equipment, study forms and manual of operations based on prior and on-going hypothermia studies. This study would encourage standard management of cooled infants regardless of randomization group, while optimizing cooling strategies as neuroprotection for neonatal hypoxic-ischemic encephalopathy.

8.0. SUGGESTED SECONDARY STUDIES

1) Fetal Heart Rate tracings and outcome (central reader for tracings)
2) aEEG amplitude and outcome during longer, deeper cooling (Van Meurs)
3) Impact of sedatives/analgesics/anticonvulsants levels on aEEG background (Pappas)

4) Economic analysis of neuroprotection with hypothermia
5) Platelet activation and aggregation with longer, deeper cooling (Rajpukar)
6) Biomarkers of brain injury during longer, deeper cooling (Everett/Shankaran)
7) Genetic markers of HIE (Schibler, Cotton)
8) Cytokines and longer/deeper cooling in HIE (Carlo)
9) Hypercoagulable states during longer deeper cooling (Shankaran)
10) Outcome following low Apgar scores (Laptook)
11) Neonatal MRI as a predictor of outcome with longer, deeper cooling (Shankaran, Pappas, Barnes, Rollins)
12) The role of hypocarbia in neonatal HIE (Pappas)
13) Renal dysfunction in neonatal HIE (Myers, Bell)
14) aEEG during Rewarming (Chalak, Sanchez, Pappas, Shankaran, Laptook, Huet)
15) Referral hospital and transport practices for neonates with HIE (Bara, Grisby, Huitema)

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Results of the survey of NICHD Neonatal Research Networks sites of number of infants receiving hypothermia at < 6 hours of age for neonatal HIE.

NICHD Neonatal Research Network Site	2006	2007	2008	2009 (As of 5/31/2009)
Case Western University	5	5	5	3
University of Texas at Dallas	14	8	17	12
Wayne State University	6	3	29	9
Emory University	0	0	21	15
University of Cincinnati	7	5	8	3
Indiana University	10	8	7	7
Yale University	5	5	4	1
Brown University	7	7	8	2
Stanford University	6	7	11	12
University of Texas at Houston	15	20	20	6
Duke University	9	22	26	14
Tufts University	0	0	3	0
University of Iowa	0	2	12	3
University of Utah	0	0	16	0
University of New Mexico	0	0	3	2
University of Alabama	24	24	12	5
Total	108	116	200	94 (for 5 months)

Note: Duke University and Wayne State University have included numbers from satellite sites in 2008 and 2009

Appendix A

Protocol Versions as Working Drafts
2007 October (concept)
April 4, 2008
August 15, 2008
September 18, 2008
November 17, 2008
June 5, 2009
July 15, 2009
October 21, 2009
December 16, 2009
December 22 2009
January 27, 2010
March 23, 2010
April 8, 2010

Appendix B

Budget and Justification: The following estimated budget is provided for the entire trial assuming enrollment of 726 subjects

	Cost per subject	**Number**	
Main Study Capitation	1660	726	1,205,160
Follow up	1200	530	636,000
HUS for first 40 patients	200	40	8,000
Training meeting	2000	16	32,000
Equipment and supplies			
Blanketrol	7900	16	126,400
Temperature Probes	60	726	43,560
Total Direct costs			2,051,120
Total Indirect costs@52%	1,881,160	52%	978,203
Total Cost			**3,029,323**

Research time: Costs will cover time to screen and determine eligibility of patients, data collection, initiating and monitoring of the cooling intervention, and transmission of all data items.

Medical supplies: Costs will cover supplies for the Cincinnati Sub-Zero Blanketrol including Blanketrol equipment, temperature probes, thermal blankets, and temperature probe adaptors.

Follow-up: Costs will cover tracking infants, incentives to participate in Follow-up and performance of follow-up at Network sites, based on survival rate of 75% in first NICHD NRN trial.

Training meeting: The study PI and coordinator from each Network site will be required to attend one training session in conjunction with the Steering Committee prior to initiation of the trial. Funds are required to cover an additional night of lodging/meals assuming this would occur during a NRN Steering Committee meeting.