A Phase II Multi-site Study of Autologous Cord Blood Cells for Hypoxic Ischemic Encephalopathy (HIE) IND 14753 (BABYBAC II)

NCT02612155

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INVESTIGATOR SIGNATURE PAGE

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I agree to conduct and supervise this clinical study in accordance with the design and specific provisions of this protocol; modifications to the study or protocol are acceptable only with a mutually agreed upon protocol amendment except when necessary to protect the safety of subjects. I agree to await IRB approval for the protocol and informed consent before initiating the study, to obtain informed consent from subjects' parent or guardian prior to their enrollment in the study. I agree to report to responsible regulatory agencies and IRB (when necessary) adverse events that occur in the course of this investigation. I agree to maintain accurate and adequate records in the case report forms as required by this protocol and maintain those records for the period of time required. I will make the study documentation available for safety oversight committee review and/or for other inspections as required. I agree to maintain study documentation for the period of time required. I agree to comply with all other requirements regarding the obligations of clinical investigators according to FDA regulations and guidance. I agree to ensure that all people assisting in the conduct of this study are informed in meeting the above commitments.

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(Investigator's printed name)

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(Investigator's signed name)

1. ABBREVIATIONS

AE	Adverse Event
BDNF	Brain-derived neurotropic factor
BSID	Bayley Scales of Infant and Toddler Development III
CBU	Cord Blood Unit
CMV	Cytomegalovirus
CPD	Citrate Phosphate Dextrose
CRF	Case report form
CTCAE	Common Terminology Criteria for Adverse Events
DMSO	Dimethyl sulfoxide
DSMC	Data Safety Monitoring Committee
ECMO	Extracorporeal membrane oxygenation
FDA	Food and Drug Administration
GDNF	Glial cell-derived neurotropic factor
HIE	Hypoxic Ischemic Encephalopathy
HIV	Human Immunodeficiency Virus
HTLV	human T-cell lymphotropic virus
ICU	Intensive Care Unit
IND	Investigational New Drug
IRB	Internal Review Board
IV	intravenous
MOP	Manual of Procedures
MRI	Magnetic Resonance Imaging
NAT	Nucleic Acid Testing
NGF	Nerve Growth Factor
NICHD	The National Institute of Child Health and Human Development
NICU	Neonatal Intensive Care Unit
NRN	Neonatal Research Network
PRBC	Packed Red Blood Cells
PT/PTT	Prothrombin time/Partial thromboplastin time
RBC	Red Blood Cells
RPR	Rapid Plasma Reagin
SAE	Serious Adverse Event
TNCC	Total Nucleated Cell Count
UCB	Umbilical Cord Blood

2. PROTOCOL SUMMARY

Title:	A Phase II Study of Autologous Cord Blood Cells for Hypoxic Ischemic Encephalopathy (HIE)
Study Phase:	Phase II
Study Sites:	Duke University and up to 14 additional sites
Study description:	The study will include two groups, randomized to receive up to two doses of cells (with a dose range of 2-5 x 10^7 cells/kg per dose) or up to two doses of placebo. The randomization will be stratified by severity of encephalopathy, as defined in the NRN's Optimizing Cooling trial.
Objectives:	Primary objective: Evaluate the efficacy of up to two infusions of autologous umbilical cord blood in a prospective, randomized, double-blind, placebo controlled multi-center study. Efficacy will be measured by one year survival and on Bayley III scores in all three domains $\geq$ 85.
Research Participant Population:	The study population includes infants with HIE who meet the NICHD cooling study enrollment criteria, (1) and have cord blood collected and available for infusion(s) in the first 48 postnatal hours
Study Design:	Prospective, randomized, double blind, placebo controlled, multi-center Phase II trial in up to 160 infants

3. INTRODUCTION

Treatment of babies with moderate to severe Hypoxic Ischemic Encephalopathy (HIE) with hypothermia results in normal outcomes in approximately 50% of the treated neonates [1-4]. Cooling targets inflammatory and excitatory neurotransmitter-related cell injury and early death [5-8]. Animal studies indicate that providing human cord blood cells after hypoxic ischemic injury also reduces the initial inflammatory response and enhances production of neurotrophic compounds and facilitates ongoing repair [9-12].

Our phase I, single center, open label study of infusion of autologous cord blood cells in infants treated with hypothermia for moderate to severe HIE has demonstrated that infusion of cells in the first 48 postnatal hours was safe. At one year, cell recipients were more likely to have survived with neurodevelopmental assessment scores in the normal range than infants with HIE concomitantly treated in our center with hypothermia alone [13, 25].

The accumulating animal studies, and the results of our phase I study justify a need for further study of safety and efficacy of autologous cord blood cells in a multi-center, double-blind, placebo controlled randomized trial in infants cooled for moderate to severe HIE. **We hypothesize that umbilical cord blood will improve the outcome of neonates with HIE associated with evidence of hypoxic-ischemic injury undergoing hypothermia at 12 months compared to neonates treated with hypothermia alone.** Our long-term goal is to optimize the use of human neonatal umbilical cord blood by understanding the mechanism by which umbilical cord blood interrupts the pathophysiologic cascade that is unleashed following hypoxic-ischemic injury. This study will move us toward our long-term goal by determining if administration of autologous umbilical cord blood will improve the outcomes of neonates with HIE who are undergoing therapeutic hypothermia.

4. STUDY OBJECTIVES

The primary objective is to assess the safety and efficacy of up to two intravenous infusions of autologous umbilical cord blood cells as compared with placebo in neonates with HIE undergoing hypothermia treatment. Efficacy will be measured by one year survival and score on Bayley III scores in all three domains $\geq$ 85.

5. BACKGROUND AND PILOT STUDY RESULTS

The composite results of whole body hypothermia studies indicates a significant reduction in risk of death or impairment when hypothermia is initiated in the first 6 postnatal hours and continued for 72 hours; however, in the four whole-body hypothermia studies, 44 – 51% of infants died or survived with disabilities, 24 - 38% of babies with HIE and were cooled died, and 13 – 28% of the survivors were later diagnosed with cerebral palsy. While cooling is helpful, the results of these trials provide strong incentive for development of adjunct therapies. Investigators are now testing multiple interventions, including inhaled xenon and erythropoietin, in pre-clinical and phase I and now phase III clinical trials [14]. Cell-based interventions have also shown promise [15].

5.1. ANIMAL STUDIES

The first study of human cord blood cells infused in a neonatal animal with a hypoxic-ischemic brain injury used the Rice-Vannucci model of unilateral carotid artery ligation followed by global hypoxia via exposure to very low oxygen concentration environment [16,17]. Neonatal rats were infused intraperitoneally with 1 x 10^7 human umbilical cord blood nucleated cells 24 hours after injury. Human cells localized to the injured hemisphere, but did not proliferate in situ. Tissue injury was reduced in cell recipients, and spastic paresis was alleviated [9]. Subsequently, another group infused cells and cells plus mannitol intraperitoneally 7 days after injury and found dendritic densities among cell recipients to be 75% of controls, and 80% of controls for those receiving cells plus mannitol 14 days after cell infusion. Dendritic densities for animals that received the vehicle solution or mannitol alone were approximately 50% of controls. These investigators also noted better neurobehavioral outcomes for cells and cells plus mannitol recipients, and higher brain tissue neurotrophic factor levels (BDNF, NGF, and GDNF). These results were obtained with a lower dose than that used in the first study (1.5 x 10^4 vs 1 x 10^7 human umbilical cord blood nucleated cells) [11].

In collaboration with Dr. Sid Tan, a series of experiments in a rabbit model of intrauterine brain injury were performed [18, 19, 24]. In these studies, pregnant rabbits had an intravascular catheter with balloon inserted in the uterine artery at day 22 of the usual 31 day pregnancy. The balloon was inflated for 40 minutes, restricting intrauterine blood flow, which results in rabbit pups born with a hypertonic-cerebral palsy-like phenotype. In our studies, human umbilical cord blood cells were intravenously infused within 4 hours after birth in animals with severe injury phenotypes. In the first experiment, we used a dose of 5 x 10^6 human cord blood cells in one milliliter of saline, and the infusion was delivered over one minute. Animals born with a severe phenotype that received cells and survived the neonatal period demonstrated improvement in motor function, but mortality was higher (although not statistically significantly higher) among cell recipients. In a subsequent replication study using the same infusion volume but half the cell amount (2.5 x10^6), the mortality was very similar for cell recipients and those receiving saline but the motor outcomes for cell recipients were improved compared to saline recipients. Studies with MRI demonstrated very sparse presence of human cord blood cells in the days after

infusion, but brain tissue samples obtained closer to the time of infusion demonstrate human DNA markers within the homogenized tissue samples [24]. This indicated that cells homed to the ischemic area but engraftment was transient. Despite this fact, a clinical effect was observed, likely due to paracrine signaling from the cord blood cells.

5.2. HUMAN CLINICAL DATA USING UMBILICAL CORD BLOOD IN INFANTS WITH HIE

We initiated a phase 1 study based on the emerging animal data and over a decade of experience at Duke University in performing allogeneic cord blood transplants for inherited metabolic disease. Our clinical experience with these allogeneic transplants noted surprising neurologic results, which included post-transplant myelination in the presence of disease that should have caused de-myelination [19]. We initiated a phase I open- label feasibility and preliminary safety study of use of autologous cord blood cells for infants with HIE in 2009 [13].

Our phase I study has enrolled 45 subjects. The initial intent was to provide up to 4 infusions of 2-5 x 10^7cells/kg each in the first 72 postnatal hours. After release of guidance for public cord blood banking in 2011, the FDA implemented a requirement for regulation of all non-homologous uses of umbilical cord blood. Accordingly, we submitted an IND application for the phase I study. In July 2011, FDA indicated that our IND was safe to proceed, and our study moved forward under an IND with a protocol modification allowing a maximum of 2 infusions of fresh cells in the first 48 postnatal hours; FDA requested the number of infusions limited until there was sufficient evidence to ensure reliable viability of unfrozen cells beyond 48 hours.

Study operations included collection of cord blood, usually with verbal assent for collection for potential use in the study, unless the family had previously provided consent for collection for public banking of cord blood. If blood was collected, and the infant met cooling criteria, and the parents provided consent for enrollment in the phase I study, cells were volume and red blood cell reduced on the Sepax device (Biosafe, International), or manually if cord blood volumes were below 30 milliliters. For the majority of enrollees (after 2011), two infusions were provided in the first 48 postnatal hours.

The improved survival and one-year neurologic outcomes of subjects who received cells and concurrent cooling compared to cooled infants who did not receive cells provide impetus to move forward to a multi-center phase II placebo controlled study. Since publication of the phase I paper in May 2014, we have accumulated and reported additional data on infants with HIE and one year outcomes [25]. Thirty-nine (39) infants have been cooled and received cells (16 since our initial report) and 146 have been cooled for HIE without receiving cells. For those receiving cells, median collection and infusion volumes were 33 mL and 5 mL. None of the cell recipients died prior to discharge while 16 (11%) of cooled non-cell recipients died prior to hospital discharge ($p = 0.03$). Ten (26%) cell recipients and 31 (21%) cooled-only infants were discharged on seizure medications ($p = 0.56$). Of the 25 cell recipients with known one year

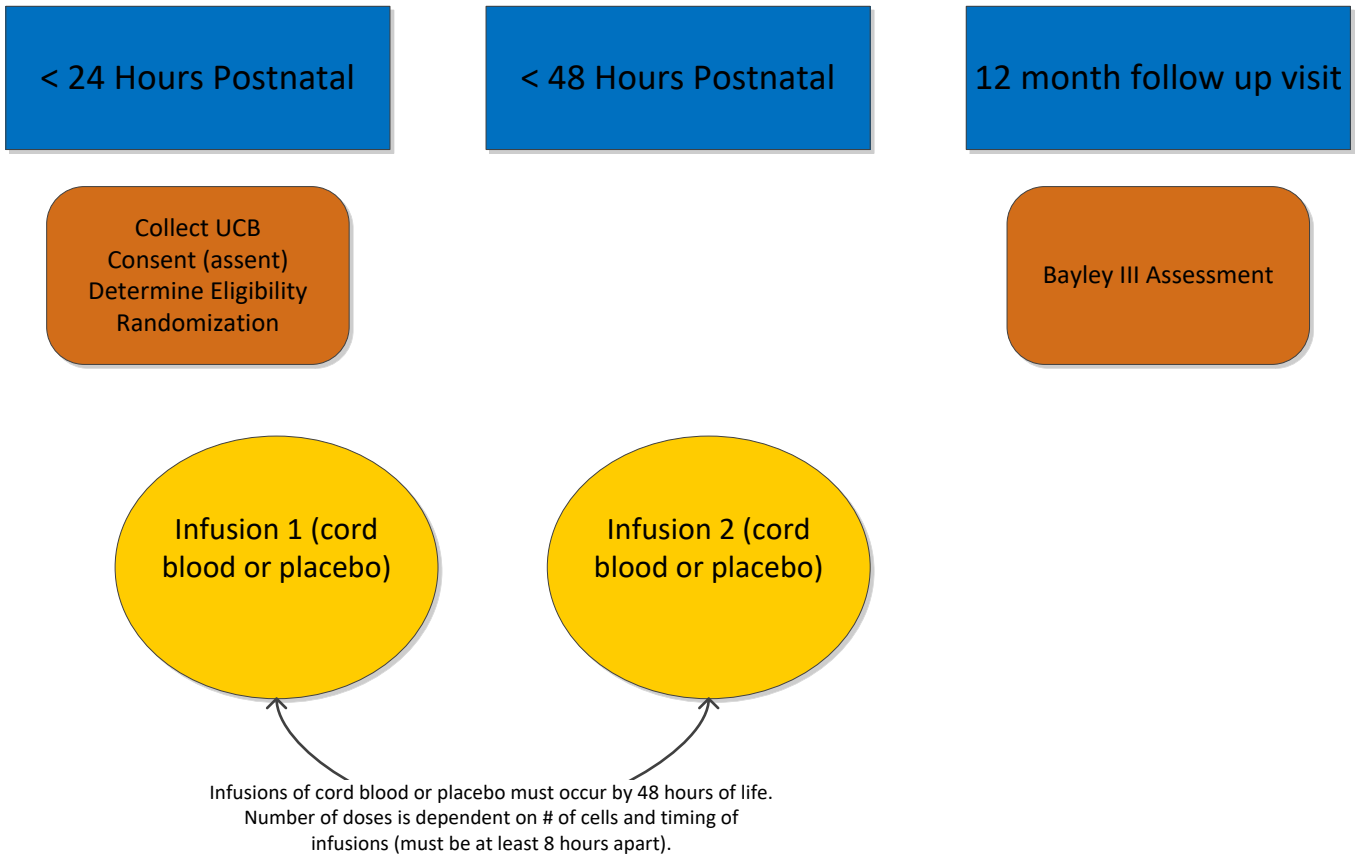
outcomes, 16 (64%) survived with Bayley III scores ≥ 85 in all three domains, and of the 63 cooled-only infants with known one year outcomes, 25 (40%) survived with one year Bayley III scores ≥ 85 in all three domains ($p = 0.04$). While the results for the cell recipients are better than for infants who were cooled, important facts must be acknowledged. More of the cell recipients were inborn, and more of the cooled only group were not seen at one year follow-up.

There were no serious adverse event directly related to study therapy. No significant infusion reactions were noted. Heart rate, mean arterial pressure, and oxygen saturation did not vary significantly before and after infusions for the first two infusions. Mean oxygen saturation was lower after the third and fourth infusion. One infant, born at 35 weeks gestation, had a cord blood pH of 6.72 and a spontaneous ileal perforation prior to the fourth infusion. One infant had a positive umbilical cord blood (UCB) culture for *E. coli*, which was also present in the mother's blood culture. The infant's own postnatal blood culture was negative and prophylactic antibiotics were started. For one subject, an infusion was stopped due to an occluded iv line but subsequent infusions were successfully completed. It was concluded the safety profile of the Phase 1 study warranted further study. **We believe that the accumulating evidence in pre-clinical studies and the initial signals of safety and feasibility in the phase I study are sufficient evidence to justify a phase II study to further assess potential efficacy of autologous cord blood cells in the first postnatal days for infants treated with hypothermia for moderate to severe HIE.**

6. STUDY DESIGN

This is a prospective, randomized, double-blind, placebo controlled multi-center Phase 2 study in up to 160 infants who meet the NICHD cooling study enrollment criteria and have cord blood collected and available for up to two infusions in the first 48 postnatal hours. We will evaluate the efficacy of the infusions of autologous umbilical cord blood. We project accrual of 60-80 subjects per year. The study will include two groups (up to 80 subjects per group), randomized to receive up to two doses of cells (with a dose range of 2-5 x 10^7 cells/kg per dose) or up to two doses of placebo. The randomization will be stratified by severity of encephalopathy, as defined in the NRN's Optimizing Cooling trial [19]. Investigators and families will be blinded to enrolled study group.

6.1. STUDY FLOW CHART



6.2. STUDY POPULATION AND SITE SELECTION

The study population will include up to 160 infants who meet the NICHD cooling study enrollment criteria[19], and have cord blood collected and available for infusion(s) in the first 48 postnatal hours.

Study sites will include Duke University and up to fourteen additional sites that are Level 3 neonatal ICUs with a standardized hypothermia protocol.

7. INCLUSION AND EXCLUSION CRITERIA

7.1. INCLUSION CRITERIA

Enrolled subjects must meet all of the following criteria:

1. Cord blood must be available for volume and red blood cell reduction before 45 hours of age. Please follow local IRB rules regarding consent/assent for cord blood collection.
2. The infant must be able to receive at least one dose of autologous cord blood before 48 hours of age
3. All infants must have signs of encephalopathy within 6 hours of age utilizing the two step (A and B) approach used in the Network's Optimizing Hypothermia Study [20].

The eligibility criteria:

- Infants will be evaluated in two steps; evaluation by clinical and biochemical criteria (*Step A, which is either A1 or A2, depending on available information and severity of blood gas abnormalities*), followed by a neurological exam (*Step B*)
- Once infant meets either A1 or A2, proceed to neurologic examination. (See part B)
- The presence of moderate/severe encephalopathy (a "2" or a "3") defined as seizures **OR** presence of signs in 3 of 6 categories in the table below. For the categories with more than one item, such as PRIMITIVE REFLEXES, the item (SUCK, MORO) with the highest score determines the level of encephalopathy assigned for that category
- The neurologic examination will be performed by a physician examiner

Steps A1 and A2. All infants will be evaluated for the following:

1. Is the baby ≥ 36 weeks gestation?
2. Is there a history of an acute perinatal event (abruptio placenta, cord prolapse, severe FHR abnormality: variable or late decelerations)?
3. Is the Apgar score ≤5 at 10 minutes or is there a continued need for ventilation initiated at birth and continued for at least 10 minutes?
4. What is the cord pH or first postnatal blood gas pH at ≤1 hour?
5. What is the base deficit on cord gas or first postnatal blood gas at ≤1 hour?

If infant meets criteria A1 or A2 and criteria B and does not meet exclusion criteria, the infant is eligible and is therefore eligible for study enrollment if cells are available

IF BLOOD GAS IS AVAILABLE:	**IF BLOOD GAS IS NOT AVAILABLE *OR* pH between 7.0 and 7.15, *OR* BASE DEFICIT 10 to 15.9mEq/L**
A1	**A2**
Answer to #1 is 'YES' AND □ Cord pH or first postnatal blood gas within 1 hour with pH ≤7.0 (#4) **OR** □ Base deficit on cord gas or first postnatal blood gas within 1 hour at ≥16 mEq/L (#5)	Answer to #1 AND □ Acute perinatal event (#2) **and either** □ An Apgar score ≤5 at 10 minutes (#3) **OR** □ Continued need for ventilation initiated at birth and continued for at least 10 minutes (#3)

Part B: Neurologic Assessment

CATEGORY	**SIGNS OF HIE IN EACH LEVEL**		
NORMAL/MILD HIE		**MODERATE HIE**	**SEVERE HIE**
1. LEVEL OF CONSCIOUSNESS	**1**	**2 = Lethargic**	**3 = Stupor/coma**
2. SPONTANEOUS ACTIVITY	**1**	**2 = Decreased activity**	**3 = No activity**
3. POSTURE	**1**	**2 = Distal flexion, complete extension**	**3 = Decerebrate**
4. TONE	**1**	**2a = Hypotonia (focal or general)** **2b = Hypertonia**	**3a = Flaccid** **3b = Rigid**
5. PRIMITIVE REFLEXES			
Suck	**1**	**2 = Weak or has bite**	**3 = Absent**
Moro	**1**	**2 = Incomplete**	**3 = Absent**
6. AUTONOMIC SYSTEM			
Pupils	**1**	**2 = Constricted**	**3 = Deviation/dilated/non-reactive to light**
Heart rate	**1**	**2 = Bradycardia**	**3 = Variable HR**
Respiration	**1**	**2 = Periodic breathing**	**3 = Apnea or requires ventilator** 3a=on vent with spont breaths 3b=on vent without spont breaths

7.1.1. EXCLUSION CRITERIA

Enrolled subjects must not meet any of the following criteria:

1. Major congenital or chromosomal abnormalities
2. Severe growth restriction (birth weight <1800 g)
3. Opinion by attending neonatologist that the study may interfere with treatment or safety of subject
4. Moribund neonates for whom no further treatment is planned
5. Infants born to mothers are known to be HIV, Hepatitis B, Hepatitis C or who have active syphilis or CMV infection in pregnancy
6. Infants suspected of overwhelming sepsis
7. ECMO initiated or likely in the first 48 hours of life
8. Mother suspected to have chorioamnionitis at time of birth. Refer to MOP-01, section 4.3 for definition.
9. ALL blood gases (cord and postnatal) done within the first 60 minutes had a pH >7.15 AND a base deficit < 10 mEq/L (source can be arterial, venous or capillary)
10. Mother with documented Zika infection during this pregnancy

7.1.2. SCREENING AND ENROLLMENT

Centers will screen for potential subjects among all deliveries of infants ≥ 35 6/7th weeks who appear to be at risk for delivery of an infant with encephalopathy. From our single site experience, the obstetric team collected cord blood at 5 – 6 deliveries of potentially encephalopathic infants for every one infant who met enrollment criteria in the Phase 1 study. If an infant whose cord blood was collected for potential enrollment in the study is subsequently not enrolled in the study, the collected cord blood will be discarded as medical waste at the study site (unless arrangements are made by the family for private banking).

7.1.3. RANDOMIZATION AND STRATIFICATION

After informed consent is obtained, eligible infants will be randomized to one of two arms (cells or placebo). Neonates randomized to receive cells will receive up to two doses of cells (with a dose range of 2-5 x 10^7 cells/kg per dose), while those randomized to the placebo arm will receive up to two doses of placebo.

Randomization will be conducted using permuted block design and stratified by stage of encephalopathy (moderate or severe). A central web based randomization system available 24 hours a day, 7 days a week, will be developed and maintained by the Data Coordinating Center at RTI International, Research Triangle Park, NC.

8. STUDY PRODUCT AND PLACEBO

8.1. STUDY PRODUCT

Autologous cord blood will be collected *in utero* during the 3rd stage of labor or shortly after delivery of the placenta from neonates at risk for the development of HIE. Sites must follow local IRB rules regarding consent/assent for cord blood collection for autologous use. . The cord blood will be collected into Pall Medical Sterile CB Collection Unit Code 791-08 containing 35 mL of citrate phosphate dextrose (CPD) anticoagulant. It is important to collect the maximum volume of cord blood. The cord blood unit (CBU) will be transported to the processing laboratory where it will be red blood cell (RBC) and volume reduced and up to 2 study doses prepared. The infusion volume will be ≤3.0 cc/kg with an infusion time of 10-20 minutes. A manual of procedures will be provided for all sites to assure consistency of processing.

If randomized to treatment arm, cell preparation will include volume- and RBC reduction after 20-30 minute incubation with 6% Hespan (hetastarch, Hospira, Lake Forest, Illinois, USA) using the Sepax automated processing system (Biosafe, Geneva, Switzerland) for units with ≥ 30mL of umbilical cord blood (UCB). A manual method will be used if the collected cord blood volume is < 30mL using procedures in the manual of procedures. A sample of processed cord blood is used to determine the cell count and decide if one or two doses are aliquotted into individual dose syringes. A sample is also removed for the gram stain.

A gram stain from the study product and placebo will be performed and resulted prior to infusion of the study product or placebo to indicate sterility. If the gram stain is positive, then the cells or placebo will not be infused and the cord blood is discarded. If sterility cultures, which will be performed on the collected cord blood at Duke University Stem Cell Laboratory (STCL), subsequently become positive, the study team will notify the clinical team of the nature of the positive culture and a follow up strategy will be developed by the clinical and research teams. In general, babies with HIE are placed on empirical antibiotic therapy shortly after birth. If the baby is on concurrent antibiotic therapy and if the antibiotic covers the organism grown in the culture obtained from the cord blood, the baby will complete a course of therapy. If, on the other hand, the baby is no longer on antibiotics or the organism identified in the culture is not sensitive to the antibiotics the baby is being treated with and is not considered a contaminant, a course of antibiotic therapy covering the organism isolated will be administered.

8.2. PLACEBO

Placebo will be prepared from a small volume of unprocessed cord blood diluted in saline. This formulation will be described in a manual of procedures. Placebo will have a similar appearance to the study product. A gram stain from the CBU must be performed and resulted prior to release of placebo (see section 8.1). Only products with negative gram stain will be released for infusion.

8.3. SHIPMENT OF REMAINING CORD BLOOD PRODUCTS TO DUKE FOR CYROPRESERVATION

The entire cord blood unit or cord blood remaining after processing and removal of cells for dosing and the remaining material in the PRBC bags and plasma bags from remote sites will be shipped to the Stem Cell Transplant Laboratory at Duke University for cryopreservation and testing for potential future autologous use by the study subject. Each infant's cord blood unit's characteristics will be recorded, including total nucleated cell counts pre- and post-processing, post-processing viable $CD34^+$ cell content, colony forming units, sterility, and viability. The manual of procedures describes packing and shipping to STCL at Duke University. Cryopreservation is dependent on receipt of cord blood or processed cord blood within 96 hours of collection at STCL at Duke University. It is best to ship as soon as possible to preserve the viability and potency of the cord blood cells.

8.4. INFUSIONS

For babies meeting entry criteria, the first dose of cells/placebo will be infused intravenously as soon as possible. Infants will be pretreated with hydrocortisone, 1 mg/kg IV 30-60 minutes prior to each infusion if the subject was not on hydrocortisone for clinical purposes. The placebo group is receiving hydrocortisone to minimize the risk of infusion-related and/or allergic reaction to the placebo - the same risk minimization as for the active stem cell recipients. Cells/placebo will be infused over 10-20 minutes, followed by a saline flush (≤ 5.0mL) to clear the intravascular line. The study allows up to two doses of cells or placebo if the cell number is adequate from the cord blood unit. There must be a minimum of 8 hours between doses and both doses need to be completed by 48 hours post delivery. Babies receiving placebo infusions will be treated in an identical fashion to those receiving cells. The infusion product will be blinded to the hospital staff administering the cells and evaluating the babies' outcomes.as well as the family.

8.5. MONITORING DURING AND AFTER INFUSION

8.5.1. VITAL SIGNS

Vital signs will be assessed pre- infusion and for 15 minutes post infusion and per hospital routine thereafter.

8.5.2. METABOLIC STATUS

Daily chemistries are routinely obtained in infants with moderate to severe HIE when they are treated with hypothermia. Serum electrolytes, CBC, BUN, and creatinine, and frequently liver function tests are monitored at baseline, and then daily. We will record results of metabolic laboratories collected for clinical purposes during the cooling period and the first 24 hours post re-warming in the case report forms.

8.5.3. RESPIRATORY STATUS

A daily blood gas is standard in infants with moderate to severe HIE. Results from daily blood gases obtained for clinical purposes during cooling and for the first 24 hours after re-warming will be collected in the case report forms.

8.5.4. NEUROLOGIC STATUS

A neurological assessment will be performed at baseline, daily during cooling, and at discharge. This will be performed by a trained examiner.

8.5.5. HEMATOLOGIC STATUS

Monitoring of coagulation studies is considered routine for infants with HIE. PT/PTT results obtained for clinical purposes during cooling and for the first 24 hours after re-warming will be recorded in the case report forms.

8.5.6. FOLLOW UP VISITS

Subjects will return at one year (12 mo - 16 mo) corrected age. The following assessments will be recorded: History and physical exam, Bayley Scales of Infant Development III (BSID), neurological exam, adverse events and concomitant medications. Infants not testable on the BSID due to Cerebral Palsy, blindness or deafness will be assigned scores of 54, 46 and 46 respectively on the cognitive, language and motor of the BSID scale.

9. SCHEDULE OF EVENTS

	First Dose<40 hours postnatal if two doses are feasible <48 hours if only one dose is feasible	Second dose if feasible < 48 hours postnatal	Rewarming through postnatal day 21	Visit 1 12-16months
Screening	X			
Maternal consent or assent	X			
Collect UCB	X			
Labs[1]	X			
Blood Gas[2]	X			
Neurological Assessment[3]	X	X	X	X
Infusion and vitals[4]	X	X (if cells are available)		
History and Physical Exam	X			X
Adverse events and concomitant meds	X	X	X	X
MRI			X	
PT/PTT[5]	X		X	

[1] Serum Electrolytes, BUN, CBC, Creatinine, liver function obtained as part of routine care for HIE (section 8.5.2)
[2]Collected daily (section 8.5.3)
[3] Collected at baseline, daily during cooling and prior to discharge (section 8.5.4)
[4] Assessed pre-infusion and for 15min post infusion and per hospital SOC (section 8.5.1)
[5] Collected during cooling and 24 hours after re-warming if obtained as part of routine care (section 8.5.5)

10. OTHER TREATMENTS/TESTS/CONCURRENT MEDICATIONS

Anti-seizure medication and sedation ideally will not be administered prior to hypothermia or study infusions, unless clinically indicated. All medications will be listed in concomitant medications in the case report forms.

11. PRIMARY OUTCOME MEASURES

11.1.BAYLEY SCALES OF INFANT AND TODDLER DEVELOPMENT- III

The Bayley Scales of Infant and Toddler Development-III (BSID) [23] will be administered with all children at 12-16. Months corrected age. The Bayley-III is an individually-administered examination that assesses the current developmental functioning of infants and young children from birth to 42 months of age. This instrument has been shown to have adequate reliability and validity, and is considered the gold standard of infant and toddler assessment tools. The Bayley is a standardized, norm-referenced measure that assesses development in Cognitive, Language and Motor domains. Composite standard scores can be derived that have a mean of 100 and a standard deviation of 15.

All Bayley examiners for this study will be trained and certified to a criterion of 80% accuracy for administration and scoring. Certification will be completed via submission of a DVD of administration of the Bayley-III Scales with a non-study child of the target age for the study. The DVD will be reviewed and the accuracy of administration and scoring will be determined by the study gold standard examiner within two weeks of submission of the DVD. Additional DVDs will be submitted by the examiner until the criterion of 80% accuracy is reached for both administration and scoring. Each examiner must be certified once per year. No study participants may be assessed by an examiner who has not been certified.

For children with sensory impairments, an attempt should be made to administer Bayley items and a decision about validity of assessment will be made subsequently with consultation with the gold standard Bayley examiner. However, some children with sensory impairment cannot be reliably assessed with the Bayley-III. This includes children who are: 1) legally blind (< 20/200 with glasses). 2) Hearing impaired and have no amplification/hearing aids. If a child does have hearing aids, the Bayley must be completed with the hearing aids in place. If the child has profound hearing loss and is expected to undergo a cochlear implant, the child will not be able to be tested. 3) Child is both vision and hearing impaired (Deaf/Blind). These children cannot be tested with the Bayley. Infants not testable on BSID due to CP, blindness or deafness be assigned respective scores of 54, 46 and 46 on the cognitive, language and motor components of the BSID III.

Non-English language learners will be assessed by a bilingual examiner who has been certified or with the assistance of a qualified interpreter. Interpreters must be instructed to translate the Bayley instructions to the child verbatim and to not repeat instructions unless instructed to do so by the examiner. Language scores will not be derived for non-English language learners as the normative developmental progression of language milestones in other languages may not be accurately reflected on the Bayley Language Scales.

11.2.MRI

An MRI is routinely obtained on HIE infants between 7 – 28 postnatal days. Ideally the MRI will be done optimally at 10 days of life (but with an acceptable window between 7 days and 21 days of life as suggested by the 2014 revised guidelines for infants with HIE, to assess extent of injury) [21]. Results from the standard of care MRI will recorded for the study.

11.3.NICU DISCHARGE

Tracking information will be recorded at discharge, and will include phone numbers and home address. All surviving infants will be followed for one year for the in-person assessment.

An attempt will be made at each site to obtain an autopsy in case of death both prior to and following NICU discharge. Autopsy results, if available, will be included in the case report forms.

11.4. SHORT TERM/HOSPITAL OUTCOMES/STUDY ENDPOINT

Short term outcomes will be considered secondary outcomes for the study. We will compare adverse outcomes, including mortality, seizures, need for nitric oxide and need for ECMO, need for G-tube feeding at discharge, and discharge on anti-epileptic medications. This information will be collected in case report forms.

11.5.LONG TERM OUTCOMES/PRIMARY OUTCOME/STUDY ENDPOINT

The Primary outcome of the study will be survival at one year with Bayley III scores in all three domains $\geq$ 85.

12. RISKS AND BENEFITS

12.1.RISK AND BENEFITS OF STUDY

Infusion of a neonate's own cord blood cells during routine cooling is hoped to improve survival and neurological outcome as compared to infants that are only cooled and receive red blood cells. Autologous cells should eliminate any risk of graft vs host reaction. If cord blood cells are collected and not infused, cord blood cells will be cryopreserved for later autologous use for the subject. Complications of cord blood infusions could include infection, volume overload, hypertension, allergic or anaphylactic reactions, hyperviscosity or pulmonary edema and will be

monitored during and after infusion. The medication hydrocortisone given prior to the infusions may increase blood sugar, blood pressure and fluid retention.

13. SAFETY AND ADVERSE EVENT REPORTING

Adverse Events (AE) Adverse events and serious adverse events parameters are listed below. The parameters for events are derived from prior studies of thawed/washed cord blood cells used for allogeneic transplant, in combination with expected physiologic responses to therapeutic hypothermia, which by definition and inclusion criteria, will be concurrently underway when the study infusions are administered. The physiologic response to hypothermia leads to decreased heart rate during cooling (the mean Heart Rate during cooling in the NICHD NRN pivotal trial was 109); therefore a lower level to define significant bradycardia is used (Shankaran 2005). Other AE/SAE definitions are taken from the NICHD Optimizing cooling trial, to account for the concurrent use of therapeutic hypothermia, and the AE/SAE category definitions developed for the NICHD Pediatric Trials Network (Shankaran 2014 ;England 2016). Definitions for each AE parameter are listed in MOP-01 Section 10.1.

SAEs may include:

- Acidosis
- Allergic reaction/hypersensitivity
- GI Pathology
- Hemoglobinuria
- Hyperglycemia
- Hypoglycemia
- Hypertension
- Hypotension, and fever in the absence of neutropenia
- Hypoxia
- Infectious Disease
- Polycythemia
- Pulmonary Hypertension, within the first 24 hours
- Sinus bradycardia
- Sinus tachycardia
- Dyspnea
- Any other cardiac rhythm disturbances,
- Any other reported SAE, including death

13.1.1. ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of the investigational product, whether or not considered related to the investigational product.

13.1.2. SERIOUS ADVERSE EVENTS (SAE)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

13.1.3. GRADE/SEVERITY

Grade/Severity will be performed per CTCAE guidelines.

13.1.4. SUSPECTED ADVERSE REACTION

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the investigational product caused the adverse event. 'Reasonable possibility' means there is evidence to suggest a causal relationship between the investigational product and the adverse event.

13.1.5. CAUSALITY

The Investigator will use the following question when assessing causality of an adverse event to the investigational product: Is there a reasonable possibility that the drug caused the event? An affirmative answer designates the event as a suspected adverse reaction.

13.2.ADVERSE EVENT REPORTING

All adverse events reported or observed during the study beginning at the time of the autologous cord blood infusion must be recorded and maintained in the study participant's paper files or electronic case report forms. Information to be reported includes when the site became aware of the event, investigator-specified assessment of severity and relationship to study therapy, whether there is an alternative etiology, seriousness, as well as any required treatment or evaluations, and outcome. In general, investigators should report adverse events as diseases or syndromes whenever possible, instead of reporting individual component symptoms, signs, laboratory abnormalities, and sequelae.

Severe adverse reactions (fatal or life threatening) that are unexpected and related will be reported to the FDA by Duke University within seven calendar days of receipt of the information by telephone, mail or fax, following FDA guidelines. All non-life threatening serious, related, and unexpected adverse events will be reported to the FDA via a written report within 15 days of receipt of the information (21 CFR 312.32) by Duke University. If the principal investigator assesses the event to be unrelated to the study, then the event will not require expedited reporting but will be included in a summary report.
Investigators are requested to notify the Principle Investigators at Duke University within 24 hours of all SAEs. The Principal Investigator or Sub-Investigator at each site is responsible for informing their Institutional Review Board (IRB). Copies of SAE correspondence with all Principal Investigators or Sub-Investigators, governing authorities, ethics committees, and the sponsor must be submitted to Duke University for filing.

13.3. UNBLINDING

Safety or other clinical or legal considerations including requests from a child's family may, on rare occasions, require unblinding of the infant with regard to the actual intervention received. If such events make it necessary, the lab staff, responsible for preparation of study product at each site will be able to unmask the study drug product given to an individual infant. Before unblinding the lab staff must contact the Center's Investigator and the overall study Principal Investigators prior to releasing this information, and a protocol violation form will be completed.

13.4.INTERIM MONITORING FOR SAFETY OUTCOMES

The intervention for this trial will be conducted during the first 48 hours of life for the study subjects, while the primary outcome will be assessed at 12 (12 – 16) months of age. Thus, the primary focus of interim monitoring for this trial will be based on monitoring adverse events within 24 hours of cord blood infusion. Adverse events monitored will include the following, plus a composite (any of the listed events), on which statistical comparisons between the treatment groups will be based:

- Acidosis
- Allergic reaction/hypersensitivity
- GI Pathology
- Hemoglobinuria
- Hyperglycemia

- Hypoglycemia
- Hypertension
- Hypotension, and fever in the absence of neutropenia
- Hypoxia
- Infectious Disease
- Polycythemia
- Pulmonary Hypertension, within the first 24 hours
- Sinus bradycardia
- Sinus tachycardia
- Dyspnea
- Any other cardiac rhythm disturbances,
- Any other reported SAE, including death

In keeping with the Bayesian nature of the trial design, we will use a Bayesian interim monitoring approach to determine whether systematic differences are emergent among the two treatment groups in the incidence of any of the adverse events listed above. Specifically, after every 40 babies enrolled in the trial have spent 24 hours post their final study transfusion, we will calculate the posterior probability that one group has higher incidence of any adverse events than another. In statistical terms, 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 (in terms of the above adverse events) is greater than η ; in other words if the predictive probability $P(\theta > 1 \mid X) > \eta$, where θ denotes the relative risk favoring the treatment group and X is the data available. In order to allow for a liberal safety monitoring regime to ensure patient safety, we propose to take η = 80%. However, we will also present the 95% credible interval for θ, as well as the entire posterior distribution for θ in a graphical fashion to the DSMC so that they have a full appreciation for the range of possible values of θ. All interim analyses will adjust for baseline level of encephalopathy, the stratification variable for this trial, whenever computationally feasible.

The choice of a prior distribution is essential and controversial in Bayesian analyses. Thus, we can compute the posterior probability of treatment harm and the posterior distribution of the associated relative risk under two sets of priors – (a) a non-informative prior that does not assume any substantive prior information about treatment differences in terms of the adverse events listed above, and (b) an informative prior based on treatment differences for these adverse events observed in the Phase I trial. However, we realize that the standard Bayesian approach of running the analysis with different priors provides only a partial solution, as the results on stopping are often inconclusive, especially when few data have accumulated. Instead of worrying about the selection of a single correct prior, we will also explore the use of a robust Bayesian approach which replaces a prior distribution with a class of priors and calculates the corresponding posterior probabilities for decision making[26].

13.5. INTERIM MONITORING FOR EFFICACY OUTCOME

The relatively rare condition that this trial addresses means that it may take considerable time for this trial to enroll the requisite number of babies. Thus, even though the primary outcome is only available at 12 months corrected age, the enrollment period for the trial may make some interim efficacy data on the primary outcome available for monitoring by the DSMC. Note that because of the Bayesian nature of the trial design, interim efficacy looks at the data will not have any adverse Type I error consequences that are normally the case for a conventionally designed (or, 'frequentist') trial.

We propose to conduct one formal interim analysis of the accruing primary outcome data, examining both efficacy and futility, at a third of primary outcome accrual for this trial, provided that recruitment and intervention have not been completed by that point in time. The analysis for both efficacy and futility will adjust for baseline level of encephalopathy, the stratification variable for this trial, if computationally feasible

13.6. INTERIM ESTIMATION OF TREATMENT EFFICACY

This interim efficacy analysis will first evaluate whether the intervention is beneficial, equivalent, or harmful relative to usual care. A Bayesian logistic regression model will be fit to the data to obtain adjusted estimates of interim treatment efficacy, adjusting for the trial stratification variables.

A prior distribution is chosen in Bayesian analyses to provide an estimate of initial beliefs concerning the size of the potential treatment benefit. Thus, if one hopes that the results from the trial will influence the treatment of future patients, the prior distribution should represent the level of skepticism that is expressed by those clinicians that one seeks to influence. There are three principal types of prior we could use: (i) the uninformative, or reference prior, (ii) the skeptical prior and (iii) the enthusiastic prior. The uninformative prior represents a lack of clinical opinion as to the likely treatment difference, and in that sense contains no information about prior beliefs or other prior knowledge (eg. a relative risk for treatment benefit set at 1.0). A skeptical prior would be that overall effect of cord blood is actually harmful in this setting (i.e., a relative risk set higher than 1). An enthusiastic prior would be that the treatment is beneficial, as in the Phase I trial (relative risk set lower than 1).

It is generally useful to present the results of Bayesian analyses under several alternative prior distributions, so that the impact of differing levels of prior belief on the observed data may be reviewed. The uninformative prior has the advantage of corresponding roughly to the classical frequentist approach, and so this together with the skeptical and enthusiastic priors gives a broad and useful overview of the implications of terminating a clinical trial[27]. Accordingly, we will use

all three prior distributions to present adjusted mean posterior relative risk (RR) estimates of treatment efficacy relative to survival free of cognitive impairment, along with corresponding 95% credible intervals for the same. These priors will be set at RR = 1.1 (skeptical prior), 1.0 (uninformative prior) and 0.625 (enthusiastic prior).

The DSMC may consider proposing termination of the trial for benefit if the upper 95% credible limit for the mean posterior RR of treatment benefit, calculated using a skeptical prior, is less than 1.0. We propose to use a skeptical prior for this consideration to provide a more conservative basis for trial termination, and also to convince even those who strongly believed at the outset that the intervention will not succeed in improving survival free of cognitive impairment. In addition, we note that early stopping of a trial based on treatment benefit generally produces a biased and sometimes misleading estimate of the treatment effect [28, 29].

13.7. INTERIM ESTIMATION OF TREATMENT FUTILITY

In addition to providing estimates of interim treatment efficacy, we will also conduct interim futility analyses for treatment efficacy. For this purpose, at each interim safety look, we will use the available data to calculate the posterior probability of concluding that the probability of survival free of cognitive impairment in the cord blood group would be higher than in the placebo group (i.e., the mean posterior RR for treatment benefit is greater than 1.0), if the trial continued until 120 patients were randomized and had their primary outcomes evaluated. In order to provide a more conservative basis for this estimate, and also to convince even those who strongly believed in this intervention at the outset, we will primarily use an optimistic prior for this calculation, with the prior belief set at a level of treatment benefit observed in the Phase I trial. However, in order to provide a broad overview and to explore the robustness of this estimate with respect to prior assumptions, this probability will also be calculated and presented for the two other prior distributions (skeptical and uninformative).

The DSMC may consider proposing termination of the trial for futility if this posterior probability of observing any treatment benefit, if the trial continued to full primary outcome accrual, is less than 10%.

14. DATA SAFETY MONITORING COMMITTEE

A DSMC will be responsible for monitoring the safety of the trial. The DSMC will have a minimum of three members including a biostatistician, a neonatologist, and an expert in cord blood stem cell transplant. The DSMC members will be independent from any enrolling center and guided by a DSMC charter.

15. STATISTICAL CONSIDERATION

15.1.STUDY DESIGN

This study is a prospective Phase II prospective, randomized, double-blind, placebo controlled, multi-center trial designed to assess the safety and efficacy of two infusions of autologous UCB cells or placebo in newborns born in distress.

15.2.ESTIMATES OF AVAILABLE AND REQUIRED SAMPLE SIZE

In our phase I study to date, 16 of 25 (64%) cell recipients with known one year outcomes have survived with all three Bayley III assessments (Cognitive, Motor, and Language) $\geq$ 85, and 25 of 63 (40%) of the cooled-only infants with known one year outcomes has survived with all three Bayley III domain scores $\geq$ 85. (13) We acknowledge the limitations of a one year Bayley score, but would note that our rate of survival with Bayley scores $\geq$ 85 among cooled-only infants is consistent with the previously reported Whole Body Hypothermia studies rates of survival at 12–24 months without moderate or severe impairment (1 -4).

For the phase II study, rather basing the sample size on effect size, we would propose a Bayesian approach as used in the NRN Late Hypothermia study. Because this is phase II where our aim is to detect a trend favoring cell recipients, and to accumulate data that will inform design and sample size calculations for a phase III study of efficacy, we anticipate that two years of enrollment would provide approximately 120-160 infants, 60-80 in each group.

Control	Cell Recipients	95% Confidence Interval	Power
0.6	0.30	0.45	0.92
0.6	0.35	0.48	0.81
0.6	0.40	0.51	0.62
0.6	0.45	0.54	0.38
0.6	0.60	0.54	0.056

The Bayesian analysis will permit calculation of a probability of treatment benefit. Within the range of an active group proportion of 0.3 to 0.45, the average 95% credible interval width ranges from 0.45 to 0.54 when the control group has a proportion having the primary outcome (death or Bayley III scores < 85) of 0.6. If the decision rule for declaring a win is the posterior 95% credible interval must exclude 1, then the design has power ranging from 0.92 to 0.38 with a Type 1 error of about 0.056.

15.3. STATISTICAL ANALYSIS PLAN

All statistical analyses, including interim monitoring, will be conducted using the intent to treat principle, unless explicitly specified otherwise for certain secondary and/or post-hoc analyses. A Bayesian approach will be used for conducting the primary analysis in this trial. 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 160 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 = 0.625) priors as well. In all analyses, MCMC methods will be used to sample from the Posterior distribution.

All analyses will involve estimating a Posterior distribution based on a log-binomial regression model with covariates and MCMC methods. In addition to estimating the treatment effect on the log relative risk scale, the log-binomial regression will adjust for level of encephalopathy. For the intercept and the encephalopathy terms, Normal (mean = 0, sd=100) priors will be used. For the term representing the log relative risk, skeptical, neutral, and enthusiastic priors will be used to estimate posterior distributions for the relative risk.

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 0.625) will also be produced to give a broad overview of the implications of the trial's efficacy results.

In addition to the analyses specified above, we will also conduct equivalent frequentist analyses using robust Poisson regression to obtain relative risk estimates of the treatment effect adjusted for level of encephalopathy at baseline. Moreover, we will also conduct secondary analyses stratified by inborn or outborn status to estimate the treatment effect separately within each of these two groups.

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