Pharmacokinetics and Safety of Caffeine in Neonates with Hypoxic-Ischemic Encephalopathy

NCT number NCT03913221
Document Date 06/26/2019

Pharmacokinetics and Safety of Caffeine in Neonates with Hypoxic-Ischemic Encephalopathy

Phase I Trial

IND 143335

National Clinical Trial (NCT) Identified Number: pending

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Funded by: Thrasher Research Fund

Version Number: v.1.2

26 June 2019

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Statement of Compliance

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

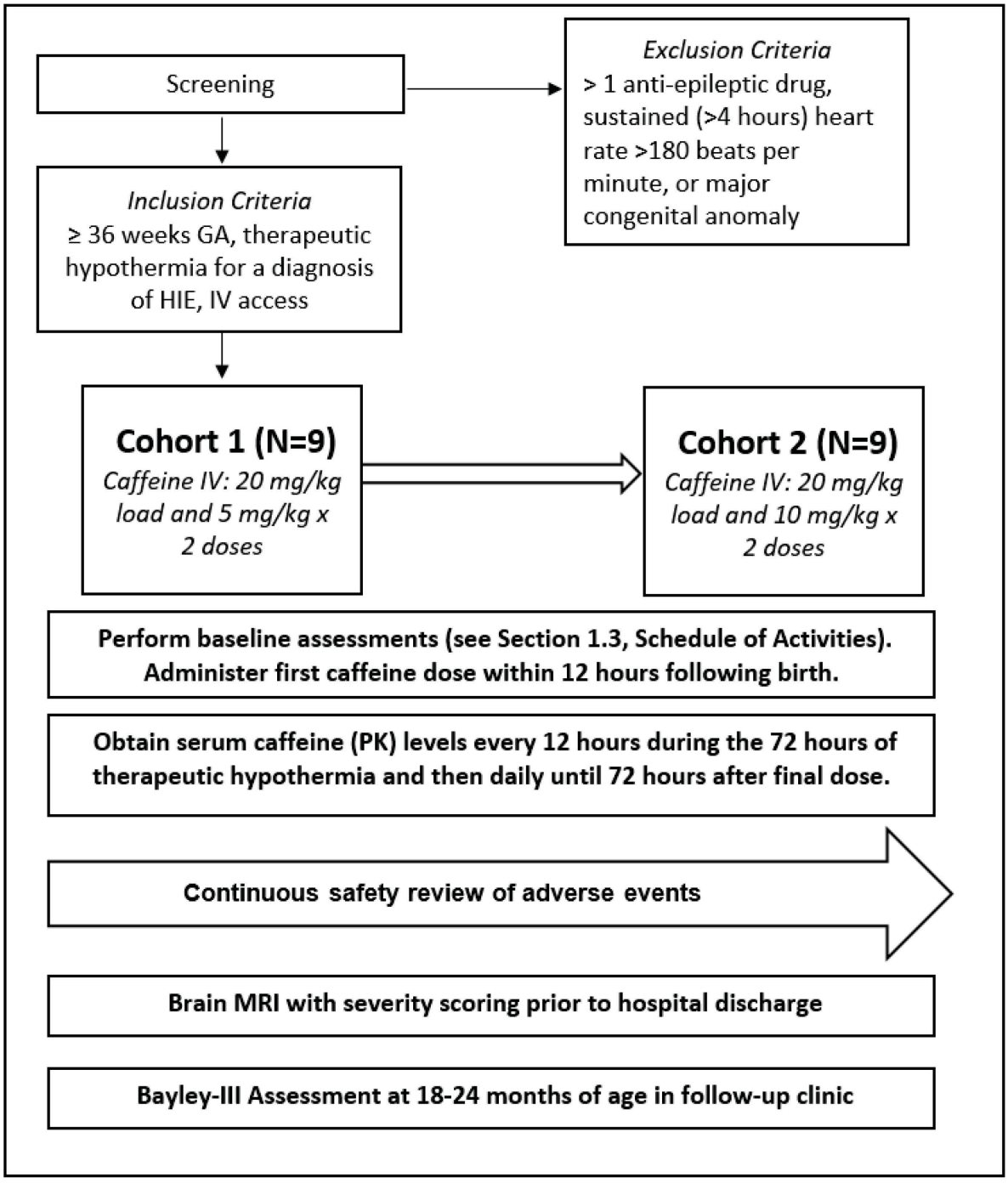
The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 Synopsis

Title:	**Pharmacokinetics and Safety of Caffeine in Neonates with Hypoxic-Ischemic Encephalopathy**
Study Description:	This is a phase I, open-label, dose-escalating pharmacokinetics (PK) and safety study of caffeine in neonates with hypoxic-ischemic encephalopathy undergoing therapeutic hypothermia. We hypothesize that drug clearance will be ≥ 20% lower than expected for gestational age and birth weight and that the incidence of adverse events will be within 20% of published results of neonates treated with therapeutic hypothermia in the NICHD Total Body Hypothermia for Neonatal Encephalopathy trial.
Objectives:	Primary Objective: Characterize the PK of caffeine in neonates undergoing therapeutic hypothermia for hypoxic-ischemic encephalopathy (HIE). Secondary Objectives: Characterize the safety of caffeine in neonates undergoing therapeutic hypothermia for HIE. Evaluate the preliminary effectiveness of caffeine in reducing brain injury in neonates with HIE.
Endpoints:	Primary Endpoint: PK: Caffeine clearance Secondary Endpoints: Safety: Adverse events Note: Seizures and necrotizing enterocolitis will be AEs of special interest Preliminary effectiveness: Death, abnormal MRI findings, neurodevelopment at 18-24 months
Study Population:	**Inclusion Criteria** 1. Documented informed consent from parent or guardian 2. ≥ 36 weeks gestational age at birth 3. Receiving therapeutic hypothermia for a diagnosis of HIE 4. Intravenous (IV) access 5. Postnatal age < 24 hours **Exclusion Criteria** 1. Receiving > 1 anti-epileptic drug for seizures 2. Sustained (>4 hours) heart rate > 180 beats per minute 3. Known major congenital anomaly 4. Any condition which would make the participant, in the opinion of the investigator, unsuitable for the study
Phase:	1
Description of Sites/Facilities Enrolling Participants:	Up to 3 US Neonatal Intensive Care Units, including The University of North Carolina at Chapel Hill Newborn Critical Care Center
Description of Study Intervention:	**Cohort 1 (N = 9):** Caffeine IV 20 mg/kg loading dose, followed by 5 mg/kg q 24 hours x 2 doses. **Cohort 2 (N = 9**): Caffeine IV 20 mg/kg loading dose, followed by 10 mg/kg q 24 hours x 2 doses. Note: An interim safety analysis will be conducted following the follow-up period in the final cohort 1 participant and prior to enrolling in cohort 2.
Study Duration:	24 months
Participant Duration:	24 months

1.2 Schema


Screening
Exclusion Criteria
> 1 anti-epileptic drug, sustained (>4 hours) heart rate >180 beats per minute, or major congenital anomaly
Inclusion Criteria
≥ 36 weeks GA, therapeutic hypothermia for a diagnosis of HIE, IV access
Cohort 1 (N=9)
Caffeine IV: 20 mg/kg load and 5 mg/kg x 2 doses
Cohort 2 (N=9)
Caffeine IV: 20 mg/kg load and 10 mg/kg x 2 doses
Perform baseline assessments (see Section 1.3, Schedule of Activities). Administer first caffeine dose within 12 hours following birth.
Obtain serum caffeine (PK) levels every 12 hours during the 72 hours of therapeutic hypothermia and then daily until 72 hours after final dose.
Continuous safety review of adverse events
Brain MRI with severity scoring prior to hospital discharge
Bayley-III Assessment at 18-24 months of age in follow-up clinic

1.3 Schedule of Activities (SoA)

	Screen/ Baseline	Treatment	Follow-up Period	Discharge/Transfer /Death	Final study assessment
Time point	< 24 hours postnatal age	Study day 1-3	7 days following last caffeine dose		18-24 Months
Informed consent	X				
Demographics	X				
Neurological Exam	X			X	X
Weight	X	X	X	X	X
Medical history	X				X[1]
Respiratory Support	X[2]	X[2]	X[2]	X	
Laboratory evaluations[3]	X	X	X		
Urine output	X	X	X		
Caffeine administration[4]		X			
Concomitant medications		X	X		
Adverse events[5]		X	X		
PK sampling[6]		X	X		
Epigenetic sampling[7]		X	X		
Clinical events of interest[8]		X	X		
Head imaging[9]		X	X	X	
Hearing screen				X	
Surgical procedures		X	X	X	
Bayley-III composite scores and GMFCS					X

. [1] Follow-up visit will include information on hearing impairment requiring hearing aids, blindness, and cerebral palsy diagnosis. [2] Highest respiratory support (room air, oxyhood, nasal cannula/CPAP, or mechanical ventilation) and maximum FIO2 exposure during each 24-hour period. [3] See Section 8.2.1. [4] Includes exact start, stop, and flush times for all caffeine doses. [5] Seizures requiring > 1 anti-epileptic drug and necrotizing enterocolitis are adverse events of special interest. [6] Date and time for each sample. [7] Obtained from scavenged blood samples, if available. [8] Includes death, sepsis, disseminated intravascular coagulopathy, hypotension, hypertension, hepatic dysfunction, acute kidney injury, persistent pulmonary hypertension, and thrombocytopenia. [9] Includes cranial ultrasounds and head CT, if done per standard of care, and brain MRI.

2 INTRODUCTION

2.1 Study Rationale

Hypoxic-ischemic encephalopathy (HIE) due to perinatal asphyxia is a common and often fatal disease in neonates. HIE occurs in nearly 1% of live births.[1] Neonates with HIE experience a temporary interruption in blood flow to the brain and body around the time of delivery. This results in tissue hypoxia with subsequent damage to the brain and other vital organs.[2] Encephalopathy manifests as abnormalities in the neurological exam, which can include seizures and alterations in levels of consciousness, muscle tone, and breathing patterns. The severity of encephalopathy is categorized into three stages (mild, moderate, and severe) based on the Sarnat grading scale.[3]

Therapeutic hypothermia reduces morbidity and mortality in neonates with HIE. Therapeutic hypothermia reduces the risk of death or neurodevelopmental impairment (NDI) at 18-24 months in neonates with moderate to severe HIE (number needed to treat is 7).[4-10] Even with therapeutic hypothermia, mortality or NDI remains ~60% in neonates with HIE.[10] As a result, there is an **urgent, unmet public health need** to develop adjuvant therapies to improve neurodevelopmental outcomes in this population.

Adjuvant therapies are being investigated to improve outcomes in neonates with HIE (Table 1). These agents primarily target the effects of reperfusion injury in the brain which occurs 6-48 hours following the initial insult as a result of inflammation, oxidative stress, and excitotoxicity leading to cell death.[11] All studies, with the exception of erythropoietin and allopurinol, are early phase trials and none of these therapies are currently approved or have demonstrated definitive benefits for HIE. Caffeine has not yet been investigated for neuroprotection in neonates with HIE.

Table 1. Adjuvant therapies under investigation for HIE

Therapy	Clinicaltrials.gov
Erythropoetin[12,13]	NCT01913340; NCT02811263*
Darbepoetin alfa[14,15]	NCT01471015
Autologous cord blood stem cells[16,17]	NCT02612155; NCT02434965
Melatonin[18]	NCT02621944
Xenon[19,20]	NCT00934700; NCT01545271
Allopurinol[21,22]	NCT03162653**
Topiramate[23]	NCT01241019; NCT01765218
Clonidine	NCT01862250

Anticipated Phase III completion: *September 2022; **December 2020

2.2 Background

Caffeine is an appealing therapeutic for neuroprotection in neonates with HIE. Caffeine given for apnea of prematurity reduces NDI at 18-21 months.[24,25] In addition, methylxanthines (the same drug class as caffeine) also reduce the incidence of acute kidney injury in neonates with HIE.[26-32] Caffeine competitively binds to and blocks the action of adenosine receptors in the brain. Mechanisms by which caffeine may offer neuroprotection are the reversal of hypoxia-induced delayed maturation of oligodendrocytes, the cells which produce myelin in the central nervous system,[33] and anti-inflammatory and anti-oxidative properties which protect against reperfusion injury.[34-36] Methylxanthines reduce neuronal cell death in animal models of HIE.[37-39] Thus, caffeine may improve neurologic outcomes in neonates with HIE.

The pharmacokinetics (PK) and safety of caffeine in neonates with HIE is unknown. Caffeine PK and safety are well-described in neonates treated for apnea of prematurity.[40-42] However, ischemic injury and therapeutic hypothermia alter the PK of medications, including morphine and gentamicin.[43,44] As caffeine is primarily metabolized by the hepatic cytochrome enzyme CYP1A2 and excreted in the urine, ischemic injury to the liver and kidneys may reduce its clearance. Additional areas of uncertainty regarding caffeine in the setting of HIE include optimal dosing and safety. There is evidence that caffeine at doses > 36 mg/kg may lower seizure threshold.[45-47] Therefore, PK and safety data for caffeine in the setting of HIE and therapeutic hypothermia are needed.

Brain injury on MRI is a reliable biomarker for neurodevelopmental outcome in neonates with HIE. The NICHD Neonatal Research Network validated an MRI scoring system that categorizes severity of brain injury in the therapeutic hypothermia trial.[5] Abnormal MRI findings were lower in the treatment group compared to the control group (48% vs 65%) and the results were independently associated with NDI at 18-22 months[48] and intelligence quotient at 6-7 years of age.[49]

In-hospital morbidities in a cohort of neonates receiving caffeine while undergoing therapeutic hypothermia for HIE are similar to those published in cooling trials. We reviewed data from the Pediatrix Medical Group, a national consortium of neonatal intensive care units (NICUs) with ~90,000 admissions per year.[50] We examined all inborn neonates with HIE who received therapeutic hypothermia and methylxanthine therapy (caffeine or aminophylline) in the first three postnatal days between 2007-2016. We recorded frequencies of death, mechanical ventilation, acute kidney injury (AKI; defined as creatinine rise > 0.3 mg/dL in a 48-hour period), hepatic dysfunction, receipt of anticonvulsant medication (defined as phenobarbital, fosphenytoin, or levetiracetam), and duration of hospitalization. We compared these frequencies to the results from three trials of therapeutic hypothermia.[4-6] There were 26 neonates who received methylxanthines in the setting of therapeutic hypothermia – 17 received caffeine, 8 received aminophylline, and one neonate received both. The frequency of AKI in neonates receiving caffeine was 25% (3/12) (**Table 2**).

Table 2. Mortality and morbidity in neonates with HIE who received methylxanthines and in treated (i.e., received therapeutic hypothermia) groups from therapeutic hypothermia trials.

	Caffeine group (n=18)	Aminophylline group (n=9)	Treated* Groups in Previous Trials (n=373)
Death	13 (2/16)	0 (0/6)	19-33[4-6]
Duration of hospitalization in days, median (IQR)	19 (16-32)	15 (9-16)	20[5]
Renal			
Creatinine rise > 0.3 mg/dL in 48 hour period Creatinine > 1.0 mg/dL	25 (3/12) 38 (5/13)	56 (5/9) 89 (8/9)	N/A 65[4]
Hepatic			
Prothrombin Time > 16 s AST > 200 U/L or ALT >100 U/L	100 (7/7) 56 (5/9)	86 (6/7) 38 (3/8)	41[6] 20-38[4,5]
Neurologic			
Receiving anticonvulsant in first postnatal week	44 (8/18)	44 (4/9)	38[5]

*Treated indicates infants enrolled in the trials who received therapeutic hypothermia. Column percentages unless otherwise noted. IQR: Interquartile Range; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase.

Determining the PK and safety of caffeine in neonates undergoing therapeutic hypothermia for HIE is an urgent, unmet public health need. Investigating caffeine as an adjuvant therapy for therapeutic hypothermia represents an ideal opportunity to approach the problem of the research gap of drugs in neonates with HIE because: 1) HIE is a relatively common diagnosis in the perinatal period; 2) Neonates with HIE are at increased risk of death or neurodevelopmental impairment; and 3) Preventing mortality and morbidities associated with HIE has life-long benefits. This study will provide the necessary first step to design next phase trial to evaluate the preliminary effectiveness of caffeine in reducing brain injury in neonates with HIE. If successful, caffeine will offer providers a cost-effective and safe treatment for neuroprotection in neonates with HIE.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

Risks of Blood Drawing

There are small risks to blood sampling, usually some pain/discomfort with the blood stick and blood loss. Every effort will be made to avoid additional (to standard of care) sticks for this study and will time clinical blood draws to coincide with timed samples, using existing intravenous lines when possible. A maximum of 7 plasma PK samples per participant will be collected during the treatment and follow-up periods. PK blood samples will be obtained only if laboratory tests are being collected per routine medical care. Each sample will be limited to a volume of 250 µL. The expected total maximum amount will be no more than 1.75 mL (about 1/3 of a teaspoon).

Caffeine[51]

1. Published literature reports have raised questions regarding possible association between the use of methylxanthines, such as caffeine, and the development of necrotizing enterocolitis (NEC) in premature neonates, although a causal relationship has not been established.[52,53]

2. Caffeine is a central nervous system stimulant and in cases of caffeine overdose, seizures have been reported.[54]

3. Caffeine increases heart rate and should be used with caution in neonates with cardiovascular disease.

4. Caffeine should be used with caution in neonates with impaired renal or hepatic function.

2.3.2 Known Potential Benefits

Clinical studies in neonates have demonstrated that caffeine reduces NDI at 18-21 months in premature neonates treated for apnea of prematurity.[24,25] Preclinical studies in rats have found that caffeine offers neuroprotection following perinatal hypoxic-ischemic injury.[34,38,39] Therefore, a reduction in brain injury and NDI are potential benefits for participants enrolled in the study. Conclusions drawn from this study will benefit neonates receiving caffeine in the setting of therapeutic hypothermia for HIE in the future through better understanding of dose response and characterization of the safety profile of caffeine.

2.3.3 Assessment of Potential Risks and Benefits

Given the high incidence of mortality and NDI in neonates with HIE, we propose that the value of the information to be gained in this study outweigh the potential risks of exposure to caffeine. We have minimized these potential risks by using a dose of caffeine that is equivalent to the dose ranges used on-label for apnea of prematurity in preterm neonates. We will exclude neonates with difficult to control seizures (i.e., receiving > 1 anti-epileptic drug [AED]), tachycardia (defined as heart rate > 180 beats per minute for > 4 hour), or major congenital anomalies, including congenital heart disease. We will collect data on seizures and necrotizing enterocolitis as safety events of interest. We will review safety data prior to dose escalation between cohorts.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Characterize the pharmacokinetics (PK) of caffeine in neonates undergoing therapeutic hypothermia for hypoxic-ischemic encephalopathy (HIE).	We will measure serum caffeine levels every 12 hours during the 72 hours of therapeutic hypothermia and then daily until 72 hours after the last dose.	We will develop a population PK model using serum caffeine measurements. Using the final population PK model, empirical Bayesian estimates of clearance (CL), volume of distribution (V), half life, and exposure metrics (e.g. AUC, maximum concentration) will be generated for each participant.
Secondary		
Characterize the safety of caffeine in neonates undergoing therapeutic hypothermia for HIE.	We will review adverse events through hospital discharge. Seizures requiring > 1 AED and NEC will be adverse events (AE) of special interest.	Due to the potential for decreased seizure threshold at high doses of caffeine, we will focus on the incidence of seizure activity as an AE of special interest. NEC is listed on the FDA label for its potential association with caffeine.
Preliminary effectiveness of caffeine in reducing mortality and NDI	• Death prior to discharge • Abnormal brain MRI findings according to the scoring strategy used in the NICHD trial[5,49]: o **Score 0:** Normal T2 MRI o **Score 1A:** Minimal cerebral lesions only with involvement of basal ganglia, thalamus o **Score 1B:** Extensive cerebral lesions o **Score 2A:** Basal ganglia thalamic, anterior or posterior limb of internal capsule, or watershed infarction o **Score 2B:** 2A with cerebral lesions o **Score 3:** Hemispheric devastation	Every neonate with HIE who receives therapeutic hypothermia has a brain MRI prior to discharge per standard of care. MRI findings are widely used in clinical settings and well-validated for the prediction of NDI in neonates with HIE. Bayley-III testing is the gold-standard for assessing NDI in infants 18-24 months.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	• We will collect cognitive, language, and motor scores from the Bayley Scales of Infant and Toddler Development- Third Edition (Bayley-III)[55] at 18-24 months of age to assess developmental delay. We will also include available data on the following outcomes at 18-24 months: cerebral palsy (using the Gross Motor Function Classification System), hearing impairment requiring hearing aids, and blindness.	
Epigenetics	• CpG methylation signatures	Exploratory aim to examine feasibility of evaluating differential methylation in genes encoding for cytochrome P450 enzymes involved in caffeine metabolism.

4 STUDY DESIGN

4.1 Overall Design

This is a phase I, open-label, dose-escalating PK and safety study of caffeine in neonates with HIE undergoing therapeutic hypothermia. There will be two cohorts, with two caffeine dosing regimens (see **Section 6.1.2, Dosing and Administration**). Each cohort will consist of 9 neonates and the intervention duration will be 48 hours. An interim safety analysis will be conducted following the follow-up period in the final cohort 1 participant (see **Section 9.4.4, Safety Analyses)** to assess for safety prior to enrollment in cohort 2. We hypothesize that drug clearance will be ≥ 20% lower than expected for gestational age and birth weight and that the incidence of adverse events will be similar to those in published trials of therapeutic hypothermia.

4.2 Scientific Rationale for Study Design

The PK and safety of caffeine in the setting of HIE are unknown. Given that ischemic injury to the liver and kidneys and therapeutic hypothermia likely alters the metabolism and elimination (i.e., PK) of caffeine, a phase I study is essential to determine optimal dosing for larger, randomized controlled trials to investigate caffeine as a therapeutic agent to reduce NDI associated with HIE. This study will provide the necessary first step to design a larger phase trial to evaluate the safety and preliminary effectiveness of caffeine in reducing brain injury in neonates with HIE.

4.3 Justification for Dose

The two dosing regimens are based on available preclinical data in animals which suggest that methylxanthine doses of 20 mg/kg reduce hypoxic brain damage.[38,39] In one study using a hypoxic-ischemic injury model in newborn rats, 20 mg/kg/day of caffeine for three doses resulted in a decrease in apoptosis of neuronal cells in the hippocampus and parietal cortex compared to controls.[39] A second study using a similar model found that a theophylline dose of 20 mg/kg prior to injury resulted in reduced brain injury compared to higher doses or controls.[38] Furthermore, the FDA caffeine label for apnea of prematurity trial recommends a loading dose of 20 mg/kg followed by a daily maintenance dose of 5 mg/kg. The higher maintenance doses in cohort 2 of our study is consistent with the dosing range we use to treat apnea of prematurity at UNC, and is also consistent with doses used in a large caffeine trial.[41]

4.4 End of Study Definition

A participant is considered to have completed the study if he or she has completed all phases of the study including the neurodevelopmental follow-up visit at 18-24 months, listed as the final study assessment in the Schedule of Activities (SoA), Section 1.3.

5 STUDY POPULATION

5.1 Inclusion Criteria

1. Documented informed consent from parent or guardian
2. ≥ 36 weeks gestational age at birth
3. Receiving therapeutic hypothermia for a diagnosis of HIE
4. Intravenous (IV) access
5. Postnatal age < 24 hours

5.2 Exclusion Criteria

1. Receiving > 1 anti-epileptic drug for seizures
2. Sustained (>4 hours) heart rate > 180 beats per minute
3. Known major congenital anomaly
4. Any condition which would make the participant, in the opinion of the investigator, unsuitable for the study

5.3 Lifestyle Considerations

Not applicable.

5.4 Screen Failures

Not applicable.

5.5 Strategies for Recruitment and Retention

We anticipate that 18 neonates will be recruited from up to 3 US NICUs, including the University of North Carolina at Chapel Hill (UNC) Newborn Critical Care Center (NCCC). Enrollment will begin at UNC and expand to other sites, if needed. The decision to provide therapeutic hypothermia will be at the discretion of the clinician. A neonate will be eligible for screening and enrollment in this study as soon as the decision is made by the clinician to provide therapeutic hypothermia for HIE. The parents of neonates undergoing therapeutic hypothermia will be approached for enrollment by study coordinators in a timely fashion to ensure that the first dose of study drug is given prior to 24 hours postnatal age. For neonates enrolled in the study, retention of subjects will be enhanced by close follow-up in the Special Infant Care Clinic, which is the standard of care for all neonates undergoing therapeutic hypothermia for HIE at UNC, or equivalent follow-up clinic at other sites.

6 STUDY INTERVENTION

6.1 Study Intervention(s) Administration

6.1.1 Study Intervention Description

The study drug is caffeine citrate injection for intravenous administration, which is commercially available and labelled by the FDA for the short term treatment of apnea of prematurity in neonates between 28 and <33 weeks gestational age.[51]

6.1.2 Dosing and Administration

Table 3. N and dosing scheme

	N	Caffeine Loading Dose	Caffeine Maintenance Dose
Cohort 1	9	**20 mg/kg** IV prior to 24 hours postnatal age	**5 mg/kg** IV q24 hours x 2 doses (first dose 24 hours after loading dose)
Cohort 2	9	**20 mg/kg** IV prior to 24 hours postnatal age	**10 mg/kg** IV q24 hours x 2 doses (first dose 24 hours after loading dose)

The first 9 participants will be enrolled in cohort 1 and receive the doses as described in **Table 3.** All doses will be intravenous as the presence of a peripheral or central venous catheter is standard of care for neonates undergoing therapeutic hypothermia. Caffeine loading doses will be administered over 30 minutes and maintenance doses will be administered over 15 minutes via an infusion pump, followed by a normal saline flush administered over 5 minutes via an infusion pump.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and Accountability

Detailed information will be part of the MOP.

6.2.2 Formulation, Appearance, Packaging, and Labeling

The study drug (caffeine citrate) is the commercially available intravenous formulation. This protocol will not specify the brand of product. Detailed information will be part of the MOP.

6.2.3 Product Storage and Stability

Detailed information will be part of the MOP.

6.2.4 Preparation

The pharmacy at each site will prepare and distribute the study drug and the drug will be administered by the bedside nurse.

6.3 Measures to Minimize Bias: Randomization and Blinding

Not applicable.

6.4 Study Intervention Compliance

Adherence to the protocol will be assessed by use of a participant drug log to be completed by the bedside nurse at the time of drug administration. We will record exact start and stop times of caffeine dose over the IV pump, as well as start and stop time of flush.

6.5 Concomitant Therapy

All drug and/or treatments are permitted while on study. All concomitant medications and treatments will be reported during the study drug administration period.

6.5.1 Rescue Medicine

Not applicable.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

Participants may be withdrawn from the study at any time. Participants withdrawn from the study due to an AE, whether serious or non-serious, must be followed by the investigator until the clinical outcome from the AE is determined. Any participant who experiences an AE may be withdrawn at any time from the study at the discretion of the investigator. The AE(s) should be noted on the appropriate case report forms (CRFs), and the participant's progress should be followed until the AE is resolved or considered stable. If the AE may relate to overdose of study treatment, the package insert should be consulted for details of any specific actions to be taken.

Discontinuation from caffeine does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an AE.

7.2 Participant Discontinuation/Withdrawal from the Study

The participant's parent or guardian may withdraw voluntarily from participation in the study at any time. The participant's parent or guardian is not obligated to state the reason for withdrawal. The reasons for withdrawal, or failure to provide a reason, must be documented by the investigator on the completion/ withdrawal section of the electronic case report form (CRF). We will also document missing values in the database for subjects who withdraw from the study.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- If any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation (e.g., prescribed a second anti-epileptic drug for seizures)

7.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for developmental follow-up at 18-24 months and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The investigator or coordinator will attempt to contact the participant and reschedule the missed visit and counsel the participant's parent or guardian on the importance of

maintaining the assigned visit schedule and ascertain if the parent or guardian wishes to and/or should continue in the study.

- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 Study Procedures

8.1.1 Screening/Baseline

Research staff will screen for eligibility requirements. After the parent or guardian has provided informed consent and after it has been determined that the participant satisfies all inclusion and no exclusion criteria, the following evaluations will be recorded in the CRF:

1. Participant demographics
    a. Maternal race/ethnicity
    b. Maternal age
    c. Inborn/outborn
    d. Infant sex
    e. Gestational age (weeks)
    f. Postnatal age (hours)
2. Neurological exam
    a. Level of consciousness
        i. Hyperalert or irritable
        ii. Lethargic or poorly responsive
        iii. Minimal or no responsiveness
    b. Spontaneous activity
        i. Slightly decreased
        ii. Decreased
        iii. Absent
    c. Posture
        i. Mild distal flexion
        ii. Distal flexion, complete extension
        iii. Decerebrate
    d. Tone
        i. Hypertonic
        ii. Hypotonic
        iii. Flaccid
    e. Primitive Reflexes
        i. Suck
            1. Normal
            2. Weak or bite
            3. Absent
            4. Unable to assess
        ii. Moro
            1. Low threshold to elicit
            2. Weak or incomplete
            3. Absent
            4. Unable to assess
    f. Autonomic

i. Pupils
    1. Equal and reactive
    2. Constricted
    3. Dilated and either fixed or sluggishly reactive; asymmetric
    4. Unable to assess
ii. Respiration
    1. Normal
    2. Periodic breathing
    3. Intubated or ventilated

3. Birth weight (grams)
4. Medical history
    a. All Apgar scores (0-10)
    b. Chest compressions in the delivery room (yes/no)
    c. Lowest pH among cord arterial, cord venous, and arterial blood gas taken before 1 hour of age
    d. Sentinel event, defined as placental abruption, shoulder dystocia, uterine rupture, or prolapsed cord
    e. Severity of encephalopathy based on Sarnat staging[3] (mild, moderate, or severe)
    f. Start time of therapeutic hypothermia
5. Respiratory support at time of enrollment (room air, oxyhood, nasal cannula/CPAP, or mechanical ventilation) and maximum FIO2 exposure during each 24-hour period
6. Concomitant medications (see Section 6.5)
7. Laboratory evaluations (see Section 8.2.1)

8.1.2 Treatment

The first dose of caffeine must be given prior to 24 hours postnatal age. The day of the first caffeine dose will be considered study day 1, with subsequent doses given 24 hours apart on study day 2 and 3. During this treatment period, the following evaluations will be recorded in the CRF:

1. Date, time (start time, stop time, and flush time), and dose of each caffeine administration
2. Daily weight (grams)
3. Highest respiratory support (room air, oxyhood, nasal cannula/CPAP, or mechanical ventilation) and maximum FIO2 exposure during each 24-hour period
4. Concomitant medications (see Section 6.5)
5. Laboratory evaluations (see Section 8.2.1)
6. Urine output (in mL/kg/hour)
7. PK sampling (date and time) (see Section 8.2.2)
8. Epigenetic sampling (date and time) (see Section 8.2.2)
9. Clinical events of interest: death, sepsis, disseminated intravascular coagulopathy, hypotension, hypertension, hepatic dysfunction, acute kidney injury, persistent pulmonary hypertension, and thrombocytopenia.
10. Adverse events (see Section 8.3), including seizures requiring > 1 AED and NEC

11. Head imaging results (including cranial ultrasounds and head computed tomography [CT], if obtained per standard of care, and brain MRI) (see Section 8.1.6)

8.1.3 Follow-up Period

The following assessments will be recorded in the CRF for the follow-up period (7 days following the final dose of caffeine):

1. Daily weight (grams)
2. Highest respiratory support (room air, oxyhood, nasal cannula/CPAP, or mechanical ventilation) and maximum FIO2 exposure during each 24-hour period
3. Concomitant medications (see Section 6.5)
4. Laboratory evaluations (see Section 8.2.1)
5. Urine output (in mL/kg/hour)
6. PK sampling (date and time) (see Section 8.2.2)
7. Epigenetic sampling (date and time) (see Section 8.2.2)
8. Clinical events of interest: sepsis, disseminated intravascular coagulopathy, hypotension, hypertension, hepatic dysfunction, acute kidney injury, persistent pulmonary hypertension, and thrombocytopenia.
9. Adverse events (see Section 8.3), including seizures requiring > 1 AED and NEC
10. Head imaging results (including cranial ultrasounds and head CT, if obtained per standard of care, and brain MRI) (see Section 8.1.6)

8.1.4 Discharge/Transfer/Death

At the time of the participant's discharge from the hospital, transfer, or death, the following assessments, when available, will be recorded in the CRF:

1. Head imaging results (including cranial ultrasounds and head CT, if obtained per standard of care, and brain MRI) (see Section 8.1.6)
2. Hearing screen results (pass or referred)
3. Neurological exam
    a. Normal
    b. Moderate; any abnormal findings such as mild head lag
    c. Severe; significant neurological abnormalities, such as:
        i. Increased peripheral tone
        ii. Poor suck
        iii. Marked head lag
4. Surgical procedures during hospitalization
    a. Gastrostomy tube
    b. Tracheostomy
    c. Peritoneal drain
    d. Abdominal laparotomy
    e. Inguinal hernia repair
    f. Neurosurgical procedure
    g. Other
5. Respiratory support

8.1.5 Final Study Assessment

At the follow-up visit at 18-24 months, the following assessments will be recorded in the CRF:

1. Head imaging results (if obtained following discharge)
2. Neurological exam
    a. Normal
    b. Moderate; any abnormal findings
    c. Severe; significant neurological abnormalities
3. Bayley-III composite scores
    a. Cognitive
    b. Language
    c. Motor
4. Gross Motor Function Classification System scores
5. Medical history
    a. Cerebral palsy diagnosis
    b. Hearing impairment requiring hearing aids
    c. Blindness

8.1.6 Efficacy Assessments (Preliminary)

While this early phase trial will not be powered to detect a difference in efficacy compared to our historical comparison group, we will assess the preliminary efficacy of caffeine in reducing NDI associated with HIE. This assessment will be based on brain MRI findings during initial hospitalization and the final study assessment at 18-24 months in the Special Infant Care Clinic, or other follow-up clinic. MRI findings have been shown to be a biomarker of 18-24 month neurodevelopmental outcomes in neonates with HIE.[49]

Brain MRI

Per standard of care, all neonates undergoing therapeutic hypothermia receive a non-contrast brain MRI, generally at 7-10 postnatal days. For the purposes of this study, a board-certified neuroradiologist will review all brain MRIs and assess brain injury using a scoring strategy developed for the NICHD Total Body Hypothermia for Neonatal Encephalopathy trial:[5,49]

- **Score 0:** Normal T2 MRI
- **Score 1A:** Minimal cerebral lesions only with involvement of basal ganglia, thalamus
- **Score 1B:** Extensive cerebral lesions
- **Score 2A:** Basal ganglia thalamic, anterior or posterior limb of internal capsule, or watershed infarction
- **Score 2B:** 2A with cerebral lesions
- **Score 3:** Hemispheric devastation

We will record the raw score and dichotomize outcome to normal (0) or abnormal (1). The results of the MRI will be disclosed to the neonate's parent or guardian, along with appropriate counseling by a neonatologist.

Neurodevelopmental Follow-Up at 18-24 Months

All neonates enrolled in the study will be followed up in the newborn follow-up clinic and screened for developmental delay by a certified examiner at 18-24 months using the Bayley Scales of Infant and Toddler Development- Third Edition (Bayley-III).[55] We will record the composite cognitive, language, and motor scores on the Bayley-III. Additionally, we will record whether the infant has hearing impairment requiring hearing aids or blindness at the time of the 18-24 month visit and assess the presence of cerebral palsy using the Gross Motor Function Classification System.

8.2 Safety and Other Assessments

Safety will be assessed through the neonate's initial hospitalization and it will be assessed by frequency and incidence of AEs and SAEs.

8.2.1 Laboratory Evaluations

All infants receiving therapeutic hypothermia for HIE receive laboratory evaluations per unit guidelines. An example of this lab schedule used at UNC is shown in **Table 4**. Following completion of therapeutic hypothermia, there are no guidelines at UNC, so additional labs are obtained per discretion of the clinician. We will define abnormal lab values using reference ranges used in the Pediatric Trials Network.[56] We will record the following labs during the baseline, treatment, and follow-up periods:

1. Arterial blood gas: We will record the temperature-corrected pH, PCO2, PaO2, and base deficit/excess on blood gases obtained every 12 hours during the treatment period and then daily, if available, during the follow-up period. If blood gases are drawn more frequently than daily, we will use the blood gas with the lowest pH for that day. If an arterial blood gas is not available, we will record venous/capillary gases, if available.
2. For serum chemistry (chem 10): sodium (mmol/L), potassium (mmol/L), Blood Urea Nitrogen (BUN) (mg/dL), creatinine (mg/dL), bicarbonate (mmol/L), calcium (mg/dL), chloride (mmol/L), magnesium (mg/dL), phosphorus (mg/dL), and glucose (mg/dL).
3. For CBC with differential: white blood cell count, hematocrit, and platelet count..
4. For liver function tests: AST (U/L), ALT (U/L), Albumin (g/dL), Alkaline phosphatase (U/L), Gamma-glutamyl Transferase (GGT) (U/L)
5. For Neobili: Total bilirubin, direct bilirubin (mg/dL).
6. For coagulation studies: Prothrombin time (PT) (seconds), partial thromboplastin time (PTT) (seconds), international normalized ratio (INR), and fibrinogen (mg/dL).
7. Microbiology: Cultures from sterile body fluids

Table 4. Laboratory Evaluation Schedule for Therapeutic Hypothermia at UNC

LABORATORY EVALUATION SCHEDULE *All hours refer to hours post start of cooling, not hours of life*						
HOUR 0	**HOUR 12**	**HOUR 24**	**HOUR 36**	**HOUR 48**	**HOUR 60**	**HOUR 72**
*** Blood gas** (If not previously obtained)	**Blood gas** (POC or lab gas) iCa Glucose	**Blood gas** (POC or lab gas) iCa Glucose	**Blood gas** (POC or lab gas) iCa Glucose	**Blood gas** (POC or lab gas) iCa Glucose	**Blood gas** (POC or lab gas) iCa Glucose	**Blood gas** (POC or lab gas) iCa Glucose
Chem 10	Chem 10	Chem 10		Chem 10		Chem 10
CBC with differential		CBC with differential		CBC with differential		
	Neobili	Neobili		Neobili		Neobili
Liver Function Tests AST ALT Total Protein Albumin Total bilirubin Direct bilirubin Alk Phosphatase GGT		**Liver Function Tests** AST ALT Total Protein Albumin Total bilirubin Direct bilirubin Alk Phosphatase GGT				**Liver Function Tests** AST ALT Total Protein Albumin Total bilirubin Direct bilirubin Alk Phosphatase GGT
Coagulation Studies PT PTT INR Fibrinogen		**Coagulation Studies** PT PTT INR Fibrinogen				

**** For blood gas results, always look at the temperature corrected values.***

8.2.2 Special Assays

PK Samples

A maximum of 7 plasma PK samples per participant will be collected during the treatment and follow-up periods. PK blood samples will be obtained only if laboratory tests are being collected per routine medical care. Each blood sample will be limited to a volume of 250 µL. PK samples should not be drawn during infusions or during the flush. Caffeine plasma concentrations will be measured in the clinical laboratories at each site using a validated bioanalytical assay requiring 250 µL of whole blood for analysis. Therapeutic drug monitoring of caffeine per routine medical care can be performed at any time by the treating physician. This can replace a planned study sample. All information obtained from therapeutic drug monitoring of caffeine will be incorporated in the final data analysis, even if the time of collection does not follow the study collection schedule.

Epigenetic Samples

Epigenetic samples will be obtained from either leftover cellular components of plasma caffeine samples or whole blood samples scavenged from standard of care labs. A maximum of 2 samples will be collected per participant. The samples will be identified by a code number, and all other identifying information will be removed. A targeted epigenetic testing approach evaluating CpG methylation on genes encoding for cytochrome P450 enzymes involved in caffeine metabolism will be conducted and related to exposure. Once this testing is completed, any remaining epigenetic samples will be destroyed. Caregivers of participants will not be informed of epigenetic results.

8.3 Adverse Events and Serious Adverse Events

8.3.1 Definition of Adverse Events (AE)

An adverse event (AE) is any untoward medical occurrence in humans, whether or not considered drug-related, which occurs during the conduct of a clinical trial. (Any change in clinical status, routine labs, x-rays, physical examinations, etc.), that is considered clinically significant by the study investigator is considered an AE.

8.3.2 Definition of Serious Adverse Events (SAE)

A serious adverse event (SAE) as determined by the investigator is any event that results in any of the following outcomes:

1. Death
2. Life-threatening AE ("life-threatening" means that the study participant was, in the opinion of the investigator or sponsor, at immediate risk of death from the reaction as it occurred and required immediate intervention)
3. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
4. Inpatient hospitalization or prolongation of existing hospitalization
5. Important medical event that may not result in one of the above outcomes, but may jeopardize the health of the study participant or require medical or surgical intervention to prevent one of the outcomes listed in the above definition of serious event

8.3.3 Classification of an Adverse Event

8.3.3.1 Severity of Event

The investigator should use the following definitions when assessing severity of an adverse event:

1. **MILD:** Participant is aware of symptoms or has minor findings but tolerates them well, and no or minimal intervention required
2. **MODERATE**: Participant experiences enough symptoms or findings to require intervention
3. **SEVERE**: Participant experiences symptoms or findings that require significant intervention

8.3.3.2 Relationship to Study Intervention

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Related** – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.

- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

The investigator will use the following question when assessing causality of an adverse event to study drug, where an affirmative answer designates the event as a suspected adverse reaction: Is there a reasonable possibility that the drug caused the event? ‘‘Reasonable possibility’’ means that there is evidence to suggest a causal relationship between the drug and the adverse event.

8.3.3.3 Expectedness

The investigator will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 Time Period and Frequency for Event Assessment and Follow-up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during the 7 day follow-up period.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician’s assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant’s condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The investigator will record all reportable events with start dates occurring any time after informed consent is obtained until time of discharge from the initial hospitalization.

8.3.5 Adverse Event Reporting

All reportable events as defined above, determined to be an AE based on physical examination, laboratory findings, or other means, will be reported in the electronic case report form (e-CRF) in the REDCap (Research Electronic Data Capture) database platform. The investigator will provide the date of onset and resolution, intensity, frequency, action(s) taken, changes in study drug dosing, relationship to study drug, and outcome.

8.3.6 Serious Adverse Event Reporting

The study clinician will immediately report to the investigator any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis).

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the investigator deems the event to be chronic or the participant is stable.

The investigator will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. In addition, the sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

8.3.7 Reporting Events to Participants

Not applicable

8.3.8 Events of Special Interest

1. Seizures will be defined by the receipt of > 1 AED, as we are interested in identifying seizures which are difficult to control.

2. Necrotizing Enterocolitis - Bell Stage II or III[57]

8.3.9 Reporting of Pregnancy

Not applicable

8.4 Unanticipated Problems

8.4.1 Definition of Unanticipated Problems (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB within 7 days of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB within 30 days of the investigator becoming aware of the problem.

All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within 30 days of the IRB's receipt of the report of the problem from the investigator.

8.4.3 Reporting Unanticipated Problems to Participants

Not applicable

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

- **Pharmacokinetics**

We hypothesize that drug clearance will be ≥ 20% lower than expected for gestational age and birth weight as a result of HIE and therapeutic hypothermia.

- **Safety**

We hypothesize that frequency of seizures in both treatment cohorts will be within 20% of published results of neonates treated with therapeutic hypothermia in the NICHD Total Body Hypothermia for Neonatal Encephalopathy trial.[5]

- **Preliminary effectiveness**

We hypothesize that the risk ratio for an abnormal MRI prior to discharge will be favorable, i.e., between 0.7 and 1.1 in treated neonates compared to published results of neonates treated with therapeutic hypothermia in the NICHD Total Body Hypothermia for Neonatal Encephalopathy trial.[5]

9.2 Sample Size Determination

- **Pharmacokinetics**

A sample size of 9 neonates in each dosing cohort will be powered (≥ 80%) to target a 95% confidence interval within 60% and 140% of the geometric mean estimate of clearance (assuming 36% between subject variability in clearance, which is based on existing literature of caffeine PK in neonates).[58-60]

- **Safety**

The frequency of seizures in the treated group of the NICHD trial was ~40%.[5] Estimation of the probability of seizures in our study will be based on the combined sample of 18 patients. For comparison with historical data, the null hypothesis that the frequency of seizures is 40% will be rejected if 11 or more adverse events (in 18 patients) are observed. The event probability will be no wider than 21% on each side of the point estimate and this will provide a power of 86%. The alternative hypothesis is that the frequency of seizures is >61% or <19% in our study (i.e., > 21% on either side of the point estimate of 40%).

- **Preliminary effectiveness**

This is an exploratory aim and will not be sufficiently powered to detect a significant reduction in brain injury on MRI. However, we estimate that there will be a trend toward risk reduction of brain injury in enrolled neonates compared to treated neonates in the NICHD trial.

9.3 Populations for Analyses

All participants enrolled and dosed will be included in the safety population and the safety analyses. All participants who had at least one interpretable PK sample will be included in the PK analysis.

9.4 Statistical Analyses

9.4.1 General Approach

Analyses will be presented by treatment cohorts. There are 2 planned treatment cohorts: 1) participants receiving caffeine dosing in Cohort 1; and 2) participants receiving caffeine dosing in Cohort 2.

Descriptive statistics
Descriptive statistics such as number of observations, mean, median, standard deviation, standard error, minimum and maximum will be presented by treatment groups for continuous variables (such as age, weight, etc.). Other descriptive statistics such as counts, proportions, and/or percentages will be presented by group to summarize discrete variables (such as race, sex, etc.)

PK analysis plan
PK parameters will be estimated by population PK approach using nonlinear mixed effects modeling in NONMEM (Icon Development Solutions, Elliot City, MD). The influence of covariates on PK parameters, such as gestational age, postnatal age, serum creatinine and transaminases, oliguria, and concomitant medications will be explored.

Safety
The number and percent of AEs and SAEs within each cohort will be summarized overall as well as by each Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. Laboratory data will be tabulated by dose groups. Summary statistics for changes from baseline will be presented.

9.4.2 Analysis of the Primary Endpoint(s)

- **Pharmacokinetics**

We will perform a population PK analysis using nonlinear mixed effects methodology implemented in the software NONMEM (Icon Development Solutions, Elliot City, MD) to analyze caffeine concentration data. We will compare clearance to expected values based on gestational age and birth weight.[59,60] Since the PK of caffeine is well-described in the literature, we will focus our analysis on the effects of HIE and therapeutic hypothermia on caffeine PK. Significant covariates (e.g., gestational age, postnatal age, serum creatinine and transaminases, oliguria, concomitant medications) will be evaluated using forward addition ($P<0.05$) with backward elimination ($P<0.01$) approach.

9.4.3 Analysis of the Secondary Endpoint(s)

- **Preliminary Effectiveness**

We will use MRI brain images from enrolled neonates to assess the severity of brain injury according to the scoring strategy used in the NICHD trial: 0 (normal MRI), 1A (minimal cerebral lesions), 1B (extensive cerebral lesions), 2A (basal ganglia thalamic injury), 2B (2A with cerebral lesions), and 3 (hemispheric devastation). We will dichotomize these results into normal and abnormal and use logistic regression to compare neonates in cohorts 1 and 2 to treated neonates in the NICHD trial to calculate a risk ratio for abnormal MRI with 95% confidence

intervals, adjusting for potential confounders such as severity of encephalopathy and presence of seizures.

We will use the composite cognitive, language, and motor scores on the Bayley-III at the 18-24 month follow-up visit to assess NDI. We will define NDI as any of the following: composite cognitive, language, or motor score < 85, hearing impairment requiring hearing aids, a diagnosis of blindness, or GMFCS level ≥ 3. We will use logistic regression to compare neonates in cohorts 1 and 2 to treated neonates in the NICHD trial to calculate a risk ratio for NDI with 95% confidence intervals.

9.4.4 Safety Analyses

We will collect information on adverse events (AEs) during and after study drug administration. Seizures will be AE of special interest and defined by the receipt of > 1 AED. We will summarize AEs by organ system and report descriptive statistics of laboratory values. We will correlate caffeine exposure with seizure activity and NEC following the first dose of caffeine.

Following the follow-up period in the final cohort 1 participant, there will be an interim safety analysis performed by an independent safety monitor (ISM) with training in neonatology and experience in conducting clinical trials who is not otherwise associated with the study. We will halt enrollment during this analysis. If there is > 1 serious adverse events (SAEs) related to the drug in any cohort, the study will be temporarily halted for an *ad hoc* safety review.

9.4.4.1 Clinical Definitions

1. Sepsis- Positive blood culture and ≥ 7 days of antibiotic treatment
2. Disseminated Intravascular Coagulopathy: Transfusion of platelets, fresh frozen plasma, or cryoprecipitate
3. Hypotension: Requiring inotrope or vasopressor support
4. Hypertension: Requiring antihypertensive medication
5. Hepatic dysfunction: AST > 200 IU/L or ALT > 100 IU/L
6. Acute Kidney Injury (AKI), stage 1-3 classified using Kidney Disease: Improving Global Outcomes (KDIGO) system (**Table 5**). We will use the highest serum creatinine per 24-hour period. A participant will be assigned a stage if they meet either serum creatinine or urine output criteria listed below.

Table 5. AKI KDIGO Classification[61]

Stage	Serum Creatinine (SCr)	Urine Output
0	No change in SCr or rise <0.3 mg/dL	≥ 1.0 mL/kg/hour
1	SCr rise ≥ 0.3 mg/dL within 48 hours or SCr rise ≥ 1.5-1.9 X reference SCr[a] within 7 days	> 0.5 and ≤ 1 mL/kg/hour

2	SCr rise ≥ 2.0-2.9 X reference SCr[a]	> 0.3 and ≤ 0.5 mL/kg/hour
3	SCr rise ≥ 3 X reference SCr[a] or SCr ≥ 2.5 mg/dL or receipt of dialysis	≤ 0.3 mL/kg/hour

[a] Reference SCr will be defined as the lowest previous SCr value

7. Persistent Pulmonary Hypertension: Requiring inhaled nitric oxide and fraction of inspired oxygen >0.50

8. Thrombocytopenia: Platelet count < 100,000 per µL

9. Seizures will be defined by the receipt of > 1 AED, as we are interested in identifying seizures which are difficult to control.

10. Necrotizing Enterocolitis - Bell Stage II or III[57]

9.4.5 Baseline Descriptive Statistics

Not applicable.

9.4.6 Planned Interim Analyses

Not applicable

9.4.7 Sub-group Analyses

Not applicable

9.4.8 Tabulation of Individual Participant Data

Individual participant data will be listed by measure and time point.

9.4.9 Exploratory Analyses

Epigenetic markers
We will relate the CpG methylation signatures of genes encoding for cytochrome P450 enzymes to caffeine exposure and metabolism.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Informed Consent Process

10.1.1.1 Consent/Assent and Other Informational Documents Provided to Participants

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant's parent or guardian and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials are submitted with this protocol: consent form in English.

10.1.1.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's parent or guardian agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and one of the participant's parents or guardians will be asked to read and review the document. The investigator will explain the research study to the parent or guardian and answer any questions that may arise. A verbal explanation will be provided in terms suited to the parent's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. The parent or guardian will have the opportunity to carefully review the written consent form and ask questions prior to signing. The parent or guardian should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The parent or guardian will sign the informed consent document prior to any procedures being done specifically for the study. Parents/guardians must be informed that participation is voluntary and that they may withdraw their neonate from the study at any time, without prejudice. A copy of the informed consent document will be given to the parent or guardian for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to the parent or guardian that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) or Investigational Device Exemption (IDE) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

10.1.3 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

10.1.4 Future Use of Stored Specimens and Data

Data collected for this study will be analyzed and stored at the University of North Carolina at Chapel Hill. During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the study is completed.

10.1.5 Key Roles and Study Governance

Principal Investigator	**Independent Safety Monitor**
Wesley Jackson, MD, MPH	*TBD*
The University of North Carolina at Chapel Hill	
101 Manning Dr. Chapel Hill, NC 27599	
Phone: 984-215-3449	
Email: Wesley.jackson@unc.edu	

10.1.6 Safety Oversight

Safety oversight will be under the direction of an ISM with expertise in neonatology and clinical trials. The monitor will be independent from the study conduct and free of conflict of interest. The monitor will perform a safety analysis following the treatment period in the final cohort 1 participant and prior to enrollment in cohort 2, or on an *ad hoc* basis if there is > 1 SAE related to the drug in any cohort.

10.1.7 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s). See the clinical monitoring plan (CMP) for more detail.

10.1.8 Quality Assurance and Quality Control

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.9 Data Handling and Record Keeping

10.1.9.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff under the supervision of the site investigator. Data collection and entry at UNC will be performed by Jennifer Talbert. If additional sites are added, the site investigator will designate research staff for data collection and entry. Quality review and staff training at all sites will be conducted by research staff from the UNC Neonatal-Perinatal Medicine Division. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) in REDCap derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into REDCap, a 21 CFR Part 11-compliant data capture system provided by the North Carolina Translational and Clinical Sciences Institute, using a data dictionary. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.9.2 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.1.10 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 7 working days of identification of the protocol deviation, or within 7 working days of the scheduled protocol-required activity. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

10.1.11 Publication and Data Sharing Policy

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers 5 years after the completion of the primary endpoint by contacting the PI.

In addition, this study will comply with the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. Large-scale data include genome-wide association studies (GWAS), single nucleotide polymorphisms (SNP) arrays, and genome sequence, transcriptomic, epigenomic, and gene expression data.

10.1.12 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 Additional Considerations

Not applicable

10.3 Abbreviations

AE	Adverse Event
AED	Anti-epileptic Drug
AKI	Acute Kidney Injury
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AUC	Area Under the Concentration Time Curve
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
Cc	Cubic Centimeters
CFR	Code of Federal Regulations
CL	Clearance
CMP	Clinical Monitoring Plan
CONSORT	Consolidated Standards of Reporting Trials
CPAP	Continuous Positive Airway Pressure

CRF	Case Report Form
eCRF	Electronic Case Report Forms
CT	Computed Tomography
dL	Deciliter
EEG	Electroencephalogram
FDA	Food and Drug Administration
FIO2	Fraction of Inspired Oxygen
GA	Gestational Age
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transferase
GLP	Good Laboratory Practices
GMFCS	Gross Motor Function Classification System
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HIE	Hypoxic-Ischemic Encephalopathy
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
INR	International Normalized Ratio
IQR	Interquartile Range
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IU	International Units
IV	Intravenous
KDIGO	Kidney Disease: Improving Global Outcomes
Kg	Kilogram
L	Liter
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mL	milliliter
µL	microliters
MOP	Manual of Procedures
MRI	Magnetic Resonance Imaging
NCCC	Newborn Critical Care Center
NCT	National Clinical Trial
NDI	Neurodevelopmental Impairment
NEC	Necrotizing Enterocolitis
NICHD	National Institute of Child Health and Human Development
NICU	Neonatal Intensive Care Unit
NIH	National Institutes of Health
NONMEM	Nonlinear Mixed Effects Modelling
OHRP	Office for Human Research Protections
PaO2	Partial Pressure of Oxygen in Arterial Blood
PCO2	Partial Pressure of Carbon Dioxide
PI	Principal Investigator
PK	Pharmacokinetics
PT	Prothrombin Time

PTT	Partial Thromboplastin Time
q	Every
QA	Quality Assurance
QC	Quality Control
REDCap	Research Electronic Database Capture
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCr	Serum Creatinine
SNP	Single Nucleotide Polymorphisms
SOA	Schedule of Activities
SOC	Standard of Care
SOP	Standard Operating Procedure
U	Units
UNC	University of North Carolina at Chapel Hill
UOP	Urine Output
UP	Unanticipated Problem
USA	United States of America
V	Distribution

10.4 Protocol Amendment History

Version	Date	Description of Change	Brief Rationale
1.1	03/11/2019	Sections 1.3; 3; 7.2; 8.1; 8.2; 9.2; 9.4.3; 9.4.4; 9.4.6; 10.1.9.1; 10.3	Suggested revisions from Scientific Review Committee
1.2	6/26/2019	Section 6.1.2 – change from 10 to 30 min administration time for load dose; changed version history and date in header and title page	Changed IV caffeine laoding dose administration run time on pump to 30 minutes to match what is in the package insert and added infusion times for maintenance doses

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