

INTERMITTENT HYPOXIA AND CAFFEINE IN INFANTS BORN PRETERM (ICAF)

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonization 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)

All National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of this study 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:	Intermittent Hypoxia and Caffeine in Infants Born Preterm (ICAF)
Study Description:	Our proposal will address the critical question: is persisting intermittent hypoxia (IH) in preterm infants associated with biochemical, structural, or functional injury, and is this injury attenuated with extended caffeine treatment? We will study the effects of caffeine on IH in 220 preterm infants born at ≤ 30 weeks + 6 days gestation. Infants who are currently being treated with routine caffeine, and who meet eligibility criteria, will be enrolled between 32 weeks + 0 days and 36 weeks + 6 days PMA. At enrollment, infants will be started on continuous pulse oximeter recording of O ₂ saturation and heart rate. If, based on standard clinical criteria, the last dose of routine caffeine is given on or before the day the infant is 36 weeks + 5 days PMA, then on the day following their last dose of routine caffeine treatment, infants will be randomized (110/group) to extended caffeine treatment or placebo. Randomized infants should begin receiving study drug (i.e. 5 mg/kg/of caffeine base, or equal volume of placebo) on the day of randomization, but no later than the third calendar day following the last dose of routine caffeine. Prior to 36 weeks + 0 days PMA, study drug will be given once daily (i.e. 5mg/kg/day) and beginning at 36 weeks + 0 days PMA, study drug will be given twice daily (i.e. 10 mg/kg/day). The last dose of study drug will be given at 42 weeks + 6 days PMA. Pulse oximeter recordings will continue 1 additional week after discontinuing

study drug. Two caffeine levels will be obtained, the 1st at one week after beginning study drug, and the 2nd at a target date of 40 weeks + 0 days PMA, but no later than the last day of study drug, whether in hospital or at home. Inflammatory biomarkers will be measured at study enrollment and again at 38 weeks + 0 days PMA, or within 2 calendar days prior to hospital discharge, whichever comes first. Quantitative MRI/MRS should be obtained between study enrollment and 3 calendar days after starting study drug and again at a target date of 43 weeks + 0 days, but no later than 46 weeks + 6 days PMA.

Objectives:

Primary Objective 1: Compare the extent of **IH** exposure, from randomization through 42 weeks + 6 days PMA (within each gestational week and overall), in infants randomized to extended caffeine treatment to infants assigned to receive placebo.

Primary Objective 2: Compare changes in a panel of inflammation-related cytokines and chemokines, from enrollment to the target age of 38 weeks + 0 days PMA, in infants randomized to extended caffeine treatment to infants assigned to receive placebo.

Primary Objective 3: Compare changes in quantitative MRI structural, microstructural and metabolic biomarkers of acute injury, from enrollment to 43-46 weeks PMA, in infants randomized to extended caffeine treatment to infants assigned to receive placebo.

Secondary Objective 1: Examine the association between salivary caffeine concentrations and **IH** outcomes at the one week post randomization and 40 weeks + 0 days PMA assessments.

Secondary Objective 2: Determine whether caffeine effects on changes in inflammatory or MRI biomarkers from baseline to follow-up are mediated by caffeine-related reduced **IH**.

Endpoints:	<p>Primary:</p> <ul style="list-style-type: none"> • Extent of IH as measured by seconds below 90% saturation per 24 hours of recorded oximetry data within each week PMA, and overall • Plasma concentration changes in inflammatory biomarkers between baseline and 38 weeks + 0 days PMA • MRI changes in microstructural measures between baseline and end of study (between 43 weeks + 0 days and 46 weeks + 6 days PMA). <p>Secondary</p> <ul style="list-style-type: none"> • #IH events per 24 hours of recorded oximetry data, # sec < 80% saturation per 24 hours of recorded oximetry data, nadir saturation during events, and the duration of events and area under of curve during events below 90% and 80% threshold saturation • Changes in inflammatory biomarkers and MRI measures of regional tissue volume between baseline and end of study in relation to IH measures
Study Population:	<p>Sample size: 220 infants, 110 caffeine and 110 placebo-treated Gender: Male and female Age: Enrollment at PMA 32 weeks + 0 days - 36 weeks + 6 days Demographic Group: Infants born at ≤ 30 weeks + 6 days gestation General Health Status: Clinically stable, on caffeine treatment and expected to receive last dose of routine caffeine treatment no later than 36 weeks + 5 days PMA Geographic location: NICU or step down unit at one of 8 participating hospitals, with final weeks at home following NICU discharge</p>
Phase:	3
Description of Sites/Facilities Enrolling Participants:	Neonatal Intensive Care Units (NICU) in 8 hospitals in six metropolitan areas in the U.S. While inpatient the research will take place in the hospital. Once discharged the patient will return to specified outpatient locations for performance of the follow-up MRI and for a study termination visit (these visits may be combined).
Description of Study Intervention:	Enrolled infants will be randomized to receive caffeine or equal volume placebo. The caffeine base dose will be 5 mg/kg once daily when the infant is at age 35 weeks + 6 days PMA or less. When the infant is at 36 weeks + 0 days PMA or more the caffeine base dose will be 5 mg/kg twice daily, based on the expected increased rate of caffeine metabolism that occurs as infants age.
Study Duration:	Enrollment is scheduled to start in December, 2018 and will continue through the end of 2021, with completion of follow-up and data analyses extending through May of 2022.

Participant Duration: The maximum time between study enrollment to study completion for an individual participant would be 15 weeks if enrollment was as early as 32 weeks + 0 days and the final visit was at the latest time permitted at 46 weeks + 6 days. Since enrollment is permitted as late as 36 weeks + 6 days, and the final study visit may occur as early as 43 weeks + 6 days, a participant may complete the full study in as few as 7 weeks.

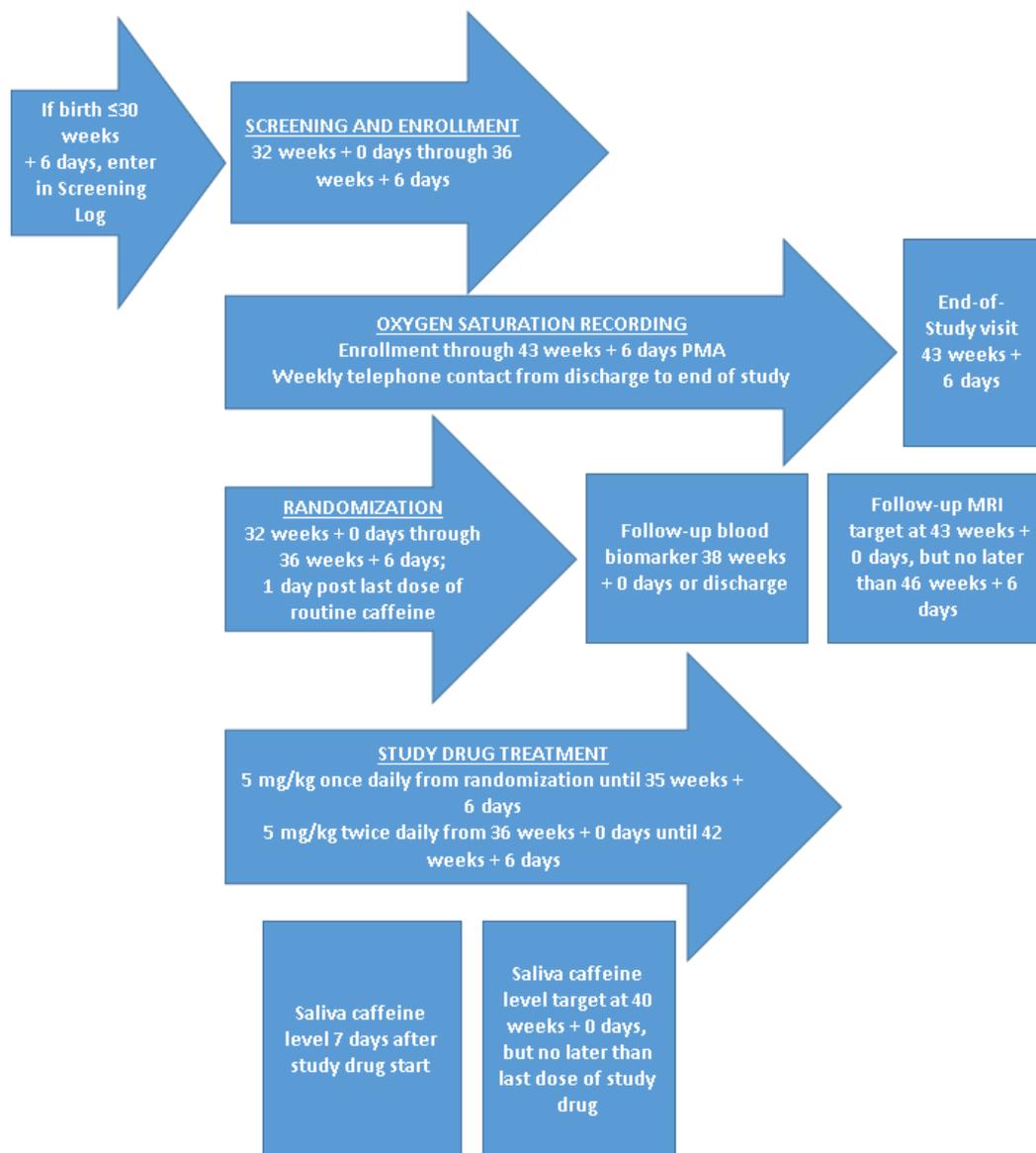
Hospital discharge most commonly occurs between 34 and 40 weeks PMA, and we expect most infants to have roughly half their time in the study as in-patient and half as outpatient, though this may vary widely.

Infants will have an end of study visit and an MRI visit scheduled between 43 weeks + 0 days and 46 weeks + 6 days in an appropriate outpatient location. These visits may be combined.

1.2 STUDY SCHEMA

Our goal is to randomize 220 infants born at a gestational age of 30 weeks + 6 days or less, who get their last dose of routine caffeine treatment between 32 weeks + 0 days and 36 weeks + 5 days PMA, to receive either extended caffeine treatment or placebo to 42 weeks + 6 days. As illustrated below, all NICU admissions of infants born at 30 weeks + 6 days or less will be noted in the screening log. Formal screening for eligibility will begin at 32 weeks + 0 days, with enrollment once infants meet all eligibility requirements, and the infant is at 36 weeks + 6 days or less PMA. Between enrollment and randomization, baseline data will be collected. Randomization will be performed within 24 hours following the last dose of routine caffeine, as long as randomization is by 36 weeks + 6 days PMA. Outcome measures will include oxygen saturation recordings, plasma biomarkers and brain MRI

FLOW DIAGRAM - ICAF



1.3 SCHEDULE OF ACTIVITIES (SOA)

Study Procedures	≤30 weeks + 6 days thru 36 weeks + 6 days	32 weeks + 0 days thru 36 weeks + 6 days	1 day post last routine caffeine dose)	1 week on study drug	36 weeks + 0 days PMA	Hospital Discharge	40 weeks + 0 days PMA	42 weeks + 6 days PMA	43 weeks + 6 Days PMA
Screening	X								
Informed Consent		X							
Enrollment		X							
Baseline Data Collection		X							
Pulse oximetry recording		X							
Randomization			X						
Once daily study drug			X ¹						
Twice daily study drug					X ¹				
Drug log			X						
Saliva for caffeine level				X			X ²		
Biomarker blood sample		X ³				X ³			
MRI/MRS		X ⁴							X ⁴
Pre-discharge checklist						X ⁵			
Weekly parent phone contact						X ⁶			
Infant sleep form								X ⁷	
End of study visit									X ⁸
Concomitant medications		X ⁹							
Adverse events		X ⁹							

- Study drug (caffeine base 5 mg/kg QD or placebo, weight adjusted weekly while in hospital) begins 1 day after last day of routine caffeine, but no later than the 3rd calendar day after last dose of routine caffeine. Dose is increased to 5 mg/kg BID at 36 + 0, finally the last dose of study drug is at 42 + 6. Note that initial dose will be BID if enrolled at 36 + 0 or later.
- 2nd saliva caffeine level should be done at 40 + 0, but no later than last day of study drug, whether in hospital or at home.
- 1st biomarker blood sample should be obtained as close as possible to the day of randomization. Whenever possible this blood sample should be timed with a clinically required blood draw. To facilitate obtaining this sample at the time of a clinical blood draw, this sample may be obtained at any time after consent, but no later than the 2nd calendar day after the first dose of study drug. The second sample is to be obtained at 38 + 0 or within 2 calendar days prior to hospital discharge whichever comes first.
- 1st MRI/MRS done in hospital sometime between enrollment and 3 calendar days after starting study drug. 2nd MRI/MRS is targeted at 43 + 0, but should be no later than 46 + 6. MRI visit may be combined with the end of study visit.
- Includes documentation of tracking information, and parent training including: use of oximeter, drug administration, use of drug administration log, salivary sample collection, communication expectations and follow-up plans.
- Weekly phone calls from study staff to parent(s) begin 1- 3 days after hospital discharge and continue through end of study follow up visit. A phone follow-up form will be completed to collect clinical data, adverse events, and document family questions.
- Sleep Assessment (BISQ) done over the phone with parent at 42 weeks.
- The end of study visit is targeted for 43 + 6 days, or one week after the last dose of study drug, whichever comes first, but should be completed no later than 46 + 6. Study staff will collect the study drug log, used bottles, unused drug, study oximeter, and frozen salivary sample.
- Collection of concomitant medications and adverse event monitoring and reporting are continuous throughout study.

2 INTRODUCTION

2.1 STUDY RATIONALE

Caffeine treatment for apnea of prematurity and related **intermittent hypoxia (IH)** is almost universal, with treatment most commonly discontinued at 33-35 weeks PMA. However, **IH** continues to occur in infants after routine discontinuation of caffeine, and persists after hospital discharge. This **IH** is not apparent clinically and accurate detection requires continuous high resolution pulse oximetry recordings. Studies confirm that the **IH** occurring during spontaneous breathing after stopping routine caffeine is a manifestation of immature breathing control. **It is unknown if this persistent IH results in acute injury, and in particular if extending the duration of caffeine treatment attenuates or prevents this acute injury.** This study will address 3 critical questions: **1)** will extending caffeine treatment from 32-35 to 42 weeks PMA reduce extent of **IH**, **2)** is persisting **IH** associated with biochemical, structural, or functional indicators of acute injury, and **3)** does caffeine treatment continued to 42 weeks PMA attenuate this acute injury? This study has the potential to (1) identify early brain injury mediated by **IH** and mitigated by caffeine at appropriate dosing, and (2) help inform optimum strategies for later definitive neurodevelopmental assessments based on nature of the acute brain injury. This study thus has high potential to justify future neurodevelopmental outcome studies that will further improve outcomes of infants born preterm, change clinical practice, and have significant public health benefit.

2.2 BACKGROUND

Acute morbidities can contribute to adverse neurodevelopment outcomes in preterm infants born at ≤ 30 weeks gestation, but neural damage occurring after resolution of acute morbidities may be more subtle and related to cycles of inflammation and repair in the developing brain. One possible contributor to these more subtle injuries is **intermittent hypoxia (IH)**, defined as repetitive cycles of hypoxia and re-oxygenation, which occur commonly in convalescing premature infants. **Caffeine** treatment can improve both motor and cognitive neurodevelopmental outcome in premature infants, especially at **higher doses**, but mechanisms are unclear.¹⁻⁸ The Caffeine for Apnea of Prematurity (CAP) Trial in infants born preterm at <1250 g reported **1)** shorter duration of positive pressure ventilation and reduced rate of bronchopulmonary dysplasia (BPD) in infants treated with caffeine during the early postnatal weeks prior to 34-35 weeks postmenstrual age (PMA), **2)** improved motor function and reduced rates of developmental coordination disorder at 5 years, and **3)** diffusion changes by MRI consistent with improved white matter microstructural development.^{7,9,10,11} Although potential mechanisms for this caffeine effect were not studied in these reports, a recent study of very preterm infants in postnatal weeks 1-10 showed for the first time a **direct link between IH and motor, cognitive and language impairment** at 18 months corrected age (adjusted risk gradient $p < 0.005$).¹² Notably, the greatest risk gradient was at postnatal ages 9-10 weeks, consistent with a contributory role of later **IH** present after stopping routine caffeine treatment. These data emphasize the potential importance of recurrent episodes of **IH**, as convalescing infants approach term-equivalent age, on later cognitive, language and motor impairments.

Studies confirm that **IH** during the early postnatal weeks of life in very preterm infants may be due to other mechanisms, including ineffective ventilation or other acute morbidities. However, **IH** in spontaneously breathing convalescing infants is due to ventilatory immaturity with associated respiratory pauses or brief apneas, and has a characteristic pattern of brief desaturation from a **normoxic baseline**

followed by reoxygenation and return to normoxia.¹³⁻¹⁸ Our study will assess **IH** only during spontaneous breathing in infants after resolution of acute morbidities or need for supplemental O₂, and approaching term-equivalent age, a time when **IH** has been shown by us and others to be the consequence of immature breathing regulation.

IH during spontaneous breathing related to ventilatory immaturity requires continuous high resolution pulse oximetry recordings for detection, and consists of brief, repetitive cycles of O₂ desaturation from a normoxic baseline, followed by return to baseline saturations. These repetitive cycles of reoxygenation following each **IH** episode are pro-inflammatory and cause oxidative stress, free radical production, and release of pro-inflammatory cytokines.^{19,20} Studies show increased levels of inflammatory biomarkers in animal models of **IH**-associated obstructive sleep apnea (**OSA**) and in human subjects with **OSA**.^{19,21-25} Although inflammatory biomarkers may be elevated in the first 2-3 postnatal weeks in very preterm infants who develop BPD and neurodevelopmental sequelae, it is **unknown if later IH during spontaneous breathing in convalescing preterm infants is associated with inflammation or other biochemical, structural or metabolic acute injury or adverse consequences**.²⁶⁻³¹

We have reported that clinically unrecognized **IH** events are still common after discontinuing routine caffeine treatment, typically at 34-35 weeks PMA.¹⁸ Except for 1 study, however, the potential **adverse consequences of IH have not been investigated** in human infants.^{12,18,32-34} In obstructive sleep apnea, however, even modest amounts of chronic **IH** have been associated with significant neurocognitive morbidity.^{23,24} Worrisome evidence from animal models also shows that **IH** has significant and long lasting effects on multiple physiological control mechanisms^{20-24,35-40} and neurological outcomes.⁴¹⁻⁴³ *We hypothesize that persistent **IH** in spontaneously breathing preterm infants after stopping routine caffeine treatment is associated with acute adverse consequences.*

The relationship between **IH**, adenosine, caffeine and brain development is complex and not fully understood. At clinically effective doses, caffeine exerts effects in the brain by blocking adenosine (Ado) A1 and A2A receptors, resulting in respiratory stimulation and increased alertness, vigilance and arousal.⁴⁴⁻⁶⁰ Ado A1 receptor activation contributes to hypoxia-induced reduction in cerebral myelination and ventriculomegaly. Caffeine treatment attenuates the effects of hypoxia, presumably through blockade of Ado A1 receptors.^{45,58,59} It is thus reasonable to hypothesize that similar mechanisms may be active in the human preterm infant. **Caffeine may thus be neuroprotective through two major mechanisms: 1)** reducing incidence and severity of **IH** due to its respiratory stimulatory effects, and **2)** reducing pre- and immature oligodendrocyte injury.

Brain development progresses through a highly programmed series of events.⁶¹⁻⁶⁴ Myelination in the cerebral hemispheres begins to accelerate at ~30-32 weeks and continues to term and beyond, and disturbances in these late gestation developmental processes often result in failure of normal brain growth, abnormal cortical organization, impaired myelination, and connectivity, commonly observed in surviving preterm infants.⁶⁴⁻⁷⁵ Persisting **IH** thus has even greater potential for later neurodevelopmental disability than the **IH** associated with obstructive sleep apnea.^{23,24,76-84} Since **IH** can be attenuated with extended caffeine, **persisting IH may thus be a modifiable cause of a previously unrecognized additional risk for disabilities** associated with preterm birth.^{7,18,41,42,45,55,73,85-88}

In summary, the period from 32-35 to 42 weeks PMA is a **critical time for brain development**, and is also a time when **significant IH during spontaneous breathing is present, but the adverse effects of this IH are unknown**. As the first step in understanding acute injury from IH, we address a fundamental and critically important question with high potential public health benefit: **does continued caffeine treatment after receiving the last dose of routine caffeine between 32 weeks + 0 days PMA and 36 weeks and 5 days PMA reduce extent of IH and attenuate indicators of acute injury at 43-44 weeks PMA?** We will assess injury in 4 domains: biochemical (inflammation), structural (MRI), functional and metabolic (MRS). Our proposed study thus has the potential to have major impacts on clinical practice: **1) how clinicians assess and interpret IH, and 2) duration of pharmacological treatment with caffeine. This will be the first study in human infants to assess the effects of continuing caffeine treatment in attenuating acute injury indicators associated with IH.**

2.2.1 KNOWN POTENTIAL RISKS

- **Study drug:** Caffeine is one of the most frequently used medications in the NICU for infants born very preterm. At the time of enrollment, infants will be tolerating routine caffeine treatment without any adverse clinical effects. However, known potential side effects of caffeine, would include:
 - Transient decreased weight gain
 - Tachycardia
 - Gastrointestinal symptoms, including feeding intolerance
 - Jitteriness
 - Hypertension
 Each of these side effects would be expected in fewer than 5% of participants.
- **Pulse oximeter recordings:** Pulse oximetry is routinely used in the NICU and sometimes also indicated for home use. The study oximeter will be in addition to the comparable oximeter routinely used clinically. We will follow routine clinical routines for frequent site relocation of the oximeter sensors (probes), to decrease the risk of significant skin irritation.
- **Blood sampling for inflammatory biomarkers:** There will be 2 blood samples obtained, one at enrollment and the 2nd prior to NICU discharge, and the blood volume for each sampling will be <1 ml. Whenever possible, these blood samples will be obtained as part of a clinically indicated routine heel stick or venipuncture, thus decreasing the need to cause infant discomfort beyond that required for routine clinical management.
- **Brain MRI:** All MRIs performed for this study will be done without sedation, thus eliminating the most important potential risk beyond that associated with a clinically-indicated brain MRI. However, the time required for each study MRI will be more than that required for a routine brain MRI.
- **Salivary samples:** The 2 salivary caffeine samples for each subject will be obtained using a commercially available salivary collection kit identical to that used in our recent pilot study. These salivary collections are easily obtained and have not been associated with any infant discomfort or parental concern.
- **Loss of confidentiality** is also a potential risk. However, all demographic and clinical information will be entered in a secure password-protected database accessible only to the research staff at that clinical site and the data coordinating center (DCC) at Boston University Medical Center. Clinical site research staff will enter data via web access. Identifiers that have been collected will be stored separately from research data. The data will be in a separate locked/password protected database linked only by a subject ID number. Identifiers collected for the purpose of performing follow-up will be stored in a separate database that will be destroyed after the study is completed. The data will be

split as it is loaded; personal identifiers (names, addresses, telephone numbers) will be entered into the tracking database along with a unique numeric identifier, and other data (excluding the participants' names and address) will be stored in the analytic database. It will be possible to link information in these two databases only through a coded numeric identifier; it will not be possible to identify individual participants in the analytic database. The separation of names and addresses from personal data adds an important level of security and helps to assure that participant confidentiality will be maintained. Follow-up data will be processed in a similar manner. The analytic database will contain no identifiers and will be the exclusive source for all data analyses performed at the DCC. Database files will be stored at the DCC on network servers located in a data center at the Slone Epidemiology Center at Boston University. Slone Epidemiology Center data center is a separate secure room monitored by key access. Activity on the Slone network and servers is monitored by a University security team. In addition, access to the Slone network is restricted by specific Virtual Private Network group access and router access control lists. Servers are protected by software firewalls and enterprise antivirus software. Administrators to the servers are limited to a core group of individuals who oversee server health and security. Network access to the servers is protected by requiring a user to login to an Active Directory system using the assigned username and Kerberos password. Access to directories and files are restricted by user membership to network security groups. Network security group membership is granted only if a user is affiliated with a research study or group. When a user is not affiliated with any research study or group, access is denied and removed. Backup data is taken at regular intervals. This backup data is encrypted and stored in a secure off-site facility for three years. Any request for file restoration is examined, monitored, and documented. After three years, backup data is verified and certified for destruction. Any websites used by Slone for data collection are protected with SSL protocols—commonly referred to HTTPS. These protocols provide encrypted communication and secure identification of web servers using certificates. Certificates are signed by trusted certificate authority.

- Recurrence of Apnea-related symptoms in placebo-treated subjects: We have not observed any recurrent apnea-related symptoms in preliminary studies after stopping routine caffeine.^{18,90} However, if a subject has apnea-related clinical symptoms after stopping routine caffeine and starting study drug, nasal CPAP or nasal cannula may be used as needed at the discretion of the clinical team. After the last dose of study drug at 42 weeks + 6 days PMA, pulse oximeter recordings will be continued for 1 additional week, to 43 weeks + 6 days PMA. No significant alarms have occurred after stopping extended caffeine treatment in our pilot studies, and none are anticipated in this clinical trial since at this PMA the extent of IH no longer differs from healthy term infants.^{15,16} However, the oximeter alarms can be activated for this final study week if deemed necessary by the clinical site investigator.

2.2.2 KNOWN POTENTIAL BENEFITS

Direct Potential Benefits:

- Infants randomized to receive extended caffeine treatment may benefit directly by having less intermittent hypoxia and hence less inflammation and improved brain MRI findings
- Placebo-treated infants will benefit from the additional support and information provided by research staff, and will have the potential for benefit from information obtained from the MRI evaluations, which would not have been done absent enrollment in this study. Except for the inflammatory biomarkers and brain MRIs, placebo-treated enrollees are equivalent to “usual care” clinical practice in NICUs today.

General Future Potential Benefits:

- This study will fill several major gaps in our current knowledge: 1) The impact on neurodevelopmental outcome of the frequency and severity of **IH** in the NICU and at home until 43 weeks PMA, assessed by continuous pulse oximeter recordings of oxygen saturation and heart rate, 2) the frequency and severity of **IH** in preterm infants receiving caffeine vs. placebo while in the NICU, 3) the frequency and severity of **IH** persisting at home after discharge, and 4) the efficacy of continuing caffeine treatment until 42 weeks + 6 days PMA with regard to reducing **IH** and improving neurodevelopment.
- Infants randomized to caffeine may benefit from improved neurodevelopmental outcome related to attenuated **IH** during the period of exposure to study drug.

2.2.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

- The risk: benefit ratio is favorable
- Extending caffeine treatment to 42 weeks + 6 days PMA has the potential of improving brain structure and function as determined by MRI, and improving neurodevelopmental outcomes.
- Infants randomized to placebo will be receiving what is currently the standard of care when their last routine caffeine dose is between 32 weeks + 0 days PMA and 36 weeks and 5 days PMA.
- The potential risks are primarily related to blood sampling and the brain MRIs. As discussed above, however, the blood samples will be obtained as a part of clinically indicated blood sampling, and the MRIs will be performed without sedation.
- In summary, the potential benefits exceed the potential risks of participation in this study, and there are no increased risks if randomized to receive placebo since placebo infants will otherwise be receiving the same clinical care as comparable infants not enrolled in this study.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
<p>Primary Objective 1: Compare the extent of IH exposure, from randomization through 42 weeks + 6 days PMA (within each gestational week and overall), in infants randomized to extended caffeine treatment to infants assigned to receive placebo.</p> <p>Primary Objective 2: Compare changes in a panel of inflammation-related cytokines and chemokines, from enrollment to the target age of 38 weeks + 0 days PMA, in infants randomized to extended caffeine treatment to infants assigned to receive placebo.</p>	<p># of sec of IH <90% at each PMA week</p> <p>Change in plasma concentration of each inflammatory biomarker from baseline to 38 weeks PMA.</p>	<p># of sec of IH <90% the most representative measure to quantitate extent of IH</p> <p>Measuring biomarker concentrations at 38 weeks will be a sufficient interval for changes since baseline, and will be the interval during which differences in extent of IH will be maximal.</p>

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
<p>Primary Objective 3: Compare changes in quantitative MRI structural, microstructural and metabolic biomarkers of acute injury, from enrollment to 43-46 weeks PMA, in infants randomized to extended caffeine treatment to infants assigned to receive placebo.</p>	<p>Difference between neuroimaging measures at baseline and at 43-46 weeks PMA, including diffusion tensor imaging (DTI) and proton magnetic resonance spectroscopy (MRS).</p>	<p>Quantitative MRI is a sensitive modality for assessing structural, biochemical, and functional changes consequent to IH and extent of improvement with extended caffeine treatment. Assessing at study end will provide maximal time for adverse MRI changes consequent to IH since starting study drug.</p>
Secondary		
<p>Secondary Objective 1: Examine the association between salivary caffeine concentrations and IH outcomes at the one week post randomization and 40 weeks + 0 days PMA assessments.</p> <p>Secondary Objective 2: Determine whether changes, from baseline to follow-up, in inflammatory or MRI biomarkers are mediated by caffeine-related reduced IH.</p>	<p>Extent of IH (# of sec <90%) paired with saliva caffeine level during PMA week of oximetry recording.</p> <p>Change in plasma concentration for each inflammatory biomarker, from baseline to 38 weeks PMA, in relation to IH measures.</p> <p>Change in each MRI parameter, from baseline to 43-46 weeks PMA in relation to IH measures.</p>	<p># of sec of IH <90% is the most representative measure of extent of IH during the week corresponding in time to the caffeine level.</p> <p>See above</p>

4 STUDY DESIGN

4.1 OVERALL DESIGN

HYPOTHESES:

Hypothesis 1: Caffeine will reduce extent of **IH** compared to placebo, and this caffeine effect will persist to 42 weeks PMA.

Hypothesis 2: Extended caffeine treatment will be associated with less acute inflammation at 37-38 weeks compared to placebo, and these effects will be mediated by attenuated **IH**.

Hypothesis 3: Quantitative MRI analyses in the active caffeine group, compared to the placebo group, will be associated with **1)** greater white matter microstructural organization, **2)** improved neuronal metabolism, and **3)** greater regional brain volume, and that these effects will be mediated by attenuated **IH**.

TYPE/DESIGN OF TRIAL:

Randomized, placebo-controlled, double-blinded clinical trial

METHODS TO BE USED TO MINIMIZE BIAS:

All clinical team personnel and all research staff (except research pharmacist) will remain blinded to study group until study completion. Caffeine and equal volume placebo will be prepared by research pharmacist and will be equivalent in color and consistency. The protocol has been designed such that there should be no need to break the code for any individual subject.

NUMBER OF STUDY GROUPS/ARM & STUDY INTERVENTION DURATION:

Infants will be randomized in a 1:1 ratio to 1 of 2 treatment arms, caffeine base (Arm 1) and placebo (Arm 2). Infants from multiple births will be cluster randomized. The duration of the intervention will be from randomization between 32 weeks + 0 days PMA and 36 weeks + 6 days PMA through 42 weeks + 6 days PMA, the age at which extent of **IH** no longer differs from healthy infants born at term.

MULTI-SITE STUDY:

There will be enrollment at 8 hospitals located in 6 metropolitan areas in the US (Boston MA, Lebanon NH, Philadelphia PA, St. Petersburg FL, Washington DC, Bethesda, MD, and Worcester MA).

NAME OF STUDY INTERVENTION:

Caffeine versus placebo

INTERIM ANALYSIS: None planned

STRATIFICATION:

A site-stratified randomly-permuted blocked randomization design will be used to ensure balance at each site. To ensure balance by gestational age at birth, randomization will also be stratified into 2 birth gestational age categories: 1) <28 weeks 2) 28 weeks + 0 days through 30 weeks + 6 days. The randomization scheme will be developed by the DCC using specialized software, and clinical sites will be provided appropriate enrollment logs and randomization procedures from the research pharmacy.

SUB-STUDIES: No ancillary studies planned at this time

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Our pilot studies have demonstrated that **IH** associated with immature breathing pattern in infants born preterm can be attenuated with caffeine. It is unknown, however, whether persisting **IH** after stopping routine caffeine treatment in the NICU, which may only be evident with continuing high resolution pulse oximetry, results in any long-term injury. The results in the group randomized to extended caffeine treatment (Arm 1) will be compared to placebo (Arm 2) to determine if extended caffeine treatment is superior to placebo in attenuating or eliminating the adverse effects of persisting **IH** on inflammation and brain MRI structural, microstructural, and metabolic markers of injury.

4.3 JUSTIFICATION FOR DOSE

Infants will be started on oral caffeine base at 5 mg/kg/day or equivalent volume of placebo (Sterile Water, and Cherry Syrup, in a ratio of 5:2:4). At 36 weeks + 0 days PMA, study drug dose will be increased to 5 mg/kg BID (total daily dose 10 mg/kg) or equivalent volume of placebo. The dose will be weight-adjusted weekly until NICU discharge. The starting dose and dose escalation at 36 weeks + 0 days PMA reflect increasing clearance rates that have been established in pilot studies as being appropriate to achieve caffeine levels within the most likely therapeutic range. All subjects will be on enteral medications when enrolled.

4.4 END OF STUDY DEFINITION

Each subject will be considered to have completed the study after pulse oximeter recording data is provided at 43 weeks + 6 days PMA, inflammatory biomarker samples are provided, and if applicable, has completed the outpatient brain MRI between 43 weeks + 0 days and 46 weeks + 6 days PMA, as shown in the Schedule of Activities (SOA), Section 1.3.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

INCLUSION CRITERIA:

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Male and female infants born preterm at ≤ 30 weeks + 6 days postmenstrual age
2. Current treatment with routine caffeine
3. PMA 32 weeks + 0 days - 36 weeks + 6 days
4. Anticipated last dose of routine caffeine will be by 36 weeks + 5 days
5. Breathing room air with no ventilatory support or nasal air flow therapy
6. Able to tolerate enteral medications
7. It is feasible to administer the first dose of study drug no later than 36 weeks + 6 days PMA

5.2 EXCLUSION CRITERIA

EXCLUSION CRITERIA:

1. Intraventricular hemorrhage Grade III-IV or cystic periventricular leukomalacia
2. Current or prior treatment for seizures
3. Current or prior treatment for cardiac arrhythmias
4. Known renal or hepatic dysfunction that in the opinion of the investigator would have a clinically relevant impact on caffeine metabolism
5. Major malformation, inborn error of metabolism, chromosomal abnormality
6. Presence of a condition for which survival to discharge unlikely
7. Social, mental health, logistical or other issues that, in the opinion of the investigator, would impact the ability of the family to complete the study.

5.3 LIFESTYLE CONSIDERATIONS

- Parents and clinical care providers will be asked to avoid exposure to caffeine for subjects.
- Open label treatment with a methylxanthine will be documented.
- There will be no restrictions on maternal diet even if breast feeding.

5.4 SCREEN FAILURES

- Screened and potentially eligible infants for enrollment will be screen failures if they reach 36 weeks + 6 days PMA and are still receiving routine caffeine or, for whatever reason, cannot be enrolled by 36 weeks + 6 days PMA.
- Enrolled infants will be screen failures, and will not be randomized, if they do not continue to meet eligibility criteria at the time that routine caffeine treatment is being discontinued.
- Analysis will be by “intention to treat,” and all randomized subjects will remain included in their Arm for analysis.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

RECRUITMENT:

- Potential enrollees will all be inpatients in the NICU at one of the 8 participating hospitals.
- All inpatients in the NICU born at ≤ 30 weeks + 6 days postmenstrual age will be captured in the screening log, and beginning at 32 weeks + 0 days PMA, if they are being treated with routine caffeine, will be screened for potential eligibility. Screening will be discontinued whenever infants meet a specified exclusion criterion for enrollment or if they reach 36 weeks + 6 days PMA without meeting all inclusion criteria. It is estimated based on pilot studies that 30-50% of all screened infants will meet 1 or more exclusion criteria or fail to meet inclusion criteria.
- The families of infants who are meeting enrollment criteria will be approached by study staff regarding possible enrollment. Based on pilot studies, it is estimated that about 30-35% of families approached for consent will consent to participate.

RETENTION:

- As infants are approaching the time of discharge staff will utilize the pre-discharge checklist to:
 - Obtain detailed tracking information that provides information regarding where the infant will be cared for post-discharge and multiple methods to contact key family members

- Provide and document comprehensive training of families regarding use of the oxygen saturation recorder
- Provide and document training of families regarding giving study drug to their infant and using the home drug log
- Provide and document training of families regarding obtaining and storing the saliva sample for caffeine levels
- Provide and document training of families related to study expectations for future communication (i.e. telephone contacts) and study visits for the MRI, if necessary, and the end of study visit
- Following discharge families will be contacted within 1 – 3 days after discharge and then at least weekly to support families in performing study procedures including drug administration, use of oximeter, and ascertain any concerns about these procedures or the clinical status of the infant, including the occurrence of adverse events.
- The parents of each subject will receive gift cards for a total of \$100, at completion of their participation in the study. The purpose is to defray travel expenses for the return visit and for baby supplies, indicating our appreciation for their support of the collective protocol requirements.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

- The intervention for this study is the extended treatment of infants with caffeine, for evaluation of the efficacy of extended caffeine in ameliorating persisting **IH** and its adverse consequences including inflammation and brain MRI structural and functional injury. Caffeine is a standard treatment in preterm infants in the NICU, but currently is commonly discontinued between 32 and 36 weeks PMA. The FDA has granted an IND Exemption for the extended treatment with caffeine to 42 weeks + 6 days.

6.1.2 DOSING AND ADMINISTRATION

- The dose of caffeine base will be the same caffeine dose per kg as determined in our recent pilot study, both the QD starting dose and the BID dose at 36 weeks + 0 days PMA to adjust for increasing rates of metabolism.
- The caffeine doses used have been shown in our pilot study to be sufficient to achieve and maintain effective systemic concentrations for significant attenuation of **IH**
- The equal volume placebo will be a sterile water and cherry syrup solution and be identical in appearance to the caffeine
- Due to the relatively long half-life of caffeine, timing of doses may have some variance to best accommodate routine care and feeding schedules. The time of the 1st dose will be approximately 24 hours after the last routine clinical dose, and the subsequent QD doses will be given at the same approximate time each day, as consistent with daily care schedules. Timing of the BID doses starting at 36 weeks + 0 days PMA will be determined as consistent with daily care schedules, and will then be continued at the same times every day. After discharge, timing of the doses may initially be modified to be most consistent with home care schedules, and then repeated at the same approximate time(s) each day.

- All study drug will be given prior to a feeding.
- Study drug will be continued from the 1st dose, which will be given sometime between 32 weeks + 0 days and 36 weeks + 6 days PMA, through the last scheduled dose at 42 weeks + 6 days PMA.
- Study drug will be administered by clinical care personnel in the same manner as for routine caffeine use. Clinical care and/or research staff will teach parents how to administer so that administration at home will be comparable to that in the NICU.
- For home use, families will be instructed how to draw up the proper dose from the study drug vials supplied by research staff.
- Missed QD doses will be given as soon as possible after the missed dose, but that dose will be eliminated (and details recorded) if missed for more than 12 hours. For BID doses, missed doses will be given as soon as possible after the missed dose, but that dose will be eliminated (and details recorded) if missed for more than 6 hours.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

Each clinical research site is responsible for procuring the following required supplies needed to prepare blinded doses for both inpatient and outpatient doses. See Pharmacy Manual for specific compounding ingredients and instructions.

- Instructions will be provided to the parents/caregivers to administer the blinded oral doses just prior to the morning feeding and just prior to a late afternoon or early evening feed (breast or bottle). A diary will be provided for recording all administered doses.
- All used bottles and unused study solution will be returned to the research pharmacy (IDS) at study end.

6.2.2 DOSE PREPARATION, AND LABELING

Inpatient Doses: **Caffeine base or Placebo**

Caffeine base: IDS pharmacies will prepare a bulk oral solution containing caffeine base (anhydrous) 20 mg/mL, according to the instructions in the Pharmacy Manual. A bulk solution can be prepared individually for a patient or can be used for multiple patients (preferred) who are assigned/randomized to receive active treatment.

A 24 hour supply is prepared from the bulk solution and dispensed in a suitable-sized unit-dose, oral syringe. All oral syringes prepared and administered in the inpatient setting are to be labeled in a blinded fashion, using specific departmental procedures for labeling of blinded syringes and reflecting blinded study drug information on the medication administration record (e.g. eMAR). All syringes must be labeled as “For Investigational Use Only” (or alternative site specific verbiage to identify a study medication). Syringes can be prepared as long as 4 days prior to administration (BUD 4 days) and should be stored refrigerated.

Example: Caffeine base 20 mg/1 mL or Placebo; Caffeine base 20 mg or placebo (1 mL)

Placebo: Research pharmacies will prepare a bulk oral solution containing dilute cherry oral solution according to instructions in the pharmacy manual. A bulk solution can be prepared individually for a patient or can be used for multiple patients (preferred) who are assigned/randomized to receive placebo treatment.

A 24 hour supply is prepared from the bulk solution and dispensed in a suitable-sized unit-dose, oral syringe. All oral syringes prepared and administered in the inpatient setting are to be labeled in a blinded fashion, using specific departmental procedures for labeling of blinded syringes and reflecting blinded study drug information on a medication administration record (e.g. eMAR). All syringes must be labeled as “For Investigational Use Only” (or alternative site specific verbiage to identify a study medication). Syringes can be prepared as long as 4 days prior to administration (BUD 4 days) and should be stored refrigerated.

Example: Caffeine base 20 mg/1 mL or Placebo; Caffeine base 20 mg or placebo (1 mL)

Outpatient Doses:

Doses intended for outpatient administration, starting at time of discharge and continuing until 42 weeks + 6 days PMA, are prepared by the IDS pharmacy at each clinical site and issued as a bulk solution containing a sufficient quantity of either the active drug (caffeine base) or placebo for 30 days (BUD 30 days), and then provided a 2nd bottle of study drug as needed to complete treatment up to 42 weeks + 6 days PMA.

6.2.3 PRODUCT STORAGE AND ACCOUNTABILITY

IDS pharmacies are temperature controlled and have access limited to pharmacy personnel only. Compounding and accountability records are maintained by the IDS pharmacy to reflect drug preparation and dispensing for patients receiving both the active and placebo solutions, prepared for both inpatient and outpatient treatment. All supplies used to prepare both the active and placebo oral solutions are to be stored in the IDS/research pharmacy, in accordance with temperature parameters indicated on the product label.

Sites may use/develop their own accountability forms; specific information to be documented on the drug accountability record includes: patient initials, patient study ID number, individual dose (mg per mL), lot number and beyond use date of the prepared solution. For outpatient dosing, the quantity dispensed (mLs), the dose and the dispensing date must also be recorded. The use of an electronic record (e.g., Vestigo) is acceptable as long as the required information is captured, including patient-returned medication.

Any remaining doses of study drug not administered at home are to be returned by the family member during the end-of-study outpatient visit, and eventually returned to the IDS pharmacy for on-site destruction following reconciliation and accountability of the unused doses. Used bottles are to be returned to the IDS pharmacy; if remaining solution is present, it is to be measured and recorded as a return. Empty bottles are to be recorded as zero mL. Once the information for the returned bottle has

been recorded, the solution and bottle can be disposed of as non-hazardous pharmaceutical waste, according to institutional procedures.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Randomization:

- Ideally one day following, but up to the third calendar day following the last routine caffeine treatment dose, enrolled infants will be randomized in a 1:1 ratio to 1 of 2 treatment arms, caffeine base or placebo.
- Infants from multiple births will be cluster randomized.
- A site-stratified randomly-permuted blocked randomization design will be used to ensure balance at each site.
- To ensure balance by gestational age at birth, randomization will also be stratified into 2 birth gestational age categories: 1) <28 weeks and 2) 28 weeks + 0 days through 30 weeks + 6 days gestation.
- A randomization scheme will be developed by the DCC using specialized software, and clinical sites will be provided appropriate enrollment logs and blinded randomization procedures.

Blinding:

- Treatment assignments will be blinded until study completion for all study personnel, except for the clinical site research pharmacy dispensing study drug.
- The study pulse oximeter will not have visual displays or alarms for respiration or heart rate activated during the study period ending at 43 weeks + 6 days. The **IH** which is the focus of study typically does not result in overt apnea or bradycardia, and therefore we do not anticipate a need for unblinding based on oximeter results
- Any requests for unblinding will be adjudicated by one of the study Principal Investigators, and can only be authorized by them.
- Two salivary caffeine concentrations are being obtained from all subjects, but the results of the caffeine concentrations will be blinded until all infants have completed the study protocol.
- Potential caffeine side effects, such as mild tachycardia, irritability or feeding difficulties are frequent in both caffeine-treated non-treated infants. If such concerns arise, however, at the discretion of the clinical care team, study drug may be held for the days necessary, and noted in study records, but would generally not be a reason to request unblinding.
- If apnea-related symptoms requiring treatment occur after starting study drug, the clinical care team may institute treatment as needed until cessation of these symptoms, and similarly, this also should not require unblinding.

6.4 STUDY INTERVENTION COMPLIANCE

- Approximately the 1st half of study duration will be in the later weeks of the NICU admission. During this time, compliance with protocol requirements will be closely monitored by research staff and documented in the database.
- To maximize compliance with study protocol during the several weeks at home, families will be instructed in all aspects of protocol adherence while in the NICU, and reinforced just prior to discharge.
- Once discharged, research staff will contact each family within the first 2-3 days home to address parental concerns or questions, and encourage compliance with study procedures. Staff will re-contact each family at least weekly thereafter to support families and encourage full home compliance with protocol requirements.

- Two salivary caffeine concentrations will be obtained in all subjects. Although results will not be available until the end of the study, the results will be useful in analyses to assess adherence to the administration of study drug, and understand the relationship between caffeine concentrations and study safety and efficacy outcomes.

6.5 CONCOMITANT THERAPY

Uniform treatment guidelines for routine treatment caffeine have been developed for the study and agreed to by sites. The Clinical Guidelines for Routine Caffeine Use in NICU (Prior to enrollment in ICAF) include: For infants with birth weight < 1250 grams, initiate caffeine treatment within the first 3 days after birth in all infants. If on CPAP only: initiate caffeine treatment within the first 24 hours. If approaching extubation in first 3 days after birth prior to any caffeine treatment, initiate caffeine treatment 6 to 8 hours prior to extubation. For infants with birth weight \geq 1250 grams, if on CPAP or on no respiratory support, initiate caffeine treatment if the infant experiences >6 apnea episodes requiring stimulation. If the infant requires mechanical ventilation, consider starting caffeine treatment prior to extubation. The initial caffeine dose is a 20mg/kg bolus dose of caffeine citrate (or equivalent caffeine base) followed by 5-10 mg/kg/day maintenance dose, beginning 24 hours after the loading dose. Levels are not required to monitor for dosage adjustment. Stop routine caffeine treatment once the infant is free of clinically significant episodes of apnea for 5-7 days off of any positive pressure (CPAP or NC > 1L) or at 34 weeks PMA, whichever comes first. If the maintenance dose is less than 5 mg/kg, infants may be allowed to “outgrow” their dose of caffeine and the dose should be stopped as noted above (i.e. no clinically significant episode for 5-7 days off any positive pressure (CPAP or NC > 1L) or 34 weeks PMA, whichever comes first.) Following randomization, open label caffeine use is discouraged, however, there are no prohibited therapies and sites will manage all participants based on their best clinical judgment, with guidance as needed by the an ICAF Principal Investigator.

6.5.1 RESCUE MEDICINE

As noted above, rescue therapy with open-label caffeine therapy is discouraged. Recurrent apnea-related symptoms have not occurred in preliminary studies after stopping routine caffeine.^{18,90} However, in the event that an infant has apnea-related clinical symptoms after stopping routine caffeine and starting study drug, study drug should be continued, and if judged to be needed by the clinical team, nasal CPAP or nasal cannula is the preferred additional therapy. If the clinical team ultimately decides that open-label caffeine must be used, we suggest the following: 1) Hold study drug; 2) begin open-label maintenance caffeine citrate at 5-10 mg/kg/day on the same day; 3) as soon as open-label caffeine is no longer deemed necessary, stop open-label caffeine and restart study drug at the next regularly scheduled time for study drug; 4) there should be no need to unblind the treatment group, since published studies have suggested that it is safe to give the suggested dose of open-label caffeine to either treatment group.³ If the attending physician thinks unblinding may be needed they should contact the clinical site principal investigators.

Pulse oximeter recordings at home will be continued for 1 week following discontinuation of study drug. The PMA at which study drug is scheduled to end is well after the clinical team judged it safe to stop routine caffeine treatment and at a PMA at which prior studies have shown that the extent of **IH** no longer differs from healthy infants born at term. However, the oximeter alarms may be activated for this final study week if deemed necessary by the clinical site principal investigators.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

The clinical site principal investigators may discontinue the participant's activity without the participant's consent if either of these criteria are met:

- A participant consistently fails to comply with study procedures
- A participant's safety or health may be compromised by further participation

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Each study participant may withdraw consent at any time during the study without penalty. In the event a participant is discontinued and thus no longer receiving the study drug, the participant will be asked to continue with follow-up assessment as described below, and not considered withdrawn from the study. If the participant refuses further follow-up, then the participant will be considered to be withdrawn. Counseling about the participant's health will be provided if the participant decides to discontinue participation in the study. Medical advice regarding what is in the best interest of the participant will be provided.

All data collected up to the time of withdrawal will be reported. The End of Study eCRF will be completed, with the reason for withdrawal specified.

7.3 LOST TO FOLLOW-UP

- A participant will be considered lost to follow-up if he or she fails to return for the end-of-study visit between 43 weeks + 0 days and 46 weeks and 6 days PMA and is unable to be contacted by the study site staff to (1) reschedule the final visit for the brain MRI (if applicable) to be completed by 46 weeks + 6 days, and (2) return all study equipment and supplies within the next 4 weeks.
- If the subject does not return for the final evaluation, the site will attempt to contact the family and reschedule the missed visit within the next 1-4 weeks, counsel the participant on the importance of maintaining the assigned visit schedule, and ascertain if the family wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the clinical site investigator or designee will make every effort to regain contact with the participant by multiple means or by contacting designated alternative contacts. These contact attempts will be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, the participant 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 EFFICACY ASSESSMENTS

8.1.1 OXYGEN SATURATION

Both in the hospital and at home, continuous recording of O₂ saturation using the study pulse oximeter is planned from enrollment through 43 weeks + 6 days PMA (i.e. one week following the last dose of study drug). The study oximeter will be the Masimo RAD-97, set at the minimum possible averaging time of 2-4 sec and a sampling rate of 1 sec (high resolution). The oximeter will be preset in the sleep lab mode, hence with no “probe off,” “low battery,” or physiologic visual displays or alarms. The internal data storage capacity of the RAD-97 oximeter is sufficient for 28 days of continuous data collection.

Inpatient Phase: During the inpatient phase, the RAD-97 will be a 2nd oximeter, in addition to the one used for standard monitoring in the NICU. Continuous oximeter recordings will be obtained whenever in the NICU. Data will be downloaded from the monitor, and archived by the DCC, at least every 4 weeks while in the NICU and at discharge.

Outpatient Phase: Prior to discharge, the family will be trained in the use of the RAD-97, and will be asked to use the oximeter during all sleep times and quiet awake periods until 43 weeks + 6 days PMA. At home, therefore, we expect at least 8 – 12 hours of use per day, so the memory capacity of the RAD-97 should be sufficient to complete the outpatient phase.

Following discharge, the family will be contacted within the first 2 – 3 days, and then at least weekly to provide support in the use of the study monitor and to support other aspects of the study protocol. As part of their support of the use the monitor at home, study site staff will assess if the use pattern is such that the oximeter memory might be close to capacity, and determine if it is necessary to schedule an additional data download.

8.1.2 INFLAMMATORY BIOMARKERS

An EDTA blood sample (0.6 – 0.8 ml) to measure a panel of inflammatory biomarkers will be obtained at enrollment prior to randomization, and again at 38 weeks + 0 days PMA or NICU discharge, whichever comes first. The blood will be centrifuged and the resultant plasma aliquoted into tubes and immediately frozen (-80 °C). The intent is to freeze a plasma volume of at least 0.3 ml. As specified by later shipping instructions, the frozen samples will be batched and shipped on dry ice overnight using shipping labels that will be provided.

Bulk analyses of inflammatory biomarker plasma concentrations will be performed at Children’s National Medical Center using a commercially available 40-plex V-Plex ELISA multi-spot assay (MesoScale Diagnostics, Rockville, MD) to measure inflammation-related proteins from different functional categories of cytokines and chemokines, including growth factors, and adhesion molecules, IFN-alpha, IL-6, Gro/CXCL1, IL-1 β , IL-4-6, IL-6 receptor, IL-8, IL-10, IL-13, ICAM-1, myeloperoxidase, CRP, MCP, IGFBP-1,

MIP-1a, RANTES, and TNF α . Analytes that show strong trends or significance with this assay may be further analyzed with individual ELISA assays, to confirm the original result.

8.1.3 QUANTITATIVE MRI STRUCTURAL, MICROSTRUCTURAL AND METABOLIC BIOMARKERS OF ACUTE BRAIN INJURY

Our goal is to obtain baseline and follow-up brain MRI in 55 subjects in each study arm. For additional information, refer to MRI Manual.

Infants will be quieted by swaddling and feeding before the MRI study. O₂ saturation, heart rate and temperature will be monitored and recorded throughout the scan. All infants will be protected from MRI scanner noise by the use of ear wax, and mini muffs (Natus MiniMuffs Noise Attenuators). MRI acquisition protocols will be standardized and field-tested across all sites in Y1 Q1-3, and MRI calibration studies will be performed to ensure that the MRI scanner properties and parameter settings during the acquisition phase are correct. Conventional MRI studies will be reviewed in a standardized fashion by a pediatric neuroradiologist at each site blinded to study randomization.

All MRI data sets will be processed at the Advanced Pediatric Brain Imaging Research Laboratory (DBRL) at Children's National Medical Center. All quantitative MRI outcome measures are continuous and will be performed by a single investigator masked to randomization. Abnormalities of brain development, maturation, the presence of focal destructive ischemic or hemorrhagic lesions, will be documented.

8.1.4 SALIVARY CAFFEINE LEVELS

Two salivary samples will be obtained for later analysis of caffeine concentrations, the 1st in the NICU 1 week after starting study drug, and the 2nd at home by parents at about 40 weeks + 0 days PMA. All samples will be pre-prandial. Salivary volumes of 200-300 μ L will be collected using commercially available kits (Salimetrics, State College, PA) and frozen at -20° C at home and then at -80° C after return to the clinical site research team (in the same freezer as the plasma samples) for bulk analysis at the end of the study. Parents will return the home salivary sample at the same time as their scheduled return to the clinical site at study end. As specified by later shipping instructions, the frozen salivary samples will be batched and shipped on dry ice overnight with the plasma samples using shipping labels that will be provided.

Salivary analyses will be performed by the Biomedical Research Lab at Walter Reed National Military Medical Center. Sample preparation and chromatography will utilize a method validated in our preliminary study.^{89,90} Chromatography will be performed using a Waters Alliance 2695 Separations Module equipped with a Waters 2996 Photodiode Array detector and Waters Symmetry column.

8.1.5 BRIEF INFANT SLEEP QUESTIONNAIRE (BISQ)

The Brief Infant Sleep Questionnaire (BISQ) is a parent-reported questionnaire on infant/toddler sleep over the prior one week. It includes 13 items in three categories (sleep duration, night wakings, and method of falling asleep). The BISQ will be completed by phone during the weekly phone call that is

scheduled between 42 weeks 0 days and 42 weeks 6 days, or in person, at study termination if this occurs prior to 42 weeks + 0 days.

8.2 SAFETY AND OTHER ASSESSMENTS

8.2.1 TELEPHONE ASSESSMENT OF STATUS AT HOME

Within 1 – 3 days following hospital discharge to home, and at least weekly thereafter, study site staff will contact families by phone and complete a phone contact log. The phone contact log will document:

- Family questions or concerns
- Assessment of completion of the drug log and issues related to administration or supply of study drug
- Assessment of extent of use of study oximeter and any questions, problems or other concerns related to study drug
- Discussion of upcoming study procedures
- Assessment for occurrence of adverse events
- Determination of use of concomitant medications

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

- Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).
- Since there will be multiple participating clinical sites, centralized safety oversight will be coordinated by the DCC in conjunction with the ICAF principal investigators.
- The study does not involve an investigational new drug, but is extending the duration of caffeine treatment beyond the customary age of discontinuing caffeine treatment in the NICU. The FDA has deemed the use of caffeine in this study as consistent with prior FDA approval, and has therefore granted an IND Exemption.
- There is no risk in randomizing to placebo; absent enrollment in this study, infants would not be treated with caffeine.

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) 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, or a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions. 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 participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.3.3 **NOTE THAT FOR THE PURPOSE OF THIS STUDY, DELAYING DISCHARGE OF AN INFANT RELATED TO ACHIEVING A SPECIFIED ‘SPELL-FREE’ NUMBER OF DAYS SHALL NOT BE CONSIDERED A ‘PROLONGATION OF EXISTING HOSPITALIZATION’ FOR THE PURPOSE OF CATEGORIZING AN EVENT AS A SERIOUS ADVERSE EVENT.** CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

The following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant’s daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant’s usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term “severe” does not necessarily equate to “serious”.

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) will have their relationship to study intervention assessed by the clinical site principal investigators based on temporal relationship and clinical judgment. The degree of certainty about causality will be graded using the categories below:

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant’s clinical condition, other concomitant events). An AE may rate as “possibly related” soon after discovery, but it can be flagged as requiring more information and later be upgraded to “probably related” or “definitely related”, as appropriate.
- **Unlikely to be related** – A clinical event whose temporal relationship to study intervention administration makes a causal relationship improbable and other drugs or underlying disease provides plausible explanations (e.g., the participant’s clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. The alternative, definitive etiology must be documented by the clinical care team.

8.3.3.3 EXPECTEDNESS

- The clinical site principal investigators 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 well-established risk information previously described for caffeine.

- Expected AE for caffeine treatment can include tachycardia, feeding intolerance, jitteriness. However, no significant AE are expected since all enrollees will have previously tolerated routine caffeine treatment at systemic concentrations similar to those achieved in this study.

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 clinical care personnel as part of routine NICU care, or by research staff or parents.
- 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 enrolled 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.
- Designated study site research staff will record all reportable events occurring in the NICU
- starting from when informed consent is obtained until the last day of study participation (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. NOTE: Last day of study participation may be any of the following:
 - end-of-study visit
 - follow-up MRI visit
 - Lost-to-follow-up determination date
- After discharge home, research staff will query parents at least weekly about the occurrence of AE/SAEs since the last contact. Events will be followed for outcome information until resolution or stabilization.

8.3.5 ADVERSE EVENT REPORTING

AEs and SAEs will be documented in the source records and recorded on the eCRFs using accepted medical terms and/or the diagnoses that accurately characterize the event. When a diagnosis is known, the AE term recorded on the eCRF will be the diagnosis rather than a constellation of symptoms. The clinical site principal investigators or their designee will assess all AEs for seriousness, relationship to investigational product, severity, expectedness, and other possible etiologies. When an event has not resolved by study closure, it will be documented on the AE eCRF as "ongoing".

The timeframe for the collection of AEs and SAEs begins at the time informed consent is obtained through 30 days after the last day of study participation.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

All SAEs must be reported promptly (within 24 hours) to the study DCC, whether or not the event is considered related to study product. Further, the clinical site principal investigators should comply with relevant clinical study site IRB requirements on reporting SAEs.

Clinical site principal investigators must submit additional information as soon as it is available on the SAE report form. The study DCC will report unexpected SAEs associated with the use of the drug as required.

Clinical site principal investigators must follow all relevant regulatory requirements as well as specific policy regarding the timely reporting of SAEs to the study DCC and the clinical study site IRB.

Reporting to the study DCC does not fulfill the clinical site principal investigator's duty to report all unanticipated problems involving risk to human subjects or others to the IRB. The clinical site principal investigators will notify the clinical study site IRB and ICAF principal investigators.

- Any AE considered serious by the clinical site principal investigator or an ICAF principal investigator, or which meets the definition of an SAE included in **Section 8.3.2, Definition of Serious Adverse Events** must be submitted on an SAE form to the Data Coordinating Center (DCC).
- The DSMB will receive expedited notification of all SAEs, even if not thought to be related to study intervention.
- All serious adverse events (SAEs) will be followed until satisfactory resolution or until the clinical site principal investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the Data Coordinating Center (DCC) and should be provided as soon as possible.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

Not Applicable

8.3.8 EVENTS OF SPECIAL INTEREST

- Although rare, infants born at ≤ 30 weeks gestation and discharged home are at risk for an apparent life-threatening event (ALTE, or brief resolved unexpected event, BRUE) or for sudden unexpected infant death (SIDS)
- Infants enrolled in this study are at no greater risk, and perhaps at even less risk, for such events than infants not enrolled

8.3.9 REPORTING OF PREGNANCY

Not applicable

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

Federal regulations (45 CFR Part 46/32 CFR 219) require that unanticipated problems involving risks to subjects or others be promptly reported to the IRB. These events encompass a broader category of events than SAEs and may include issues such as problems with loss of control of subject data or the investigational product; adverse psychological reactions; or breach of confidentiality. Risks to others (e.g., program personnel) must also be reported.

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 clinical site principal investigators will report unanticipated problems (UPs) occurring from the time the informed consent form is obtained through 30 days after the last day of study participation to the clinical study site Institutional Review Board (IRB) and to the Data Coordinating Center (DCC)/ICAF principal investigators. The UP report will include the following information:

- Protocol identifying information: protocol title and number, Clinical site principal investigator’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 will be reported to the clinical study site IRB and to the DCC and PI/Co-PI within 1 business day of the investigator becoming aware of the event.

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) promptly after the clinical study site IRB’s receipt of the report of the problem from the clinical site principal investigators.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

Primary Objective 1: Compare the extent of IH exposure, from randomization through 42 weeks + 6 days PMA (within each gestational week and overall), in infants randomized to extended caffeine

treatment to infants assigned to receive placebo. *We hypothesize that caffeine will reduce the extent of IH compared to placebo, and that this caffeine effect will persist through 42 weeks PMA.*

Primary Objective 2: Compare changes in a panel of inflammation-related cytokines and chemokines, from enrollment to the target age of 38 weeks + 0 days PMA, in infants randomized to extended caffeine treatment to infants assigned to receive placebo. *We hypothesize that continuing caffeine treatment will be associated with less inflammation compared to placebo.*

Primary Objective 3: Compare changes in quantitative MRI structural, microstructural and metabolic biomarkers of acute injury, from enrollment to 43-46 weeks PMA, in infants randomized to extended caffeine treatment to infants assigned to receive placebo. *We hypothesize that continued caffeine treatment compared to placebo will be associated with 1) greater white matter microstructural organization, 2) improved neuronal metabolism, and 3) greater regional brain volume.*

Secondary Objective 1: Examine the association between salivary caffeine concentrations and IH outcomes at the one week post randomization and 40 weeks + 0 days PMA assessments. *We hypothesize that those with higher salivary caffeine concentrations will have less severe IH outcomes.*

Secondary Objective 2: Determine whether caffeine effects on changes in inflammatory or MRI biomarkers from baseline to follow-up are mediated by caffeine-related reduced IH. *We hypothesize that caffeine effects on changes in inflammatory or MRI biomarkers will be at least partially mediated by reduced IH.*

9.2 SAMPLE SIZE DETERMINATION

Sample Size: We will randomize 220 infants, **110 in each treatment group**, with ≥ 100 infants/group available for final analyses. Sample size considerations focus on PMA week-specific differences between the extended caffeine vs. placebo infants for our primary outcome of #sec/day < 90% saturation from 36 to 42 weeks PMA. Preliminary data showed effect sizes at 37 and 38 weeks PMA of 0.87 and 0.73. With the decline in IH with increasing age, we expect that 1) the caffeine effect may decline and 2) moderate effects will be needed to achieve a meaningful reduction in IH to 42 weeks PMA. Therefore, we consider an effect size of 0.50 to represent a meaningful detectable difference between groups. Final samples of $n=100$ per group gives 82% power to detect this effect, testing at the overall 2-tailed .05 level with a familywise Type I error correction adjusting for multiple testing across weeks. Secondary outcomes of #IH events and #sec < 80% saturation/day showed effect sizes at 38 weeks PMA of 0.73 and 0.46 in our pilot data.

9.3 POPULATIONS FOR ANALYSES

The intention-to-treat (ITT) population will consist of all randomized subjects.

The modified intention-to-treat (mITT) population will include all subjects who took at least one dose of study drug and who have data available for efficacy outcomes.

The safety population will consist of all randomized subjects who receive a dose of study drug.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

Statistical significance will be declared at the two-sided $p < 0.05$ level; when appropriate, correction for multiple comparisons will be employed. For analyses of primary and secondary objectives, we will adjust for demographic and clinical differences. When employed, tests for interaction will be at the 0.1 alpha level. Results will be presented through measures of effect and 95% confidence intervals. If lost to follow-up exceeds the 10% anticipated under sample size considerations, multiple imputation on the intent-to-treat population will be used for sensitivity analyses that account for missing data. All computations will be performed using the latest available version of SAS®.

Demographic variables and subject characteristics will be summarized descriptively by treatment assignment and overall. Continuous parameters will be summarized using descriptive statistics (N, mean, median, standard deviation, minimum and maximum value). Categorical demographic parameters, such as gender, will be summarized as a percentage of the population.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINTS

Primary Objective 1: Compare the extent of **IH** exposure, from randomization through 42 weeks + 6 days PMA (within each gestational week and overall), in infants randomized to extended caffeine treatment to infants assigned to receive placebo.

Analyses for this objective will be performed on the mITT population and compare those randomized to extended caffeine vs. placebo. To be included in **IH** analyses, a subject must have had at least 10 hours of acceptable quality oximetry data recorded within at least one PMA week. A single overall baseline SaO_2 will be determined for each infant, confirmed in our pilot study to be $\geq 95\%$ for the PMA weeks being studied. An **IH** event is defined as SaO_2 decrease $\geq 10\%$ (event threshold) and lasting ≥ 5 sec; the event ends when SaO_2 increases above the event threshold. For sec of SaO_2 below threshold, all sec below the threshold are included. We will compare extended caffeine vs. placebo infants on measures of **IH**, with # of sec $< 90\%$ saturation/day our primary outcome. Our pilot data showed that # of sec $< 90\%$ optimally discriminates between infants on vs. not on extended caffeine, with high correlation between #sec $< 90\%$ saturation/day and multiple other measures to quantify **IH** which will be secondary outcomes, including #**IH** events/day, # sec $< 80\%$ saturation/day, nadir saturation during events, duration of events (Spearman's correlations $r > 0.90$, and AOC during events below 90% and 80% threshold saturation ($r=0.83$).⁹⁰ Preliminary data showed that these outcomes follow a log-normal distribution, and data will be log-transformed for analysis; non-parametric procedures will be considered as an alternative analytic approach if warranted by the distribution of the **IH** outcomes. Exploratory analyses will also compare caffeine vs. placebo on heart rate data. As a check on randomization, preliminary analyses will compare the two study groups on key characteristics including gestational age, birth weight, infant sex, morbidity (e.g., necrotizing enterocolitis, mild BPD), and severity of illness measures; variables found to differ meaningfully between groups will be controlled for in primary analyses through multiple linear regression models. Primary analyses will determine how long extended caffeine provides an advantage over placebo by testing PMA week-specific differences between study groups through multiple regression models controlling for key covariates using Holm's family-wise error adjustment to account for multiple testing across PMA weeks.

Primary Objective 2: Compare changes in a panel of inflammation-related cytokines and chemokines, from enrollment to the target age of 38 weeks + 0 days PMA, in infants randomized to extended caffeine treatment to infants assigned to receive placebo.

Analyses for this objective will be performed on the mITT population and compare those randomized to extended caffeine vs. placebo. To be included in the analyses a subject must have had a baseline/follow-up pair of blood samples for which at least one biomarker was measured on both samples. Analysis will focus on change in inflammatory biomarker levels over the study period. Preliminary analyses will examine the distribution of biomarkers, and data will be transformed (e.g., logged) as appropriate for analysis. Two approaches will be taken for the primary analyses, comparing changes in biomarkers over the study period in extended caffeine vs. placebo infants. First, we will conduct separate analyses of each inflammatory biomarker using linear regression models to compare study groups at study completion, controlling for baseline levels as well as key covariates including morbidities that might be associated with inflammation, using the Benjamini and Hochberg method to account for multiple testing by controlling the false discovery rate. Second, we will examine the structure of the biomarker data using data reduction procedures of factor analyses (identifying latent variables reflected by the set of biomarkers) and latent profile analysis (identifying subgroups of infants with similar patterns of biomarker profiles). Extended caffeine vs. placebo infants would then be compared on the summary measures of inflammation biomarkers resulting from these analyses.

Primary Objective 3: Compare changes in quantitative MRI structural, microstructural and metabolic biomarkers of acute injury, from enrollment to 43-46 weeks PMA, in infants randomized to extended caffeine treatment to infants assigned to receive placebo.

Analyses for this objective will be performed on the mITT population and compare those randomized to extended caffeine vs. placebo. To be included in the analyses a subject must have had a baseline/follow-up pair of MRI studies completed from which at least one MRI endpoint could be measured on both assessments.

Primary neuroimaging outcome measures will include microstructural measures derived from our DTI analyses (FA/MD) and proton MRS. Secondary outcomes will include regional tissue volumes. Individual end-of-study MRI parameters will be compared through analysis of covariance multiple linear regression models controlling for the MRI parameter at baseline along with key covariates including indicators of comorbidities potentially associated with brain development, using the Benjamini and Hochberg method to account for multiple testing by controlling the false discovery rate. .

Secondary Objective 1: Examine the association between salivary caffeine concentrations and **IH** outcomes at the one week post randomization and 40 weeks + 0 days PMA assessments.

Analyses will examine the association between salivary caffeine levels and **IH** outcomes, both at the end of the inpatient stay and at study completion. Caffeine levels at home will also be used to verify protocol adherence. Secondary analyses will also include relationship between individual neonatal morbidities and extent of **IH** in caffeine and placebo groups. Exploratory analyses will model the trends in **IH** parameters over time in the extended caffeine and placebo groups using mixed-effects linear regression models for longitudinal data. Should missing data become a problem, multiple imputation methods will be used to

account for potential bias in the complete case analysis.

Secondary Objective 2: Determine whether changes, from baseline to follow-up, in inflammatory or MRI biomarkers are mediated by caffeine-related reduced **IH**.

We will also examine **IH** levels during the study period as a mediator of the extended caffeine effect. Initial analyses will examine associations between **IH** parameters and inflammatory biomarkers, to determine which **IH** measures are most strongly correlated with inflammation at study completion. Linear structural equation mediational models will be used to determine whether any associations between extended caffeine treatment and changes in inflammatory biomarkers can be explained by **IH** parameters during the study period. The relationship between neonatal morbidities, Inflammation, and response to caffeine will also be assessed.

Similarly we will also examine associations between changes in MRI parameters and levels of **IH** during the study period through correlation and linear regression models with the goal of determining **IH** parameters most strongly associated with brain injury. Structural equation mediational models will determine whether any associations between extended caffeine and MRI changes can be explained by the level of **IH** activity during the study.

9.4.3 SAFETY ANALYSES

Analyses of safety outcomes will be performed on the safety population consisting of all randomized subjects who receive a dose of study drug. Descriptive data on safety outcomes will be provided by treatment group.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

- In collaboration with the clinical care team, research staff will screen all NICU patients born at ≤ 30 weeks gestation and receiving routine caffeine treatment for study eligibility
- Clinical care staff will inform parents of this ongoing research study and inform research staff of families desiring additional study information
- Parents will not be approached for formal consent before the infant is 32 weeks + 0 days PMA.

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

- The Consent Form describing in detail the study intervention, study procedures, and risks will be given to the parents of each eligible subject. Written documentation of informed consent will be obtained prior to enrolling each infant
- The Consent Form is being submitted with this protocol

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to parental agreement to participate in the study and continues throughout the duration of the study. The Consent Form will be Institutional Review Board (IRB)-approved and the parent(s) will be asked to read and review the document. The investigator will explain the research study to the parent 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. Parents will have the opportunity to carefully review the written consent form and ask questions prior to signing, and will have the opportunity to discuss the study with family or surrogates or to think about it prior to agreeing to participate. A parent will sign the informed consent document prior to any procedures being done specifically for the study. Parents will be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the parents 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 subject undergoes any study-specific procedures. The rights and welfare of the subjects will be protected by emphasizing to parents that the quality of medical care will not be adversely affected if they decline to participate in this study.

- As may be deemed necessary by the clinical site, the Consent Form will be available in Spanish as well as in English, and a Spanish translator will be available whenever needed.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. If the study is prematurely terminated or suspended, the clinical site principal investigators will promptly inform study participants, the clinical study site Institutional Review Board (IRB), and will provide the reason(s) for the termination or suspension.

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

10.1.3 CONFIDENTIALITY AND PRIVACY

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

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.

Representatives of the clinical site Institutional Review Board (IRB) or DSMB may inspect all documents and records required to be maintained by the clinical site principal investigators, 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 study 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 clinical site IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Data Coordinating Center. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical study sites and by Data Coordinating Center research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the Data Coordinating Center.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

See Section 10.1.3, Confidentiality and Privacy and Section 10.1.9, Data Handling and Record Keeping, for further information on future use of study records.

Data collected for this study will be analyzed and stored at Boston University Medical Center (Data Coordinating Center (DCC)). After the study is completed, the de-identified, archived data will be transmitted to and stored at the Slone Epidemiology Center at Boston University, for use by other researchers including those outside of the study. Permission to transmit data to the Slone Epidemiology Center at Boston University will be included in the informed consent.

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.

When the study is completed, access to study data and/or samples will be provided through the DCC.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

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Study Leadership

- This is a Multiple PI grant application, with Drs. Hunt and Eichenwald as Co-PIs.
- There will be a Steering Committee, chaired in rotating years by Drs. Hunt and Eichenwald. Other members will be Drs. McEntire (Project Director), Corwin (DCC), and Co-Investigators Darnall, Dobson, Fort, Frantz, Hartman, Heeren, Limperopoulos, Poets, Revenis, Rhein, and Stark. This group will be responsible for coordinating and managing all phases of this study. They will meet at funding onset to establish responsibilities and timelines, complete the manual of operations, and then meet biweekly in Quarter (Q) 1-2 of Year 1, and monthly thereafter. The committee will meet face-to-face initially and once/year; all other meetings will be virtual. Y1 will be used to complete all project materials, train site personnel, and obtain IRB approvals.
- Executive Committee (EC): Drs. Hunt and Eichenwald (Co-PI), Dr. McEntire (Project Director), and Dr. Corwin (DCC) will provide overall study oversight and coordination, and resolution of any site-specific issues. The EC will meet in conjunction with SC meetings, and will also meet virtually as needed, approximately biweekly in Y1 and monthly thereafter.

10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB) composed of individuals with the appropriate expertise, including content and statistical expertise. Members of the DSMB should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DSMB will meet at least semiannually to assess safety and efficacy data on each arm of the study. The DSMB will operate under the rules of an approved

charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to the executive committee and to NICHD staff.

10.1.7 CLINICAL MONITORING

There will be no formal on site clinical monitoring of data. All study data will be managed at the data coordinating center (DCC). Each subject will be assigned a unique study identifier code. Data entered by sites will be via a web-based portal and will flow directly into the main study Microsoft Access database. Standard quality control and cleaning procedures will be applied to ensure that accurate data entry has occurred. All study data will be stored on secure password-protected computer servers that are backed up automatically daily, and maintained by the Information Systems staff. The database will be programmed to produce weekly reports tracking enrollment and follow-up completion and other periodic reports as required. Virtual meetings between DCC and site investigators will be held frequently to monitor enrollment and track overall study conduct and any difficulties encountered.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

See Section 10.1.7, Clinical Monitoring.

10.1.9 DATA HANDLING AND RECORD KEEPING

See Section 10.1.7, Clinical Monitoring

10.1.9.1 STUDY RECORDS RETENTION

Study documents will be retained for a minimum of 2 years since the formal discontinuation of clinical development of the study intervention. These documents may be retained for a longer period, however, if required by local regulations.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the clinical site principal investigators, 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.

No protocol deviations will be approved. However, it will be the responsibility of the clinical site principal investigators to use continuous vigilance in order to identify and report deviations within 5-10 working days of identification of the protocol deviation, or within 5-10 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to the clinical site PI, DCC, and NICHD Program Official. 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

- The Executive Committee will be responsible for developing publication procedures and resolving authorship issues.
- This study will be conducted in accordance with the 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 has been registered at [ClinicalTrials.gov](#) (NCT03321734), and results 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 after the completion of the primary endpoint by contacting the DCC.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence 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 in conjunction with NICHD 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

None

10.3 ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
CFR	Code of Federal Regulations
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FA	Fractional Anisotropy
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
IH	Intermittent Hypoxia
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
MD	Mean Diffusivity
MOP	Manual of Procedures
MRI/MRS	Magnetic Resonance Imaging / Magnetic Resonance Spectroscopy
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
ICU	Neonatal Intensive Care Unit
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PI	Principal Investigator
PMA	Postmenstrual age
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOA	Schedule of Activities
SOP	Standard Operating Procedure
TDD	Total Daily Dose
UP	Unanticipated Problem
US	United States

10.4 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
NA			
2.0	11-24-18	p. 6 Section 1.1 Study Synopsis Enrollment start date changed to December, 2018	Estimated approval date for Version 2.0
“ ”	“ ”	p. 7 Section 1.2 Study Schema diagram changed randomization from 32+1 to 32+0	It is possible to do randomization on same day as enrollment
“ ”	“ ”	Section 5.2 Exclusion Criteria, changed wording on exclusion #2 and #3 from “history of or current treatment for...” to “current or prior treatment for...”	Clarify wording to show the exclusions are if the infant was treated for the conditions, not just diagnosed with the conditions.
“ ”	“ ”	Section 6.1.2: Delete “simple syrup” in multiple sentences. Same deletion in other sections as needed.	Simple syrup not necessary component of study drug; cherry syrup sufficient.
“ ”	“ ”	Section 6.2.1: For specific components of compounded study drug, refer to Pharmacy Manual	Pharmacy Manual the appropriate location for this detailed information
“ ”	“ ”	Section 6.2.2: Individual dose syringes can be prepared up to 4 days in advance, and stored refrigerated.	As recommended by Investigational Drug Service, according to USP guidelines
“ ”	“ ”	Section 6.2.2: Stock study drug bottles can be stored, refrigerated, for up to 30 days instead of 90 days	As recommended by Investigational Drug Service, according to USP guidelines
“ ”	“ ”	Section 6.5.1, 7.1, 8.3.3.2, 8.3.3.3, 8.3.6, 8.4.2, 10.1.2, 10.1.3, 10.1.10 changed “study PI, clinical site PI, primary care provider” to “clinical site principal investigators” throughout document, and “PI” to “ICAF PI”	Make wording consistent for “clinical site PI” and “ICAF PI” throughout document
“ ”	“ ”	Section 8.1.4 Salivary Caffeine Levels, changed to “20 degrees or colder, -80 C is okay, if more convenient to store in same freezer as blood samples”	Purpose of freezing saliva sample is to prevent evaporation, so colder temperature is okay if makes adherence to protocol easier for sites
“ ”	“ ”	Section 8.3.4 Last day of study participation may be defined as “end of study visit, follow up MRI, or lost-to-follow-up determination date”	Clarify definition of last day of study participation

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