A Randomized, Double-Blind, Controlled Study to Assess the Efficacy and Safety of Intravenous Phenobarbital in Neonatal Seizures

Protocol Number: MI-5780

National Clinical Trial (NCT) Identified Number: NCT04320940

IND Sponsor: Hikma Pharmaceuticals USA Inc.

Funded by: Hikma Pharmaceuticals USA Inc.

Version Number: 2.0

07-Dec-2021

Summary of Changes from Previous Version:

Affected Section(s)	Summary of Significant Revisions Made and Rationale
1.1 Synopsis	Updated study duration
1.3 Schedule of Events	Update language for Vital Signs, Laboratory Safety Tests, cvEEG Monitoring and Clinical Seizure Assessments during the different study Periods
1.3.1 Schedule of Events (Open Label Extension Period)	Update language for Vital signs, Laboratory Safety Tests, cvEEG Monitoring and Clinical Seizure Assessments
3.2.1 Primary Endpoint	Clarified T0
4.1 Overall Design	Clarified qualifying seizure burden for first and second loading dose (($\geq$ 30seconds/hour)
4.1.2 Screening Period	Clarified length of time for screening period; Updated description of screening period assessments to match schedule of events
4.1.3 Treatment Period	Clarified length of time for treatment period; Updated description of screening period assessments to match schedule of events
4.1.4 Follow-up Period	Clarified length of time for follow-up period; Updated description of screening period assessments to match schedule of events
4.1.5 Open-Label Extension Period	Clarified length of time for Open-Label Extension Period and when study participants can enter the period; Updated description of screening period assessments to match schedule of events
4.1.6 Subject Treatment Scenarios	Section not required due to clarifications provided in sections 4.1.2 to 4.1.5
5.2 Exclusion Criteria	Added language regarding enrollment of study participants in other neonatal clinical trials

6.1.2 Dosing and Administration	Clarified when a second loading dose of study drug can and cannot be administered.
6.2.3 Product Storage and Stability	Updated storage conditions to reference the study Pharmacy Manual
6.5.2 Second Line Anticonvulsants	Updated title from Rescue Medication to Second Line Anticonvulsants. There is no rescue medication in this study; Added the use of midazolam prior to study screening and updated time of administration from >24 h to >=8 h
8.1.4 Seizure Documentation	Added section to provide guidance on how to and when to review the cvEEG recordings
8.2.1 Demographic and Medical History	Added section to provide additional instructions on what to and how to record demographics and medical history
8.2.2 Pregnancy and Delivery History	Updated delivery presentation options
8.2.5 Physical Examination	Added neurological exam details in order to standardize across all sites
8.2.8 Continuous Video EEG Monitoring	Added section to provide guidance on how to and when to review the cvEEG recordings
12.4 Appendix IV Clinical Laboratory Tests	Added guidance on blood draw and daily blood volume; Updated lab safety tests and removed non-standard of practice exams

Clinical Protocol Approval Form

Protocol Title: A Randomized, Double-Blind, Controlled Study to Assess the Efficacy and Safety of Intravenous Phenobarbital in Neonatal Seizures

Study No: MI-5780

Original Protocol Date: 28-Aug-2020

Protocol Version No: 2.0

Protocol Version Date: 07-Dec-2021

This study protocol was subject to critical review and has been approved by the appropriate protocol review committee of the Sponsor. The information contained in this protocol is consistent with:

- The current risk-benefit evaluation of the investigational product.
- The moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki, and principles of GCP as described in 21 CFR parts 50, 54, 56 and 312 and according to applicable local requirements.

The Investigator will be supplied with details of any significant or new findings, including adverse events, relating to treatment with the investigational product.

Name and Title	Approval	Signature	Date
Sponsor Representative: Naomi Donovan Hikma Project Manager	Yes x No (check one)	DocuSigned by: Naomi Donovan C36B373BA526464...	12/8/2021
Clinical Operations: Robert Taylor Jr, PhD NEMA Project Manager	Yes x No (check one)	DocuSigned by: Robert Taylor Jr 1ABE38E3B97243C...	12/9/2021
Biometrics: Duolao Wang, PhD Biostatistician	Yes x No (check one)	DocuSigned by: Duolao Wang, PhD F8A94CFBFB2C49E...	12/9/2021
Medical Lead: Sumedha Labhsetwar, MD Medical Monitor	Yes No (check one)	DocuSigned by: B2ADB256768849C...	12/9/2021
Regulatory Affairs: Robert Colucci, PharmD Clinical Development and Regulatory Consultant	Yes x No (check one)	DocuSigned by: Robert D. Colucci, Pharm. D., FCCM, FCP 20A08135675B4CA...	
Protocol Principal Investigator: Taeun Chang, MD Neonatal Neurologist	Yes No (check one)	DocuSigned by: TaeUn Chang, MD 767C005ED7DE44C...	12/15/2021

MI-5780

A Randomized, Double-Blind, Controlled Study to Assess the Efficacy and Safety of Intravenous Phenobarbital in Neonatal Seizures

Confidentiality and Investigator Statement

The information contained in this protocol and all other information relevant to MI-5780 are the confidential and proprietary information of Hikma, and except as may be required by federal, state or local laws or regulation, may not be disclosed to others without prior written permission of Hikma.

I have read the protocol, including all appendices, and I agree that it contains all of the necessary information for me and my staff to conduct this study as described. I will conduct this study as outlined herein, in accordance with the regulations stated in the Federal Code of Regulations for Good Clinical Practices and International Conference on Harmonization guidelines and will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision copies of the protocol and any amendments, and access to all information provided by Hikma or specified designees. I will discuss the material with them to ensure that they are fully informed about MI-5780 and the study.

Principal Investigator Name (printed): _____

Signature: _____

Date: _____ Site Number: _____

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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)

Investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of clinical trials have completed Human Subjects Protection and ICH GCP Training.

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

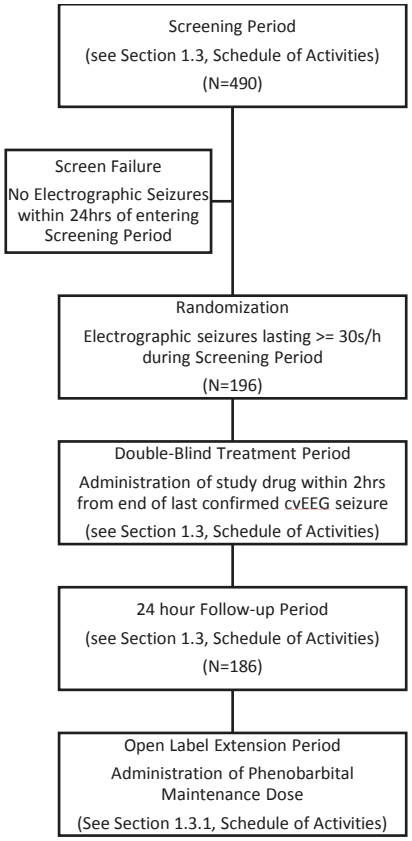
1 Protocol Summary

1.1 Synopsis

Title:	A Randomized, Double-Blind, Controlled Study to Assess the Efficacy and Safety of Intravenous Phenobarbital in Neonatal Seizures
Study Description:	This is a multicenter, randomized, double-blinded, active-controlled, parallel study in neonates (≥34 - ≤44 gestational weeks). This study is designed to evaluate the efficacy, safety and tolerability of intravenous phenobarbital in treatment of neonatal seizures diagnosed by continuous video EEG (cvEEG). This study will consist of a Screening Period, Treatment Period, Follow-up Period and Open Label Extension Period.
Objectives:	Primary Objective: The primary objective is to evaluate a potential dose-response of two different doses of intravenous phenobarbital for safety and efficacy in a neonatal population at high risk for seizures. Secondary Objectives: • To assess the safety and tolerability of intravenous phenobarbital administration in neonates experiencing seizures. • To assess the requirement of a second dose of intravenous phenobarbital in neonates with ongoing seizures. • To assess the requirement of alternative anticonvulsive therapy in neonates with ongoing seizures after the second dose of intravenous phenobarbital. • To characterize the seizure burden following administration of intravenous phenobarbital.
Endpoints	Primary Endpoint: • Percent of neonates who do not require additional seizure treatment after the first dose of phenobarbital during the first 24 hours after treatment. Secondary Endpoints: • Percent of neonates who do not require additional seizure treatment for 2 hours after the first dose of phenobarbital. • Percent of neonates who do not require additional seizure treatment after the second dose of phenobarbital within the first 24 hours of treatment.

	• Seizure burden over 24 hours following initial administration of the phenobarbital injection. • Seizure burden over 48 hours following initial administration of the phenobarbital injection. • Percent of neonates requiring alternative anticonvulsive therapy for the management of seizures at 24 and 48 hours • Time period between the first dose and second dose of intravenous phenobarbital in neonates randomized. • Time period between the second dose of intravenous phenobarbital to alternative anticonvulsant treatment in neonates randomized. • Time period between the first dose of intravenous phenobarbital to administration of an alternative anticonvulsant treatment in neonates randomized.
Study Participant Population	Male or female neonates with a gestational age of ≥ 34 - ≤ 44 weeks admitted into the NICU with a high probability of developing seizures
Phase:	Phase III
Description of Sites/Facilities Enrolling Participants	Level III/IV NICU
Description of Study Drug	Phenobarbital 20 mg/kg (first/initial dose) followed by 20 mg/kg (if required) Phenobarbital 40 mg/kg (first/initial dose) followed by 10 mg/kg (if required) The doses will be administered intravenously over a 30-minute period.
Study Duration:	Estimated Study Start Date: Q4 2020 Estimated Primary Completion Date: Q3 2023 Estimated Study Completion Date: Q4 2023
Participant Duration:	Total study duration for a single neonate is up to 5 days.

1.2 Schema



1.3 Schedule of Events

Study Procedure	Screening Period	Double-Blind Treatment Period	Follow-Up Period / Early Discontinuation/ End of Study
Study Day(s)	**-1-0**	**0-1**	**1-2**
NICU Admission	X		
Informed Consent	X		
Inclusion/Exclusion Criteria	X		
Delivery History	X		
Medical History	X		
Apgar Score	X		
Physical Examination (includes neurological exam)	X[1]		X[2]
Height/Length (include Head Circumference and Abdominal Circumference)	X		
Body Weight	X		
Vital Signs[1,3]	X	X	X
Laboratory Safety Tests (Hematology, Chemistry, Coagulation)[1,4]	X	X	X
Continuous Video EEG Assessment[5]	X	X	X
Clinical Seizure Assessment[6]		X	X
Randomization		X	
Phenobarbital Administration		X	
Phenobarbital Maintenance Administration			
Phenobarbital PK Samples[7]		X	X
Recording of Additional Anticonvulsant Medication		X	X
Prior and Concomitant Medication Review	X	X	X
Adverse Events Evaluation	X	X	X

1. All neonates will undergo a baseline physical exam (including a neurologic exam), baseline vital signs (blood pressure, pulse rate, respiration rate, oxygen saturation, and auxiliary or rectal temperature) and baseline laboratory safety tests.
2. Physical examination and neurologic exam to be completed at end of Follow-up Period and/or prior to discharge.
3. Vital signs will be monitored and recorded prior to each (loading dose and second dose) phenobarbital treatment administration and every 15±5 minutes up to 1 hour after the completion of each 30-minute dose infusion. Recording of vital signs will continue to be collected every 4-6 hours post phenobarbital administration through the Treatment Period and until completion of the Follow-up Period or at early study discontinuation. The 4-6 hours is calculated from initial loading dose start of infusion time.
4. Laboratory safety tests will be collected at the completion of the Treatment Period, at end of Follow-up Period and every 24 hours during the Open-Label Extension Period or at time of discharge/early study discontinuation.
5. cvEEG monitoring will be recorded for the study from time of informed consent or start of cvEEG (which ever comes last) and up to 48 hours after first loading dose of phenobarbital.
6. Clinical seizure assessment will be monitored and recorded prior to each phenobarbital treatment dose and at 30 and 60 minutes after the completion of each 30-minute dose infusion. At the end of Treatment and Follow-up Period, whether seizures were observed clinically will be documented in the eCRFs.
7. Collection of plasma samples for phenobarbital analysis will be obtained:
    - PK Group 1: 30 minutes, 4-6 hours post initial dose,
    - PK Group 2: 30 minutes, 10-12 hours post initial dose,
    - PK Group 3: 30 minutes, 16-18 hours post initial dose,
    - PK Group 4: 30 minutes, 22-24 hours post initial dose,
    - A sample will be collected after second dose infusion is complete (if applicable)
    - A sample will be collected at end of Follow-up Period or at time of discharge/discontinuation.
    - During the Open Label Extension Period, phenobarbital plasma concentrations will be obtained every 24 hours post initial dose up to 120 hours and or at time of discharge/discontinuation.

1.3.1 Schedule of Events (Open Label Extension Period)

Study Procedure	Open Label Extension	Open Label Discharge/End of Open Label
Study Day(s)	**2-4**	**2-4**
NICU Admission		
Informed Consent		
Inclusion/Exclusion Criteria		
Delivery History		
Medical History		
Apgar Score		
Physical Examination (includes neurological exam)		X[1]
Height/Length (include Head Circumference and Abdominal Circumference)		
Body Weight	X	
Vital Signs	X[2]	X[2]
Laboratory Safety Tests (Hematology, Chemistry, Coagulation)	X[3]	X[3]
Continuous Video EEG Assessment [4]		
Clinical Seizure Assessment	X[5]	X[5]
Randomization		
Phenobarbital Administration		
Phenobarbital Maintenance Administration	X	
Phenobarbital PK Samples[6]	X	X
Recording of Additional Anticonvulsant Medication	X	X
Prior and Concomitant Medication Review		X
Adverse Events Evaluation	X	X

1. Physical examination and neurologic exam to be completed prior to discharge.
2. Vital signs will be monitored and recorded prior to each phenobarbital maintenance treatment administration and every 15±5 minutes up to 1 hour after the completion of each 30-minute dose infusion. Recording of vital signs will continue to be collected

every 4-6 hours hour post phenobarbital administration until the completion of the Open Label Extension Period or at study discharge. The 4-6 hours is calculated from initial loading dose start of infusion time.

3. Laboratory safety tests will continue to be collected every 24 hours during Open-Label Extension Period or at time of discharge/early study discontinuation.
4. cvEEG monitoring will be recorded from time of informed consent or start of cvEEG (which ever comes last) and up to 48 hours and up to 48 hours after first loading dose of phenobarbital. If study participant enters Open Label Extension before end of Follow-up Period, cvEEG recording and monitoring will continue until the 48 hours is complete. cvEEG recording and monitoring after 48 hours is not required per this protocol and will follow site's standard of practice.
5. Clinical seizure assessment will be recorded prior to each phenobarbital maintenance treatment administration and at 30- and 60-minutes after the completion of each 30-minute dose infusion. At the end of each 24-hour Open Label Maintenance Study, whether seizures were observed clinically will be documented in the eCRFs.
6. Collection of plasma samples for phenobarbital analysis will be obtained every 24 hours post initial loading dose up to 120 hours and or at time of discharge/discontinuation.

2 Introduction

2.1 Study Rationale

This is a randomized, double-blind, parallel-group, phase 3 study to evaluate the efficacy of the administration of phenobarbital sodium injection in neonates who have suffered from electrographic or electroclinical seizure. As neonatal seizures can have long-term adverse effects, including death, placebo-controlled studies are not appropriate for this population. This study is designed to show intravenous phenobarbital is effective at preventing subsequent seizures by demonstrating greater efficacy at a higher dose compared to a lower dose.

2.2 Background

Phenobarbital is one of the first barbiturates synthesized and is one of the approximate 50 barbiturates used clinically. Phenobarbital has an extensive history with nearly 100 years of use as a sedative, hypnotic and anticonvulsant drug. It is recommended by the World Health Organization (WHO) as a first-line agent for partial and generalized tonic-clonic seizures in developing countries.

Currently, phenobarbital is one of the oldest and most utilized antiepileptic drugs worldwide. The drug is relatively safe, and provides a cost-effective alternative to other antiseizure medications (ASMs), making this drug a more popular choice. Despite other conventional ASMs including carbamazepine, sodium valproate and phenytoin replacing phenobarbital as a first-line agent and possible behavior and cognitive side effects as well as sedation from drug use, phenobarbital continues to be a second- or third-line agent in the developed world.

Phenobarbital is commonly administered orally in tablet form for prophylactic purposes but can also be used parenterally when the oral route is impossible or impractical (i.e. emergency situations). Phenobarbital is administered parenterally as a slow intravenous (IV) injection or given as an intramuscular (IM) injection. Subcutaneous routes are not recommended.

Phenobarbital sodium injection is no longer a first-line therapy for many types of acute epileptic seizures since it has been largely replaced by newer parenteral antiepileptic drugs such as lorazepam, “which is the most commonly administered benzodiazepine for status epilepticus when intravenous access is available”(1). Other intravenous antiepileptic drugs available in the USA include phenytoin, fosphenytoin, levetiracetam, sodium valproate, and lacosamide.

However, phenobarbital remains the first-line therapy for acute seizures in neonates in the USA and international survey data indicate that it remains the most commonly used agent in this setting (2). Seizures occur more often during the newborn period (2-3.5 per 1000 live births) than at any later age. Neonatal seizures can lead to frequent and serious long-term consequences in survivors, such as later epilepsy and significant cognitive and motor disabilities. A National Hospital Discharge Survey indicates that the incidence of all types of neonatal seizures is 2.84 per 1,000 live births in the United States; similar rates of neonatal seizures have been reported in population-based studies in California (0.95/1000 live births) and Texas (1.8/1000 live births) (3-5). For

hypoxic-ischemic encephalopathy specifically, similar incidence rates of 1-3 per 1000 live births have been reported in the United States and other high-income countries (6, 7). In a recent Task Force Report for the ILAE Commission of Pediatrics, phenobarbital, carbamazepine, and valproate were recommended choices for benign infantile convulsions, but the committee indicated that each was "possibly effective" and that the published data were "weak" with overall recommendations of "C" (possibly effective, ineffective, or harmful, or as useful/predictive or not useful/predictive) for all three drugs according to the American Academy of Neurology (AAN) grading system (8).

2.2.1 Clinical

In infants with seizures, phenobarbital 15−20 mg loading dose with additional 5−10 mg/kg doses to maximal plasma concentration of 40 mg/L (172 micromol/L) resulted in a plateau of the response rate. Plasma concentrations >50 mg/L (215 micromol/L) were associated with sedation and feeding difficulty (9).

The clearance of phenobarbital increases with birth weight and postnatal age, but is reduced at a concentration >50 mg/L (215 micromol/L) (10). Bioavailability is 50% after oral administration. Simulations recommend a loading dose 20 mg/kg and maintenance 2.5 − 5 mg/kg/day for intravenous administration and; loading dose 40 mg/kg and maintenance 5 − 11 mg/kg/day for oral administration to meet a target phenobarbital concentration between 15 and 30 mg/L (64.5 and 129.1 micromol/L) (11). The clinical management of neonatal seizures and the doses (both partial and cumulative) of phenobarbital administered as first line therapy have generally varied among clinicians and institutions. A total dose of 50 mg/kg can be given if required (12).

The clearance may also be reduced in infants with perinatal asphyxia undergoing therapeutic hypothermia (13-15). In term infants treated with hypothermia, an initial phenobarbital loading dose of 20 mg/kg with an additional 10−20 mg/kg if needed is recommended (15).

2.2.2 Importance of Clinical Trial

This clinical trial represents a national and international, therapeutic confirmatory study to further demonstrate the tolerability, safety and efficacy of phenobarbital use in neonatal seizures.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

2.3.1.1 Contraindications

Hypersensitivity to phenobarbital or any ingredients. Any forms of acute porphyria.

2.3.1.2 Precautions

Use with caution in renal or hepatic impairment. Dependence may develop with prolonged use – consider weaning instead of abrupt withdrawal. Therapeutic hypothermia may increase the serum concentrations of phenobarbital – monitor serum concentrations closely.

Additional precautions in preterm infants, especially those with extreme immaturity and neonates with respiratory failure.

2.3.1.3 Drug Interactions

Phenobarbital is known as an inducer of microsomal liver enzymes. Morphine, fentanyl, midazolam and other CNS depressants may cause reduced blood levels when co-administered with phenobarbital, resulting in ineffective treatment (16-18). If phenobarbital is substituted by non-CYP3A inducers, lower doses of other drugs might be appropriate to avoid excessive exposure.

Acceleration of elimination of digoxin, metronidazole, corticosteroids (e.g. betamethasone, dexamethasone), vitamin D, and beta-blockers (e.g. propranolol, sotalol), resulting in reduced plasma levels may occur if administered concurrently with phenobarbital. Concurrent administration of phenytoin with phenobarbital has variable effects on serum concentrations of either drug. Serum concentrations should be monitored for both drugs.

2.3.1.4 Adverse Reactions

Expected phenobarbital adverse reactions include drowsiness, lethargy, impaired sucking reflex and poor feeding. If administration is too rapid, expected adverse reactions include respiratory depression, apnea, hypotension, laryngospasm and bronchospasm. Other expected adverse reactions include phlebitis, tissue necrosis if extravasation occurs, GI intolerance, physical dependence and tolerance, folate deficiency, hepatitis and hypocalcaemia.

Stevens-Johnson syndrome and Toxic Epidermal Necrolysis: Serious and sometimes fatal dermatologic reactions, including Stevens-Johnson syndrome (SJS) and Toxic Epidermal Necrolysis (TEN) have been reported with phenobarbital. Post-marketing reporting rate is generally accepted to be an underestimate due to under-reporting. Recurrence of serious skin reactions following re- challenge with phenobarbital has also been reported. Therefore, if a study participant develops a skin reaction during phenobarbital treatment, consideration should be given to permanent discontinuation and replacement of the drug with alternative treatment.

2.3.2 Known Potential Benefits

Phenobarbital has been recommended as first-line treatment for neonatal seizures (19). In RCTs, phenobarbital (target plasma concentration 25 mg/L) was reported to be similarly as effective as phenytoin (target plasma concentration 3 mg/L) for control of electrical seizures (43% versus 45%) (20); and phenobarbital 20 mg/kg was reported to be more effective than phenytoin 20 mg/kg at controlling clinical seizures (72% versus 15%) (21).

2.3.3 Assessment of Potential Risks and Benefits

Overall, this is a carefully designed trial targeting to meet its objectives of providing effective neonatal seizure treatment without exposing study participants to any unnecessary risk.

2.3.3.1 Physical Harms

The study is designed to measure the effects of therapeutic doses of phenobarbital for treating neonatal seizures. The risks associated with the drug is assumed not to beyond those presented during standard of care treatment or described in the literature (12). The doses tested in this study are within the dosing guidelines available in the literature (12). The increased number of blood draws required for the study can be considered beyond that of standard of care. There is risk associated with multiple blood draws during a prespecified time period.

2.3.3.2 Psychological Harms

The risks associated with the drug are assumed not to be beyond those presented during standard of care treatment or presented in the literature (12). The doses tested in this study are within the dosing guidelines available in the literature (12). It is not expected for the study participant to experience any psychological harm during the study. Undesired changes in thought process and emotion may occur for the parents/legal guardians during the study. These changes are only expected to be transitory and not, recurrent or permanent.

2.3.3.3 Invasion of Privacy

The study does not involve any type of observation that would be seen as covert or intrusion.

2.3.3.4 Social and Economic Harms

The study does not involve any type of outcome that would be seen to affect the social or economic status of the parents/legal guardians or study participant.

2.3.3.5 Minimization of Risk

Minimization of risk will be achieved by following the listed guidelines:

- Study staff will receive complete information in the protocol regarding the experimental design and the scientific rationale underlying the proposed research, including the results of previous animal and human studies.
- A research team with sufficient expertise and experience to conduct the research will be assembled.
- Statisticians will ensure that the projected sample size is sufficient to yield useful results.
- As much data will be collected from standard-of-care procedures to avoid unnecessary risk, particularly for invasive or risky procedures (e.g., spinal taps, cardiac catheterization).
- Appropriate safeguards will be incorporated into the research design such as an appropriate data safety monitoring plan, the presence of trained personnel who can respond to emergencies, and procedures to protect the confidentiality of the data (e.g., encryption, codes, and passwords).
- The protocol has also been designed in order to meet the requirements of Part 46.205 (Protection of Human Subjects, Research involving neonates) which include:
    - Neonates of uncertain viability will not be included in the study

- Preclinical and clinical studies have been conducted and provide data for assessing potential risks to neonates
- Parents/Legal Guardians providing consent (in-person or remote) will be fully informed regarding the impact of the research on the neonate
- Study staff engaged in the protocol will have no part in determining the viability of a neonate

3 Objectives and Endpoints

3.1 Objectives

3.1.1 Primary Objective

The primary objective is to evaluate a potential dose-response of two different doses of intravenous phenobarbital for safety and efficacy in a neonatal population at high risk for seizures.

3.1.2 Secondary Objectives

- To assess the safety and tolerability of intravenous phenobarbital administration in neonates experiencing seizures.
- To assess the requirement of a second dose of intravenous phenobarbital in neonates with ongoing seizures.
- To assess the requirement of alternative anticonvulsive therapy in neonates with ongoing seizures.
- To characterize the seizure burden following administration of intravenous phenobarbital.

3.1.3 Exploratory Objectives

- To characterize the population pharmacokinetics of intravenous phenobarbital in neonates with seizures.
- To evaluate a potential dose-response of two different doses of intravenous phenobarbital for safety and efficacy in neonatal populations with different seizure etiologies.

3.2 Endpoints

3.2.1 Primary Endpoint

Percent of neonates who do not require additional seizure treatment after the first dose of phenobarbital during the first 24 hours after treatment.

Time zero (T0) will be defined as the start of the first loading dose 30-minute infusion.

3.2.1.1 Justification for Primary Endpoint

Primary endpoint is in line with recommendations provided by the US Food and Drug Administration. This measure should provide a clear delineation between the efficacy of the low and high loading doses of intravenous phenobarbital.

3.2.2 Secondary Endpoints

- Percent of neonates who do not require additional seizure treatment after 2 hours of the first dose of phenobarbital.
- Percent of neonates who do not require additional seizure treatment after the second dose of phenobarbital within the first 24 hours of treatment.
- Seizure burden over 24 hours following initial administration of the phenobarbital injection.
- Seizure burden over 48 hours following initial administration of the phenobarbital injection.
- Percent of neonates requiring alternative anticonvulsive therapy for the management of seizures at 24 and 48 hours.
- Time period between the first dose and second dose of intravenous phenobarbital in neonates randomized.
- Time period between the second dose of intravenous phenobarbital and alternative anticonvulsant treatment in neonates
- Time period between the first dose of intravenous phenobarbital and administration of an alternative anticonvulsant treatment in neonates randomized.

3.2.2.1 Justification for Secondary Endpoints

Reduction in seizure burden, seizure recurrence and administration of rescue ASDs have been identified as reliable outcomes to assess efficacy of antiepileptic drugs in late stage drug trials (22).

3.2.3 Exploratory Endpoints

3.2.3.1 Pharmacokinetic Endpoints

A population PK analysis approach utilizing sparse plasma sampling will be implemented to assess the pharmacokinetics of intravenous phenobarbital in neonatal seizures. The following phenobarbital pharmacokinetic parameters may be ***explored*** during the study:

- Observed time to reach maximum drug concentration (T_{max})
- Observed peak drug concentration (C_{max})
- Area under the plasma concentration-time curve (AUC_{0-t}), the percent AUC extrapolated from AUC_{0-t} to infinity AUC_{extrap} and infinity (AUC_{0-inf})
- Apparent terminal elimination rate constant (K_{el})
- Apparent terminal drug elimination half-life ($t_{1/2}$)
- Clearance adjusted by absorption, calculated as dose/AUC_{0-inf} (Cl/F)
- Volume of distribution adjusted by absorption, calculated as Dose/K_{el}•AUC_{0-inf} (Vd/F)

Both free and total phenobarbital will be attempted to be measured in order to assess the variability in protein binding of phenobarbital.

Relationship between potential covariates including co-medication, cooling therapy, renal function, liver function, gestational age and protein binding will be explored. Specific analyses will be detailed in the final pharmacokinetic analysis plan.

3.2.3.2 Other Exploratory Endpoints

The endpoints described in Section 3.2.1 and Section 3.2.2 will also be evaluated for different neonatal seizure etiologies. The subpopulations to be evaluated include HIE, stroke and intracerebral hemorrhage.

3.2.3.3 Justification for Exploratory Endpoints

Gestational age less than 44 weeks has different seizure etiologies, seizure pathogenesis, and hepatic/renal function, potentially affecting drug efficacy, safety, and/or drug metabolism and elimination. Pharmacokinetic data and analysis are necessary to understand these potential study participant differences (22). The data collected is also part of the overall safety assessment for the study participants.

3.2.4 Safety Endpoints

The following safety assessments will be performed during the study:

- Type, incidence, and severity of adverse events
- Physical examination (including neurologic exam)
- Vital signs (blood pressure [arterial line], respiration rate, pulse rate, oxygen saturation, and temperature)
- Clinical laboratory tests (hematology, serum chemistry)

3.2.4.1 Justification for Safety Endpoints

Safety monitoring is a standard, yet critical component of any clinical trial. The safety assessments in this study are not beyond that of standard safety assessments performed in many clinical studies.

4 Study Design

4.1 Overall Design

This is a multicenter, randomized, double-blinded, active-controlled, parallel study in neonates (defined as ≥34 - ≤44 gestational weeks). The study will consist of a Screening Period, Double-Blind Treatment Period, Follow-up Period and an Open-Label Extension Period. The study participant population is neonates being admitted to the NICU and requiring continuous video electroencephalography (cvEEG) for seizure detection. One or two parents and/or legal guardians must provide written approval of informed consent, before the neonate can be assessed, monitored and managed in accordance with the study protocol and before any study data can be collected and recorded as study data. The reviewing IRB, based on its standard operating procedures, will determine number of parents/legal guardians who will be required to sign the informed consent.

Upon NICU admission and after parents or legal guardians provide informed consent, neonates will be monitored for electrographic seizures.

Seizures requiring study drug treatment will be defined as cumulative electrographic seizure of at least 30 sec/h (22). Confirmation of electrographic seizures by principal investigator or site designees (recommended to be a pediatric neurologist and/or neurophysiologist) is required. Randomization and study drug administration should start as soon as possible after the 30 sec/h of seizure activity criteria is met ideally within 30 min (maximum two hours) (22). If study drug cannot be administered within two hours of recognition of qualifying seizure burden (≥ 30 sec/h), then study drug should not be administered and the neonate will be discontinued from the study and will be a screen failure.

Following confirmation of seizure criteria, neonates will be randomized in a 1:1 allocation to receive a phenobarbital treatment loading dose of 20mg/kg or 40mg/kg, infused over 30 minutes.

If seizures persist (≥30 sec/h of seizure activity) 60 minutes following the start of the initial dose of phenobarbital, neonates receiving an initial dose of 20 mg/kg will receive an additional 20 mg/kg dose. Neonates receiving an initial dose of 40 mg/kg will receive an additional 10 mg/kg dose. Administration of this second loading dose is required to be within 2 hours of recognition of qualifying seizure burden (≥ 30 sec/h). The time period between the first and second dose of study treatment will be determined by the principal investigator or site designee (recommended to be a pediatric neurologist and/or neurophysiologist) review of the cvEEG, but must be at least 30 minutes after infusion is completed and no more than 24 hours after the first dose of study treatment (22).

If the seizure activity continues (≥30 sec/h) is still not resolved, study participants may receive a second-line alternative anticonvulsant therapy per institutional protocol. In each treatment arm, the second-line anticonvulsant therapy will be confirmed by the principal investigator or site designee (recommended to be a pediatric neurologist and/or neurophysiologist) and recorded in the study documentation as a concomitant medication.

All phenobarbital treatment doses will be prepared by the unblinded investigational pharmacist. The investigational pharmacist will be unblinded (have access to the treatment arm labels) and will not be involved in the assessment or medical management of the neonate. All other study personnel will be blinded to the first and second phenobarbital dose treatment. Each study treatment arm will be prepared in the same total volume of administration and administered over a **30-minute infusion** in order to maintain the study blind.

Throughout the Treatment Period the neonate will be continuously monitored by cvEEG, vital signs will be recorded, clinical seizure assessment will be recorded, and blood samples will be collected for laboratory safety monitoring and phenobarbital plasma concentrations according to the schedule of activities (Section 1.3). cvEEG monitoring will continue throughout the Treatment

Period and will be discontinued at end of Follow-up Period or upon entering Open Label Extension Period.

A population pharmacokinetic approach will be implemented using sparse sampling collection. Collection of plasma samples for phenobarbital analysis will be obtained after each loading dose and will follow the sampling guidelines in the schedule of activities (Section 1.3). Additional plasma samples will be obtained at the completion of the treatment period or at early study discontinuation. In order to maintain the study blind, the phenobarbital plasma concentrations will not be made available to the study personnel.

Seizure management in the treatment period will be determined by cvEEG monitoring. Recording of all vital signs and collection of laboratory safety monitoring and collection of phenobarbital plasma samples will be dependent on the clinical management and status of the neonate. The principal investigator will be allowed to discontinue the neonate from the study protocol at any time throughout the study protocol and allowed to administer alternative anticonvulsant therapy as medically required or following standard of care guidelines.

The study treatment period for the primary study analysis will end 24 hours after the neonate meets the seizure criteria and receives the initial phenobarbital study dose (i.e. T0-24 hr). Prior to completing the Treatment Period or early study discontinuation, cvEEG assessment, clinical seizure assessment, and vital signs will be recorded, clinical laboratory tests will be performed, and a phenobarbital plasma sample will be collected. The neonate will be monitored for an additional 24 hours (Follow-up Period) after the Treatment Period is completed. During the 24-hour Follow-up Period general seizure activity, administration of additional non-study anticonvulsant medication, and adverse events will be recorded. Neonates requiring ongoing/maintenance intravenous phenobarbital therapy following study treatment completion will enter an Open Label Extension Period (up to 3 days) and will be monitored for safety and additional phenobarbital plasma samples will be obtained while they are on therapy and until hospital discharge. All study related assessments and work related to the study will be compensated by the funding Sponsor.

4.1.1 Definitions

4.1.2 Screening Period

Parental/Legal Guardian consent must be provided prior to study participant enrollment and prior to any study-related procedures being done. All screening evaluations must be completed before the study participant enters the Treatment Period. Screening Period is from time of consent or start of cvEEG (which ever comes last) and up to 24 hours (T(-)24-0 hrs).

The following procedures will be completed during the Screening Period (prior to dosing):

- Informed Consent
- NICU Admission

- Review of Inclusion/Exclusion Criteria
- Delivery History
- Medical History (includes Family History of Seizures and Delivery Complications)
- Physical Examination (including neurologic exam)
- Vital Signs (baseline assessment)
- Height/Length
- Head Circumference
- Abdominal Circumference
- Apgar Score
- Body Weight
- Collection and review of Laboratory Safety Tests (Hematology, Chemistry and Coagulation) (baseline assessment)
- cvEEG recording and monitoring
- Prior and Concomitant Medication Review
- Adverse Events Evaluation

Gestational age can be calculated using either ultrasound, last menstrual period, or neonatal assessment.

4.1.3 Treatment Period

Treatment Period is from time of first drug administration and up to 24 hours (T0-24 hrs).

The following procedures will be performed during the Treatment Period:

- Review of Inclusion/Exclusion Criteria
- Vital Signs
- Collection and review of Laboratory Tests (Hematology, Chemistry and Coagulation)
- Randomization
- cvEEG recording and monitoring
- Clinical Seizure Assessment
- Initial Phenobarbital Administration
- Additional Phenobarbital Administration
- Phenobarbital Plasma Samples
- Recording of Additional Anticonvulsant Medication
- Concomitant Medication Review
- Adverse Events Evaluation

4.1.4 24 Hour Follow-Up Period /Early Discontinuation/End of Study

Follow-Up Period is from end of Treatment Period and up to 24 hours (T24-48 hrs).

The following procedures will be performed during the Follow-up Period:

- Physical Examination (including neurologic examination)
- Vital Signs
- Collection and review of Laboratory Tests (Hematology, Chemistry and Coagulation)
- cvEEG recording and monitoring
- Clinical Seizure Assessment
- Phenobarbital Plasma Samples
- Recording of Additional non-study Anticonvulsant Medication
- Concomitant Medication Review
- Adverse Events Evaluation

4.1.5 Open-Label Extension Period

Open-Label Extension Period is from start of first phenobarbital maintenance dose and up to 72 hours. Maintenance cannot be started <24 hours after T0 (e.g., not during Treatment Period). If it is determined that maintenance phenobarbital is no longer needed, study participant will be discontinued from the Open-Label Extension Period and final study assessments will be completed based on schedule of activities (Section 1.3).

The following procedures will be performed during the Open-Label Extension Period:

- Vital Signs
- Physical Examination (including neurologic examination)
- Collection and review of Laboratory Tests (Hematology, Chemistry and Coagulation)
- Clinical Seizure Assessment
- Phenobarbital Administration
- Phenobarbital Plasma Samples every 24 hours
- Recording of Additional Anticonvulsant Medication
- Concomitant Medication Review
- Adverse Events Evaluation

4.2 Scientific Rationale for Study Design

4.3 Justification for Dose

The clinical management of neonatal seizures and the doses (both partial and cumulative) of phenobarbital administered as first line therapy have generally varied among clinicians and institutions. A total daily dose up to 50 mg/kg have been recommended for the treatment of neonatal seizures (12). Based on the published literature and current standard practices, the most frequently used initial doses of phenobarbital to treat neonatal HIE are 20 to 30 mg/kg i.v. followed by maintenance dose of 4 to 6 mg/kg per day (2). If seizures do not resolve with the initial dose, repeat boluses of 10 to 20 mg/kg may be administered with a goal of approximately 50 µg/mL phenobarbital serum level or a total 24 hour total dose of 50 mg/kg (2).

The Sponsor proposes evaluating two double-blind intravenous dose regimens of phenobarbital: Phenobarbital 20 mg/kg (initial dose) followed by 20 mg/kg (second dose, if required) [total daily dose 40 mg/kg] or phenobarbital 40 mg/kg (initial dose) followed by 10 mg/kg (second dose, if required)[total daily dose of 50 mg/kg].

If seizures do not resolve following the initial or second dose of phenobarbital, the PI or site designee will choose an alternate drug for additional dosing. The PI or site designee will be permitted to choose an alternate drug of choice if the neonate does not achieve appropriate seizure control at any time during the study.

For ethical concerns, there will not be a placebo control group.

4.4 End of Study Definition

A study participant is considered to have completed the study if he or she has completed all phases of the study including the last scheduled procedure prior to discharge shown in the Schedule of Activities (SoA).

The end of the study is defined as completion of the last procedure shown in the SoA in the trial for the last enrolled study participant.

5 Study Population

5.1 Inclusion Criteria

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

1. Male or female neonates with a gestational age of ≥ 34 - ≤44 weeks admitted into the NICU with a high probability of developing seizures (e.g., HIE, stroke, intracerebral hemorrhage, central nervous system infection)
2. Parental/Legal Guardian informed consent (in-person or remote consent)
3. Undergoing continuous video electroencephalogram (cvEEG) monitoring
4. Has evidence of electrographic seizure burden of at least 30 seconds/h

5.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Received anticonvulsant treatment, including phenobarbital, prior to randomization (with exception of lorazepam and midazolam administered ≥ 8 hours before enrollment)
2. Strong suspicion or confirmed diagnosis of brain malformation, inborn error of metabolism genetic syndrome, or major congenial malformation prior to randomization

3. Seizures responding to correction of hypoglycemia, hypocalcemia or any other metabolic disorder
4. Death appears to be imminent as assessed by the NICU attending physician
5. Is currently enrolled in another study assessing the same and/or similar primary/secondary endpoints with/without drug treatment.

Study participants who may be already participating in another neonatal clinical trial (e.g., registry or observational) that meet all MI-5780 inclusion criteria and none of the MI-5780 exclusion criteria as well as can meet study assessment requirements, can be enrolled into this study. In addition, study participants enrolled in this trial can enroll in other neonatal trials as long as 1) the inclusion criteria for study MI-5780 continue to be met 2) none of the exclusion criteria for study MI-5780 are met 3) the other study will not result in minor or major MI-5780 protocol deviations.

5.3 Lifestyle Considerations

Not applicable

5.4 Screen Failures

Screen failures are defined as study participants whom parent/legal guardian consented to participate in the clinical trial but are not subsequently randomly assigned to the study drug. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) MAY NOT be rescreened.

5.5 Strategies for Recruitment and Retention

5.5.1 Target Study Sample Size

Literature supports that lower loading doses of phenobarbital achieve seizure control in approximately 30 to 60% of treated neonates, with higher loading doses achieving seizure control up to 85% (19, 20, 23, 24). Based on 70% and 50% of study participants would achieve seizure control in high dose and low, respectively, an effect size of 20%, an alpha of 0.05 and 80% power, it is estimated that the number of completed study participants required will be 93 study participants in each group (total 186). With approximately, 5% dropout and or non-evaluable study participants, the minimum required for randomization will be 196. A screen failure rate of approximately 60% is anticipated. A total of 490 study participants would be enrolled.

5.5.2 Anticipated Accrual Rate

It is estimated that investigator sites will enroll approximately 1-2 neonates a month. This rate would achieve the necessary 490 screened over the course of approximately one year.

5.5.3 Anticipated Number of Sites

Total number of sites/institutions expected to enroll study participants is up to 30 sites.

5.5.4 Source of Study Participants

Study participants are expected to come from sites/institutions that are level 3/level 4 NICUs.

5.5.5 Recruitment Venues

Not applicable

5.5.6 Identification of Potential Study Participants

Maternity ward and hospital transfer staff will, at minimum, be informed about the study being performed at their associated NICU. The ward or transfer staff will alert the PI or delegated study staff of the transfer of the potential study participant after their birth. The ward staff should inform the study staff of the potential seizure risk, any suspected seizure activity and who identified the seizure risk/activity. At that time, it will be the responsibility of the PI or designated study staff to begin the informed consent process with the parents/legal guardians as well as ensure ward or transfer staff do not administer any prohibited anticonvulsants.

Potential study participants who are at increased risk of seizure activity are listed below. The list is not all inclusive and can be used as a guide when determining study participant eligibility.

Study participants with the following characteristics may be high risk for experiencing seizures:

- Hypoxic-ischemic encephalopathy
- Ischemic stroke
- Intracranial hemorrhage
- Epileptic encephalopathy
- Intracranial infection

Those with the following characteristics are recommended to not be included, but ultimately at the discretion of the investigator and are within the study participant eligibility requirements.

- Brain malformation
- Transient metabolic (e.g. hypoglycemia, hypocalcemia)
- Benign familial epilepsy

5.5.7 Recruitment Strategies

Specific recruitment strategies are detailed in a separate Recruitment Plan.

5.5.8 Long-term Recruitment/Enrollment Strategies

Specific recruitment strategies are detailed in a separate Recruitment Plan.

5.5.9 Justification for Vulnerable Population Recruitment

Phenobarbital is the first line therapy for neonatal seizures. In order to evaluate the efficacy of phenobarbital in a randomized, double-blind clinical trial, neonates must be included as the study population. The risks associated with enrollment are discussed in Section 2.3.

5.5.10 Compensation for Study Participation

Study participants' parents/legal guardians will not be receiving compensation for participating in the study. This information will be clearly out lined and described in the informed consent form and approved by an institutional review board (IRB).

6 Study Drug

6.1 Study Drug(s) Administration

6.1.1 Study Drug Description

Phenobarbital sodium injection is part of the class of drugs known as barbiturates. The barbiturates are nonselective central nervous system (CNS) depressants which are primarily used as sedative hypnotics and anticonvulsants in subhypnotic doses. The barbiturates and their sodium salts are subject to control under the Federal Controlled Substances Act as scheduled IV controlled substances.

Barbiturates are substituted pyrimidine derivatives in which the basic structure common to these drugs is barbituric acid, a substance which has no central nervous system activity. CNS activity is obtained by substituting alkyl, alkenyl or aryl groups on the pyrimidine ring.

6.1.2 Dosing and Administration

Following confirmation of seizure criteria, neonates will receive 1 of 2 different phenobarbital treatment dose regimens.

- Phenobarbital 20 mg/kg (first/initial dose) followed by 20 mg/kg (if required)[dose to be prepared from 90 mg/ml vials], or
- Phenobarbital 40 mg/kg (first/initial dose) followed by 10 mg/kg (if required)[dose to be prepared from 90 mg/ml vials]

The initial dose will be administered intravenously over a 30-minute period. If seizures persist and the criteria for a second dose is achieved, a second dose of phenobarbital will be administered.

The time period between the first and second dose of phenobarbital cannot occur within 30 minutes of the end of the first dose. Note: the second loading dose can only be administered while study participant is in the Treatment Period and cannot be administered during the Follow-up Period.

Any additional treatment required must be a second line anticonvulsant and be based on site/institutional protocol/standard operating procedures.

If seizures persist following the second dose of phenobarbital, additional treatment will be based on site/institutional protocol/standard operating procedures or site has the option to administer the phenobarbital maintenance dose. Note, the optional maintenance dose cannot be administered during the Treatment Period. Study participant must enter the Follow-up Period before phenobarbital maintenance dose can be administered.

If study participant qualifies for the optional phenobarbital maintenance dose, the study participant can enter the Open-Label Extension Period.

- Optional Maintenance Dose: 5 mg/kg/day administered intravenously as a single dose over a 30-minute period, no earlier than 24 hours after completion of initial loading dose (dose to be prepared from 55 mg/ml vials)

Treatment administration information should be reported to the study participants treating pediatric physician and entered into their medical records at time of study completion and unblinding of the study participant.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and Accountability

For detailed instructions regarding study drug acquisition and accountability, refer to the IP Instructions for Handling.

The PI is responsible for ensuring accountability of all study medication supplied and appropriate storage and allocation of these supplies.

The PI is responsible for ensuring that all study medication received at the site are inventoried and accountability performed, and that dispensed study medication is recorded in both the eCRF and the Study Medication Accountability Logs. The PI, or designee, will verify study medication accountability with the CRA during site visits. Any discrepancies should be investigated. The PI will not supply study medication or rescue medication to any person except those named as Sub-Is on Form FDA 1572, designated staff, and study participants in this study and will not dispense study medication or rescue medication from any sites other than those listed on Form FDA 1572. Study medication and rescue medication may not be relabeled or reassigned for use by other study participants.

All unused or damaged study medication and/or rescue medication will be returned, and unit counts will be performed whenever medication is returned. The site must account for all study medication received. The site will retain and store all study medication and rescue medication until inventoried by the monitor.

All unused or damaged (e.g., films that were torn during opening of the foil package) study medication and/or rescue medication will be returned to the CRO's return vendor along with copies of the Study Medication Accountability Logs.

6.2.2 Formulation, Appearance, Packaging, and Labeling

Phenobarbital Sodium Injection, USP is a sterile solution for intramuscular or slow intravenous administration as a long-acting barbiturate. Each mL contains phenobarbital sodium either 55 mg or 90 mg, alcohol 140 mg/mL, propylene glycol ~779mg/mL or 754mg/mL, respectively, and no benzyl alcohol in water for Injection; hydrochloric acid and sodium hydroxide added, if needed, for pH adjustment. Chemically, phenobarbital sodium is 2,4,6(1H,3H,5H)-Pyrimidinetrione,5-ethyl-5-phenyl-, monosodium salt.

The packaging and labeling will be detailed in the IP Supply Plan.

6.2.3 Product Storage and Stability

Phenobarbital vials can be stored at 15°-25°C (68°-77°F), excursions permitted between 15°-30°C (59°-86°F) are allowed provided that the mean kinetic temperature (MKT) does not exceed 25C. Transient spikes up to 40C are allowed only if they do not exceed 24 hours.

Do not use if solution is discolored or contains a precipitate.

Detailed storage conditions of phenobarbital prepared in syringes is described in the study provided pharmacy manual.

6.2.4 Preparation

All phenobarbital treatment doses will be prepared by the unblinded investigational pharmacist. The investigational pharmacist will be unblinded (have access to the randomization code) and will not be involved in the assessment or medical management of the neonate. All other study personnel will be blinded to the phenobarbital dose treatment administered. Each study treatment arm will be prepared in the same total volume of administration in order to maintain the study blind.

Further details are provided in the IP Instructions for Handling (also known as Pharmacy Manual).

6.3 Measures to Minimize Bias: Randomization and Blinding

Study participants will be assigned unique study participant numbers upon completion of the Screening Period and upon achieving the seizure criteria.

A unique randomization number will be assigned by an interactive web response system (IWRS) once eligibility for the Treatment Period of the study has been determined. Randomization codes will be prepared by an external-to-study, unblinded biostatistician.

Detailed instructions for contacting the IWRS will be provided in the Study Manual. The PI or designee will use the IWRS to determine which investigational medicinal product (IMP) will be

dispensed to a given study participant for the Treatment Period of the study. The unblinded investigational pharmacist will prepare the IMP (both first and second loading dose for each treatment arm [labeled A and B in IWRS for unblinded pharmacist]) after the informed consent is signed and provide to the PI or designee for storage in the NICU's pyxis machine, if possible. At time of randomization, the PI or designee will contact the IWRS for the randomization code and syringe code prior to first dose administration. The IWRS will be contacted again if second loading dose administration is needed.

6.3.1 Unblinding

The Investigator may unblind an individual study participant's treatment in an emergency when knowledge of such treatment may have an impact on further treatment decisions or aid in the emergency treatment of the study participant. Every effort must be made to contact the Medical Monitor prior to any unblinding of the study drug. The circumstances that lead to unblinding will be promptly communicated via telephone and in writing to the Medical Monitor.

6.4 Study Drug Compliance

The PI, or designee, will verify study medication accountability with study participants during site visits.

6.5 Concomitant Therapy

6.5.1 Allowed Concomitant Medication

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications (if applicable) and supplements (if applicable).

Only the short acting benzodiazepine lorazepam and midazolam administered ≥8 hours before enrollment will be permitted prior to study entry.

6.5.2 Second-line Anticonvulsants

Non-phenobarbital second line anticonvulsants/antiseizure medications are allowed and include:

- Fosphenytoin
- Levetiracetam
- Topiramate
- Clonazepam
- Lidocaine

7 Study Drug Discontinuation and Participant Discontinuation/Withdrawal

7.1 Discontinuation of Study Drug

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

The data to be collected at the time of study drug discontinuation will include the following:

- Vital Signs
- Collection and review of Laboratory Tests (Hematology, Chemistry and Coagulation)
- cvEEG Assessment
- Clinical Seizure Assessment
- Phenobarbital PK Samples
- Recording of Additional Anticonvulsant Medication
- Concomitant Medication Review
- Adverse Events Evaluation

7.2 Participant Discontinuation/Withdrawal from the Study

Parents/Legal Guardians are free to withdraw their study participants in the study at any time upon request.

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

- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the study participant
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Participant unable to receive study drug

The reason for study participant discontinuation or withdrawal from the study will be recorded on the Study Participant Disposition Case Report Form (CRF). Study participants who sign the informed consent form and are randomized but do not receive the study drug may be replaced. Study participants whose parent/legal guardian sign the informed consent form, and are randomized and receive the study drug, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

7.3 Lost to Follow-up

Not applicable

7.4 Study Stopping Rules

The trial DSMB will decide on terminating the trial based on stopping rules defined under Section 7.4.2. Stopping rules will be reviewed along with information regarding conduct and progress of the study, study participant accrual, protocol compliance and other study issues. After each meeting, a recommendation to continue or terminate the study will be provided. DSMB responsibilities, board process, communications and meeting format details are defined in the DSMB Charter.

The study may be prematurely terminated at any time by the Sponsor, IRB and or Investigator in the interest of the study participant safety and welfare. The Sponsor reserves the right to discontinue the trial at any time for any reason.

7.4.1 Stopping rules for study participants

7.4.1.1 Efficacy

Study participants will not be stopped in the trial if seizure cessation is not achieved with one or two doses of phenobarbital. Study participants will be placed on second-line treatments per institutional protocol/standard operating procedures and will continue to be observed. Sponsor is not providing sites with second-line anticonvulsant treatments.

7.4.1.2 Safety

Newborns who will be enrolled in this study are at high risk for several serious medical complications and death related to underlying etiology of their seizures. It is very unlikely that phenobarbital will result in death or any serious adverse event. The following adverse events not described in the investigator brochure for phenobarbital will be considered expected adverse events in this population:

- Clinical or electrographic seizures
- Multiorgan Failure
- Abnormal lab values associated with organ failure
- Death
- Any other adverse events associated with systemic asphyxia and hypoxic-ischemic encephalopathy in newborns

Any adverse events that are expected, not related to phenobarbital, and not severe in nature will be recorded in the database and reviewed at regular meetings of the DSMB. The DSMB will be able to review significant trends of adverse events at their meetings, since the DSMB will be the only party not blinded in this trial. After review of the data, the DSMB will report to NEMA regarding the risks of the study and whether the risks are consistent with what is described in the informed consent form. These reports will be provided by NEMA to the local IRBs for determining continuing review, and to the FDA for annual IND safety reports.

7.4.2 Stopping rules for Overall Trial

The DSMB may recommend stopping the study for the following reasons:

- The data show a clinically important risk of serious adverse effects in one or both of the treatment arms.
- It becomes clear that successful completion of the study is not feasible (e.g. there is an excess of study participant dropout, missing data, lack of recruitment etc).

7.4.2.1 Safety-Continuous Monitoring

Adverse Events will be continuously monitored by the investigator, clinical research associate, CRO Medical Monitor and Sponsor.

If while continuous monitoring the investigator deems an adverse event to be severe, unexpected and possibly related to phenobarbital, the investigator will report the event to NEMA within 24 hours of the event. These unanticipated adverse events will events will also be reported by the PI to the local IRB in accordance with the local IRB policies. NEMA will be responsible for reporting the unanticipated adverse events to all DSMB members within 24 hours of receiving the report, and to the FDA in an IND safety report in accordance with FDA regulations. The DSMB will determine if the adverse event changes the known risk to study participants. If the information changes the known risk to study participants, the DSMB's report regarding the change in risk will be released to all participating investigators by NEMA. The DSMB may request changes to the DSMB Charter and or potentially halt the trial.

7.4.2.2 Safety-Interim Monitoring

The DSMB will evaluate the study after the first 10% and 50% of randomized study participants.

Criteria to be evaluated are outlined in detail in the committee charter.

8 Study Assessments and Procedures

8.1 Efficacy Assessments

8.1.1 Seizure Burden

8.1.1.1 Total Seizure Burden

Total Seizure Burden summarizes the duration of all seizures on the cvEEG recording for each study participant individually and is measured in minutes.

At minimum, the start and end time of each seizure will be recorded in the case report forms.

8.1.1.2 Maximum Seizure Burden

The Maximum Seizure Burden is defined as the maximum hourly seizure burden with the cvEEG recording and is measured in minutes per hour.

8.1.2 Seizure Recurrence

The recurrence of seizures is measured by total seizure burden from the end of the defined response period until 24 h after study drug administration. If the study participant continues in the open-label period, seizure recurrence will be evaluated every 24 hours until discharge. This is contingent on maintenance of therapeutic levels of study drug.

8.1.3 Seizure Period

Seizure period is calculated as the time from the onset of the first electrographic seizure to the offset of the last electrographic seizure.

8.1.4 Seizure Documentation

The start and end time of seizures will be recorded in the case report forms based on the following guidelines:

- All seizures within the first 2 hours from the start of the first dose of study drug.
- If a second dose of study drug is given, start time of seizure that prompted a second dose will be recorded as well as the stop time of the seizure.
- If need to give a second line seizure medication, document start time for the seizure that prompted giving the second line seizure medication as well as the stop time of the seizure.
- If seizures continue, for each additional seizure medication administered, document start time for the seizure that prompted giving the seizure medication as well as the seizure stop times.

8.2 Safety and Other Assessments

8.2.1 Demographics and Medical History

Demographic information to be obtained will include date of birth, sex, Hispanic ethnicity, race.

Medical history to be obtained will include determining whether the study participant has any significant conditions or diseases relevant to the disease under study. If available, family history of seizures and delivery complications should be recorded under medical history. Ongoing conditions are considered concurrent medical conditions. Concurrent medical conditions are those significant ongoing conditions or diseases that are present at signing of informed consent. This includes clinically relevant laboratory or physical examination abnormalities noted at Screening. The condition (i.e., diagnosis) must be described.

8.2.2 Pregnancy and Delivery History

Current delivery information will be recorded for the following:

- Date of delivery
- Delivery presentation

- Occiput posterior
- Occiput anterior
- Brow
- Face
- Compound
- Transverse
- Breech
- Other (please specify)
- Unknown

8.2.3 Medical History of Mother

The medical history of the mother will not be recorded.

8.2.4 Apgar Scores

Apgar is a point system routinely used at birth to assess a neonate's vitality at one, five and ten minutes after birth. The scale ranges between 0 and 10, where a score of 10 indicates a baby in full health. Retrospective apgar assessment scores may be used if assessment can't be performed after time of consent.

8.2.5 Physical Examination

A complete physical examination will be done that is limited to the following body systems: general appearance, head and neck, eyes and ears, nose and throat, chest, lungs, heart, abdomen, extremities and joints, lymph nodes, skin, and neurological. A neurologic exam will also be performed as part of safety evaluation.

Neurological exam to include the examination of the following:

- General Exam
  - Newborn reflexes
  - Posture
  - Alertness
  - Level of activity
  - Jitteriness
  - Benign neonatal sleep myoclonus
  - Saccadic Intrusions
  - Periodic breathing
- Cranial Nerve Exam
  - Pupil reflex
  - Doll's Eyes
  - Corneal, sucking and rooting reflexes
  - Response to noise

- Gag reflex

8.2.6 Vital Signs

Temperature, blood pressure, pulse, respiratory rate and O2 saturation will be recorded. Vital signs will continue to be recorded based on schedule of assessments (Section 1.3).

8.2.7 Clinical Laboratory Determinations

Clinical laboratory tests will be conducted according to the SoA. Clinical laboratory tests to be conducted include complete blood count with differentials, liver panel, basic metabolic panel and coagulation tests (See Appendix 12.4 for list of tests). Clinical laboratory tests will be performed by a designated local or institutional laboratory. PK blood testing will be performed by a Bioanalytical Laboratory. Each site will be provided with instructions on PK specimen collection, preparation, packaging and transport. Refer to the Bioanalytical Laboratory's manual for further information regarding sample collection, handling, and labeling.

Clinical laboratory test data will be reviewed by the PI, or designee, and additional clinical laboratory tests may be ordered at his/her discretion (e.g., if the results of any clinical laboratory test falls outside the reference range or clinical symptoms necessitate additional testing to ensure safety). Any additional testing will be performed by the designated central laboratory.

The PI will review the lab results for clinical significance. Any clinical laboratory test result meeting the PI's or Sponsor's criteria for clinical significance (refer to laboratory manual) will be recorded as an AE or SAE as appropriate.

8.2.7.1 Blood Sampling for Pharmacokinetic Analysis

Phenobarbital in neonates (1-10 days old) have been reported to have a half-life of approximately 114±43 hours.

Collection of plasma samples for phenobarbital analysis will be obtained using a population pharmacokinetic approach at the following time points:

- PK Group 1: 30 minutes, 4-6 hours post initial dose,
- PK Group 2: 30 minutes, 10-12 hours post initial dose,
- PK Group 3: 30 minutes, 16-18 hours post initial dose,
- PK Group 4: 30 minutes, 22-24 hours post initial dose,

At the time of randomization to treatment, the IWRS system will also randomize study participants to the 4 PK groups with a 1:1:1:1 allocation. Collection of blood will also occur after the second dose (if applicable). During the Open Label Extension Period, phenobarbital plasma concentrations will be obtained every 24 hours post initial dose up to 120 hours or at time of early discontinuation.

Total blood volume drawn for each PK time point will be approximately 1 mL.

Total blood volume withdrawn per day for clinical labs and PK should be within institution standard of care. If total maximum daily blood volume will be exceeded, the following priority list should be followed:

1. Standard of Care/Institution's Standard of Care Clinical Labs
2. Study related/requested Clinical Labs
3. PK Blood Samples

If study related blood draws are missed, this will be captured as "minor" protocol deviations.

8.2.8 Continuous Video EEG Monitoring

Continuous video-EEG (cvEEG) monitoring is the gold standard for neonatal seizure detection and quantification and should be used whenever available for seizure detection and differential diagnosis of abnormal appearing, paroxysmal clinical events (25). It is the ideal tool to measure the exact number and duration of seizures, their site(s) of onset and spatial patterns of migration. Multichannel (minimum 8-20 channels) cvEEG monitoring is required for accurate detection of seizures to determine drug response (26).

The following are cvEEG reading **recommendations** during the Screening and Treatment Period for the site neurophysiologist or site designee:

- Review first hour of cvEEG recording as soon as possible.
- If not sure if a pattern is a seizure or study participant does not have 30 seconds/hour seizure burden, review every hour for up to 6 hours followed by every 2 hours for up to 4 hours and then every 4 hours.
- If runs of spike/sharp wave discharges or focal discharges, review every 2 hours for up to 6 hours and then review every 4 hours.
- For any other EEG background/pattern- every 4 hour (if normal not to have to wake up midnight to 6 AM).
- Shortly after reports of suspicious clinical event or 1 hour after start of a therapeutic intervention to evaluate for treatment response (assumes 30-minute infusion).

The following are cvEEG reading recommendations during the Follow-up Period and Open Label Maintenance Period:

- Site is to follow their cvEEG reading standard of practice.

It is also **recommended** that the cvEEG technologist or site designee should remain at the monitor for the first hour of recording to ensure a high-quality recording and to make note of relevant clinical signs. Thereafter, the cvEEG technologist or site designee should re-evaluate the quality of the cvEEG recording frequently and adjust recording leads as required by site's standard of practice. It is also **recommended** that the bedside research staff evaluate the quality of the recording periodically and should contact the technologist or designee if the tracing is suboptimal.

cvEEG recording and monitoring will start at time of informed consent or when study participant is hooked up (whichever is latest) and continue up to 48 hours from start time of first phenobarbital dose. cvEEG recording and monitoring after 48 hours is not required per this protocol and will follow site's standard of practice.

Skin assessments as required by institutional standard operating procedures will be allowed. It is recommended that the amount of time the leads are off the study participant are minimized.

At minimum, the entire EEG recording will need to be backed up and archived for the study. Details regarding data transfer for the cvEEG recordings and video clippings are described in the Operational Procedure Manual.

8.2.9 Clinical Seizure Assessment

Clinical manifestations of seizures will be assessed and reported based on the SoA. Research staff will observe the study participants for abnormal behaviors or movements. Research staff should observe for the following (27):

- Ocular, oral-buccal-lingual, autonomic, apnea, limb posturing and movements
- Repetitive jerking, distinct from jittering (can be unifocal or multifocal)
- Rapid isolated jerks (focal, multifocal or generalized)
- Stiffening and/or decerebrate posturing (focal or generalized)

Seizures should also be categorized as either clonic, tonic, myoclonic or subtle.

8.2.10 Assessment of Adverse Events

Adverse Events that are ongoing at the end of the study will be followed until resolution or for 30 days, whichever is shorter.

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 a drug in humans, whether or not considered drug-related (21 CFR 312.32 (a)).

An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, and does not imply any judgment about causality. An adverse event can arise with any use of the drug (e.g., off-label use, use in combination with another drug) and with any route of administration, formulation, or dose, including an overdose.

8.3.1.1 Suspected Adverse Reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32 (a)).

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, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3.3 Classification of an Adverse Event

For adverse events (AEs) not included in the protocol defined grading system, 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".

8.3.3.1 Relationship to Study Drug

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

- **Definitely Related:** There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal

laboratory test result, occurs in a plausible time relationship to study drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study drug (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory re-challenge procedure if necessary.

- **Probably Related**: There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **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). Although an AE may rate only as "possibly related" soon after discovery, 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, including an abnormal laboratory test result, whose temporal relationship to study drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study drug) and in which other drugs or chemicals 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 drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

As a guide, some questions to ask/think about when addressing drug-related causality may include:

- Is the AE a known reaction of the drug?
- Is the AE similar to other adverse events listed the investigator's brochure or consent documents?
- Has the AE occurred before in this study?
- Is the AE reasonably temporally related to the drug?
- Does the AE improve or disappear when the drug is discontinued? What happens on re-test?
- Was the AE present at the baseline assessment of the study participant or in the study participant's recent medical history?
- Can the AE be reasonably explained by the study participant's clinical disease status?
- Are there any other potential causes for the AE?

8.3.3.2 Expectedness

An unexpected adverse event is one where the nature or intensity is not consistent with the information in the Investigator´s Brochure (for compounds in development) or the USPI (for compounds marketed in the US).

Furthermore, reports which add significant information on specificity or so far unexpected severity of a known, already documented adverse reaction constitute unexpected events. For example, an event more specific or more severe than described in the USPI or Investigator´s Brochure would be considered 'unexpected'.

Expectedness will be assessed by the CRO Medical Monitor and Sponsor.

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

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

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

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

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

PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. A follow-up call at day 7 or day 30 will be conducted to confirm resolution or stabilization of adverse events. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

Any adverse event or abnormal laboratory result considered clinically relevant will be followed until it reaches a satisfactory resolution, becomes stable, or can be explained by other causes (e.g., concurrent condition or medication), and clinical judgment indicates that further evaluation is not warranted. All findings must be reported in the study participant's file.

8.3.5 Adverse Event Reporting

Adverse events reported spontaneously by site staff outside the above indicated time points will also be documented.

The occurrence of all adverse events will be documented in the electronic Case Report Form with the following information where appropriate:

- Nature of adverse event (reported adverse event term)
- When the adverse event first occurred (onset)
- When the adverse event ended and/or how long it persisted
- Whether the adverse event was serious
- Intensity of the adverse event
- Concomitant medications
- Concomitant procedures
- Definite or last observed outcome
- Relationship to IMPs

8.3.6 Serious Adverse Event Reporting

The study clinician will immediately report (within 24 hours) to the CRO any serious adverse event, whether or not considered study drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study drug caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study drug and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the CRO.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site 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)/study Sponsor and should be provided as soon as possible.

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

8.3.7 Reporting Events to Parents/Legal Guardians

Not applicable.

8.3.8 Events of Special Interest

8.3.8.1 Cardiovascular Events

The following events will be considered serious adverse events:

- Systemic Hypertension (must require treatment)
- Major venous or arterial thrombosis (clot)
- Disseminated Intravascular Coagulation (DIC)
- Pulmonary Hypertension
- Intracranial Hemorrhage
- Cardiopulmonary Arrest

8.3.8.2 Laboratory Investigations

The following events will be considered serious adverse events:

- Polycythemia

8.3.8.3 Serious Dermatological Reactions

Serious and sometimes fatal post-marketing cases of dermatologic reactions, including Stevens-Johnson syndrome (SJS) and Toxic Epidermal Necrolysis (TEN) have been reported in patients treated with phenobarbital. Post-marketing reporting rate is generally accepted to be an underestimate due to under-reporting. Recurrence of serious skin reactions following rechallenge with phenobarbital has also been reported. Therefore, if a study participant develops a skin reaction during phenobarbital treatment, consideration should be given to permanent discontinuation and replacement of the drug with alternative treatment.

8.3.9 Reporting of Pregnancy

Not Applicable

8.4 Unanticipated Problems

8.4.1 Definition of Unanticipated Problems (UP)

Sponsor considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the Data Management/lead principal investigator (PI). The UP report will include the following information:

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

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

- UPs that are serious adverse events (SAEs) will be reported to CRO study team within 24 hours
- CRO study team will review and request additional information immediately and report the information to the IRB and to the DSMB/study Sponsor within 24 hours of the CRO study team becoming aware of the event.
- Any other UP will be reported to the IRB and to the DSMB/study Sponsor within 48 hours of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), after the IRB's receipt of the report of the problem from the investigator.

8.4.3 Reporting Unanticipated Problems to Parents/Legal Guardians

Not applicable.

9 Statistical Considerations

9.1 Statistical Hypothesis

This study was designed to show that high dose phenobarbital (40 mg/kg) is more effective than low dose phenobarbital (20 mg/kg) following single dose administration in terms of study's primary endpoint (Section 3.2.1). Specifically, the following primary statistical hypotheses will be tested.

Null hypothesis H_0: there is no difference between treatments in the proportion of participants having a primary efficacy endpoint.

Alternative hypothesis H_a: The proportions of participants having a primary efficacy endpoint are different between the two treatment groups.

9.2 Sample Size Determination

Literature supports that lower loading doses of phenobarbital achieve seizure control in approximately 30 to 60% of treated neonates, with higher loading doses achieving seizure control up to 85% (19, 20, 23, 24). Based on 70% and 50% of study participants achieving seizure control in high dose and low, respectively, an effect size of 20%, an alpha of 0.05 and 80% power, it is estimated that the number of completed study participants required will be 93 study participants in each group (total 186). With approximately, 5% dropout or unevaluable study participant, the minimum required for randomization will be 196.

9.3 Populations For Analyses

1. *Intention-to-treat (ITT) population*: Study participant population consists of all randomized study participants with valid informed consent.
2. *Per-protocol (PP) population:* A Subset of the ITT population. Study participants with major protocol deviations will be excluded from PP population. Major protocol deviations will be defined in the Statistical Analysis Plan (SAP).
3. *Safety population:* A subset of the ITT population, consisting of all randomized study participants who receive at least one dose or partial dose of study drug.
4. *Pharmacokinetic (PK) population:* Study participants that received at least 1 dose from the same study arm (as per randomization or per error) are included in this population group.

9.4 Statistical Analyses

9.4.1 General Approach

Please refer to the SAP for specific details.

9.4.2 Analysis of the Primary Efficacy Endpoint(s)

The primary endpoint will be summarized by number and percent of participants with events and compared by a log binomial model (a generalized linear model [GLM]) that includes treatment as a study variable, which will generate risk ratio (RR) together with their 95% CIs of having a primary outcome between high dose and low dose groups. A study participant who is withdrawn from the study within the first 24 hours after treatment will be considered not to have achieved the primary endpoint. Treatment of such dropouts with respect to other endpoints will be specified in the SAP.

In addition, covariate adjustment will be performed by GLM model with treatment as a study variable and, gender, gestational age, birth weight, apgar scores, liver function tests, seizure etiology, therapeutic hypothermia and pre-treatment seizure burden as covariates. From this

model, adjusted RR together with their 95% confidence interval will be derived. Imputation for these baseline missing covariates will be carried out for adjusted analysis.

9.4.3 Analysis of the Secondary Endpoint(s)

The secondary outcomes will be analyzed similarly as the primary endpoint analysis using a GLM. For GLM analysis of a continuous endpoint such as quality of life, normal distribution and identity link functions will be used; for GLM analysis of a binary outcome (such as having a primary endpoint), binomial distribution and log link functions will be used.

For the analysis of time-to-event outcome, the Kaplan-Meier curves will be presented and compared by the log rank test by treatment group and hazard ratio and its 95% confidence interval will be calculated using Cox regression model with the treatment arm as the study variable.

The secondary binary outcomes with repeated measurements at different time points will be summarized using number (%) of events at each time point and analyzed using a generalized linear mixed model (GLMMIX) model, in which treatment, time, interaction between treatment and time as fixed effects and participant as cluster effect. Exchangeable covariance structure will be used. The odds ratio together with their 95%CIs at each time point will be derived.

9.4.4 Safety Analyses

Safety analysis will be based on the safety population. Assessment of safety will be based on the incidence of TEAE, TEAEs resulting in discontinuation, and serious TEAEs (SAEs). TEAE summaries will be provided showing the number and percentage of study participants who experienced at least 1 AE. These summaries will be presented by body system (system organ class) and preferred term (Medical Dictionary for Regulatory Activities). SAEs, TEAEs, ADRs, and SADRs resulting in discontinuation will be summarized separately. Laboratory data and their change from Baseline will be summarized using descriptive statistics. Vital signs data and any changes from Baseline will be summarized using descriptive statistics. Changes in physical examination will be summarized.

Serious adverse events and serious adverse drug reactions (SADR, serious adverse events at least possibly related to IMP) will be followed up and specifically analyzed (details in protocol/SAP). In case of an ongoing SADR at time of discharge, the follow-up visit should take place at the site.

9.4.5 Baseline Descriptive Statistics

Continuous variables will be summarized according to number of study participants with non-missing data (n), mean, standard deviation (SD), median, minimum, and maximum.

Categorical variables will be summarized according to the absolute frequency and percentage of study participants (%) in each category level. The denominator for the percentages is the number of study participants in the treatment arm with data available, unless noted otherwise.

9.4.6 Planned Interim Analyses

Due to the small sample size of the study population, an interim analysis for efficacy will not be performed.

9.4.7 Subgroup Analyses

Subgroup analysis for the primary outcome will be performed in a series of predefined strata, which will include gender, gestational age, birth weight, Apgar scores, liver function tests, seizure etiology, therapeutic hypothermia, pre-informed consent seizure burden and pre-treatment seizure burden. Assessment of the homogeneity of treatment effect by a subgroup variable will be conducted by a GLM with the treatment, subgroup variable, and their interaction term as predictors, and the *P*-value presented for the interaction term.

9.4.8 Tabulation of Individual Participant Data

Data collected from all randomized study participants will be presented in data listings. Both absolute values and change from baseline values for each study participant will be given where applicable. Data listings will be sorted by treatment, study participant number, and time point.

9.4.9 Exploratory Analyses

Plasma concentrations will be summarized descriptively and graphically by nominal time. Pharmacokinetic parameters will be assessed for calculation. Individual plasma phenobarbital concentrations will be listed and plotted by neonate.

10 Supporting Documentation and Operational Considerations

10.1 Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Informed Consent Process

10.1.1.1 Consent/Assent and Other Informational Documents Provided to Participants

Consent forms describing in detail the study drug, study procedures, and risks are given to the parents / legal guardians and written documentation of informed consent is required prior to starting screening and administering study drug. Electronic consent, if approved by local or central IRBs, will also be acceptable. Verbal consent, if approved by local or central IRBs, can be used to initiate screening process; however, written consent must be obtained prior to drug administration. The following consent materials are submitted with this protocol:

- Informed Consent Form

10.1.1.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the parent/legal guardian will be asked to read and review the document. The investigator will explain the research study to the parent/legal guardian

and answer any questions that may arise. A verbal explanation will be provided in terms suited to the parent/legal guardian's comprehension of the purposes, procedures, and potential risks of the study and of their rights. Parent/legal guardian will have the opportunity to carefully review the written consent form and ask questions prior to signing. The parent/legal guardians should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The parent/legal guardian will sign the informed consent document prior to any procedures being done specifically for the study. Parent/legal guardians must 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 parent/legal guardians 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 study participant undergoes any study-specific procedures. The rights and welfare of the study participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 Study Discontinuation and Closure

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

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

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

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

10.1.3 Confidentiality and Privacy

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

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

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

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

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Data Management 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 sites and by NEMA research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at NEMA Research.

10.1.4 Future Use of Stored Specimens and Data

Data collected for this study will be analyzed and stored at Data Management Facility. After the study is completed, the de-identified, archived data will be transmitted to and stored at the Sponsor's approved location. Information will be included in the informed consent.

With the parents / legal guardians approval and as approved by local Institutional Review Boards (IRBs), de-identified biological samples will be stored at the Bioanalytical Lab. These samples could be used to research the causes of special events of interest, its complications and other conditions for which individuals with special events of interest are at increased risk, and to improve treatment.

During the conduct of the study, an individual parent / guardian 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 Sponsor.

10.1.5 Key Roles and Study Governance

Principal Investigator and Medical Monitor information is available in the Operational Procedure Manual and on the Trial Team Details sheet located in the eTMF.

10.1.6 Site Principal Investigators

Site Principal Investigators will have expertise in treating neonatal seizures and can be a trained neonatologist, pediatric neurologist, pediatric neurophysiologist and/or pediatric epileptologist. Confirmation of training will be based on their current curriculum vitae.

10.1.7 Safety Oversight

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB) composed of individuals with the appropriate 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, unless noted otherwise in the charter. The DMSB 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 CRO and Sponsor. The CRO will report the results to the respective IRB(s).

10.1.8 Clinical Monitoring

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

- Monitoring for this study will be performed by trained NEMA Clinical Research Associates.
- Monitoring will be done on-site and remotely. Frequency of visits will be based on guidelines set forth in the CMP. 100% Source Document Verification will be performed.
- Sponsor will be provided copies of monitoring reports within 30 days of visit.
- Details of clinical site monitoring are documented in a Clinical Monitoring Plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.
- Independent audits will be conducted by NEMA Quality Manager or Designee to ensure monitoring practices are performed consistently across all participating sites and that monitors are following the CMP.

10.1.9 Quality Assurance and Quality Control

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

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

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

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

10.1.10 Data Handling and Record Keeping

10.1.10.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

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

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

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

Details regarding data transfer for the cvEEG recordings and video clippings are described in the Operational Procedure Manual.

10.1.10.2 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study drug. These documents should be retained for a longer period, however, if required by local regulations. No

records will be destroyed without the written consent of the Sponsor, if applicable. It is the responsibility of the Sponsor to inform the investigator when these documents no longer need to be retained.

10.1.11 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 investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

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

It is the responsibility of the PIs and delegated SubIs to use continuous vigilance to identify and report deviations within 2 working days of identification of the protocol deviation, or within 2 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to Clinical Monitor. 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.

10.1.12 Publication and Data Sharing Policy

This study will be conducted in accordance with the Sponsor’s publication and data sharing policies and regulations.

10.1.13 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the Sponsor 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

10.3 Abbreviations

AE	Adverse Event
ANCOVA	Analysis of Covariance
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
cvEEG	Continuous Video Electroencephalography
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GEE	Generalized Estimating Equation
GLM	Generalized Linear Model
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HIE	Hypoxic-Ischemic Encephalopathy
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health

NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PI	Principal Investigator
PP	Per-Protocol
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
eTMF	Electronic Trial Master File
UP	Unanticipated Problem
US	United States

10.4 Protocol Amendment History

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale
1.0	28-Aug-2020	Initial Protocol	
2.0	07-Dec-2021	See Summary of Changes in Protocol Title Page	Administrative changes and changes to assessments to align protocol more with standard of care at individual sites

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12 APPENDICES

12.1 APPENDIX I – Names of Study Personnel

Sponsor:	Hikma Pharmaceuticals.
Medical Monitor:	Sumedha Labhsetwar, MD NEMA Research Inc.
Clinical Research Organizations:	NEMA Research Inc.
Bioanalytical Laboratory:	Axis Bioanalytical

12.2 APPENDIX II – Declaration of Helsinki

https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles

for

Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly

Helsinki, Finland, June 1964

and amended by the

29th WMA General Assembly, Tokyo, Japan, October 1975

35th WMA General Assembly, Venice, Italy, October 1983

41st WMA General Assembly, Hong Kong, September 1989

48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996

52nd WMA General Assembly, Edinburgh, Scotland, October 2000

53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)

55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)

59th WMA General Assembly, Seoul, Republic of Korea, October 2008

64th WMA General Assembly, Fortaleza, Brazil, October 2013

PREAMBLE

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

GENERAL PRINCIPLES

3. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and

standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimizes possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

RISKS, BURDENS AND BENEFITS

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimize the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

VULNERABLE GROUPS AND INDIVIDUALS

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

SCIENTIFIC REQUIREMENTS AND RESEARCH PROTOCOLS

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, Sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

RESEARCH ETHICS COMMITTEES

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the Sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

PRIVACY AND CONFIDENTIALITY

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

INFORMED CONSENT

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

USE OF PLACEBO

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

POST-TRIAL PROVISIONS

34. In advance of a clinical trial, Sponsors, researchers and host country governments should make provisions for post-trial access for all subjects who still need an intervention identified as beneficial in the trial. This information must also be disclosed to subjects during the informed consent process.

RESEARCH REGISTRATION AND PUBLICATION AND DISSEMINATION OF RESULTS

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, Sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

UNPROVEN INTERVENTIONS IN CLINICAL PRACTICE

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

12.3 APPENDIX III List of Medical Concepts for Consideration of Seriousness Stratified by System Organ Class (15-Sep-2009)

Blood and lymphatic system disorders		
Agranulocytosis	Aplastic anaemia	Blast cell proliferation (myeloproliferative and lymphoprolipherative disorders)
Bone marrow depression	Disseminated intravascular coagulation (DIC)	Haemolytic anaemia
Histiocytosis	Loss of anticoagulation control	Pancytopenia
Splenic haemorrhage, infarction or thrombosis	Thrombocytopenia (<30000)	Thrombotic thrombocytopenic purpura
Cardiac disorders		
Angina unstable	Atrial flutter	Atrioventricular block complete
Cardiac arrest	Cardiac failure	Cardiac fibrillation
Cardiac tamponade	Cardiogenic shock	Cardiomyopathy acute
Coronary artery spasm	Cor pulmonale	Myocardial infarction
Torsade de pointes	Ventricular fibrillation	Ventricular tachycardia
Ear and labyrinth disorder		
Deafness	Vestibular ataxia	
Endocrine disorders		
Adrenocortical insufficiency acute		
Eye disorders		
Cataract/lens opacity	Glaucoma	Keratitis/corneal opacification
Macular degeneration	Optic neuropathy, atrophy	Papilloedema
Ptosis	Retinal artery/vein occlusion	Retinitis

Blood and lymphatic system disorders		
Scotoma	Sudden visual loss	Uveitis
Vitreous detachment		
Gastrointestinal disorders		
Colitis haemorrhagic	Gastric ulcer haemorrhage	Gastric ulcer perforation
Haematemesis	Haemoperitoneum	Ileus
Intestinal ischaemia	Intestinal perforation	Melaena
Mesenteric occlusion	Mesenteric vein thrombosis	Pancreatitis
Peritonitis		
General disorders		
Malignant hyperthermia	Drug withdrawal syndrome	
Hepatobiliary disorders		
Hepatic failure	Hepatitis fulminant	Hepatic necrosis
Hepatorenal syndrome	Portal hypertension	Reye's syndrome
Immune system disorders		
Amyloidosis	Anaphylactic reaction	Anaphylactic shock
Graft versus host disease		
Infections and infestations		
Endotoxic shock	Sepsis	Toxic shock syndrome
Transmission of an infectious agent via a medicinal product		
Injury, poisoning and procedural complications		
Transplant failure	Wound dehiscence	
Metabolism and nutrition disorders		
Diabetic coma	Failure to thrive (CTC IV)	Hypercalcaemia (CTC IV)
Hyperkalaemia (CTC IV)	Hypocalcaemia (CTC IV)	Hypokalaemia (CTC IV)

Blood and lymphatic system disorders		
Lactic acidosis	Porphyria	Shock hypoglycaemic
Tetany		
Musculoskeletal, connective tissue and bone disorders		
Aseptic necrosis bone	Fracture pathological	Muscle necrosis
Osteomalacia	Rhabdomyolysis	Systemic lupus erythematous
Systemic sclerosis		
Nervous system disorders		
Amnesia	Anticholinergic syndrome	Aphasia
Cerebral oedema	Chorea	Coma
Convulsions	Demyelination	Encephalitis
Encephalopathy	Epilepsy	Guillain Barré syndrome
Hydrocephalus	Intracranial haemorrhage	Meningitis
Multiple sclerosis	Myasthenia gravis	Myelitis
Neuroleptic malignant syndrome	Opisthotonus	Paralysis
Paresis	Parkinson syndrome	Serotonin syndrome
Stroke	Tunnel vision	
Pregnancy, puerperium and perinatal conditions		
Abortion	Eclampsia	Intra-uterine death
Psychiatric disorders		
Anorexia nervosa	Delirium	Drug abuse
Drug dependence	Homicidal ideation	Intentional misuse
Self-injurious ideation/attempt	Suicidal ideation/attempt	Suicide completed
Renal and urinary disorders		

Blood and lymphatic system disorders		
Anuria	Goodpasture's syndrome	Haemolytic uraemic syndrome
Nephritis/nephritic syndrome	Nephrotic syndrome	Oliguria
Renal failure acute	Renal tubular necrosis	Urinary obstruction/retention
Reproductive system and breast disorders		
Metrorrhagia/uterine haemorrhage	Priapism	
Respiratory, thoracic and mediastinal disorders		
Acute respiratory failure	Adult respiratory distress syndrome	Alveolitis allergic
Asphyxia	Bronchospasm	Laryngeal oedema
Pulmonary fibrosis	Pulmonary haemorrhage	Pulmonary infarction
Pulmonary vasculitis	Respiratory arrest	Status asthmaticus
Pulmonary oedema		
Skin and subcutaneous tissue disorders		
Angioneurotic oedema	Erythema nodosum	Pemphigus
Stevens Johnson syndrome	Toxic epidermal necrolysis	Vascular purpura
Vascular disorders		
Acute circulatory failure	Embolism	Malignant hypertension
Necrosis ischaemic	Thrombosis	

12.4 APPENDIX IV Clinical Laboratory Tests

All samples will be collected in accordance with acceptable laboratory procedures. The maximum volume of blood at any single visit should remain within the following guidelines.

The following chart outlines the guidelines for blood drawing and the max volume which can be safely drawn per day:

Conversions:

lbs/2.2 = kg

kg X 1.7 = mL

Kilograms	(mLs) Volume which can be safely drawn per day
2.7 – 3.6	4.6 – 6.1
4.1-4.6	7.0-7.8
5.0-6.4	8.5-10.8

The following is a list of lab tests collected for each study participant.

Blood	
Comprehensive Metabolic Panel	**Complete Blood Count w/ Differential**
Glucose	WBC Count
Calcium	RBC Count
Sodium	Hemoglobin
Potassium	Hematocrit
CO2/Bicarbonate	RBC indices (MCV, MCH, MCHC, RDW)
Chloride	Reticulocyte count
BUN	Platelet count
Creatinine	
Total Protein	
Albumin	
Bilirubin (total and direct)	
ALP	
AST	
ALT	