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

STATISTICAL ANALYSIS PLAN

Version 1.0

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SAP version history and details of interim/final analyses			
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ABBREVIATIONS

Abbreviation	Explanation
AE	Adverse Event
ATC	Anatomical-Therapeutic-Chemical
CI	Confidence interval
cvEEG	Continuous video EEG
GEE	Generalized estimating equation
GLM	Generalised linear model
HIE	Hypoxic-Ischemic Encephalopathy
IQR	Inter quarter range
ITT	Intent-to-treat
IWRS	Interactive web response system
PK	Pharmacokinetic
PP	Per-protocol
RD	Risk difference
RR	Risk ratio or relative risk
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard deviation
TEAE	Treatment emergence adverse event

1. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to define the outcome variables, statistical methods, and analysis strategies to address the study objectives of the trial: A Randomized, Double-Blind, Controlled Study to Assess the Efficacy and Safety of Intravenous Phenobarbital in Neonatal Seizures (Protocol Version 2.0 and 07- Dec-2021).

The pharmacokinetic analysis is outside the scope of this analysis plan. That analysis will be described in a separate document. Prior to unlocking of the data base a determination will be made if an exploratory pharmacokinetic analysis will be performed based on the total number of plasma samples available.

2. STUDY OBJECTIVES

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).

2.1. Primary Objective

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.

2.2. 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 after the second dose of intravenous phenobarbital.
- To characterize the seizure burden following administration of intravenous phenobarbital.

2.3. 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. STUDY DESIGN

3.1. Design

This is a multicenter, randomized, double-blinded, active-controlled, parallel study in neonates (⩾34 weeks). The study will consist of a Screening Period, Double-Blind Treatment Period, Follow-up Period and an Open-Label Period. This study will consist of a Screening Period, Treatment Period, Follow-up Period and Open Label Extension Period.

3.2. Treatments

Treatment A: Phenobarbital 20 mg/kg (first/initial dose) followed by 20 mg/kg (if required)
Treatment B: Phenobarbital 40 mg/kg (first/initial dose) followed by 10 mg/kg (if required)
The initial dose will be administered intravenously over a 30-minute period.

3.3. Eligibility

3.3.1. 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., Hypoxic-Ischemic Encephalopathy (HIE), stroke, intracerebral hemorrhage, central nervous system infection)
2. Parental informed consent
3. Undergoing cvEEG monitoring
4. Has evidence of electrographic seizure burden of at least 30 seconds/h

3.3.2. 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
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

3.4. Randomisation

Subjects will be assigned unique subject 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. A permuted block randomization list 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 IMP will be dispensed to a given subject for the Treatment Period of the study. The unblinded investigational pharmacist will prepare the IMP and provide to the PI or designee for administration.

3.5. Unblinding

The Investigator may unblind an individual subject'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 subject. 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.

3.6. 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%. Based on an effect size of 20% (from 50% in the low dose group to 70% in the high dose group), an alpha of 0.05 and 80% power, it is estimated that the number of completed subjects required will be 93 subjects in each group (total 186). With approximately, 5% dropout resulting in non-evaluable primary outcome, the minimum required for randomization will be 196.

3.7. Outcomes

3.7.1. Primary outcome

Primary outcome is defined as a binary outcome indicting whether a neonate requires additional seizure treatment after the first dose of phenobarbital during the first 24 hours after treatment (1 for yes and 0 for No), which will be summarized as the percentage of neonates requiring additional seizure treatment.

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

3.7.2. Secondary outcomes

Efficacy

- 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.

Safety

The following safety assessments will be performed during the study:

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

3.7.3 Exploratory Pharmacokinetic Endpoints

A population PK analysis approach 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 (Tmax)
- Observed peak drug concentration (Cmax)
- Area under the plasma concentration-time curve (AUC0- t), the percent AUC extrapolated from AUC0-t to infinity AUCextrap and infinity (AUC0-inf)
- Apparent terminal elimination rate constant (Kel)
- Apparent terminal drug elimination half-life (t1/2)
- Clearance adjusted by absorption, calculated as dose/AUC0-inf (Cl/F)
- Volume of distribution adjusted by absorption, calculated as Dose/Kel•AUC0-inf (Vd/F)

4. ANALYSIS POPULATIONS

The membership of each analysis set will be determined and documented by the Medical Monitor, data manager and the trial statistician, the reasons for exclusion will be given prior to database lock. A summary table will list the individual subjects sorted by treatment group and describe their protocol deviation/violation.

The analysis populations will be listed as follows:

4.1. Intention-to-treat (ITT) population:

Intention-to-treat (ITT) population: Subject population consists of all randomized subjects with valid informed consent.

Analyses for efficacy, tabulations of demographic and baseline characteristics, population disposition and important protocol deviation will utilize this analysis set.

4.2. Per-protocol (PP) population:

A subset of the ITT population. Subjects with major protocol deviations will be excluded from ITT population if they have any of the following major protocol deviations:

- Subjects who did not qualify for randomization but were inadvertently randomised into the study
- Randomized but was given treatment wrongly

This population will be used for the supportive analyses.

4.3. Safety population:

Subject population consist of all randomized subjects who receive at least one dose or partial dose of study drug.

Subjects will be analysed according to the randomised treatment unless a subject has received the incorrect treatment during the entire study period. Analysis for safety endpoints will utilize this analysis set.

4.4. 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.

5. GENERAL CONSIDERATIONS FOR DATA ANALYSES

5.1. Reporting guidelines

We will follow the Consolidated Standards of Reporting Trials (CONSORT) 2010 statement: updated guidelines for reporting parallel group randomised trials (http://www.consort-statement.org/).

5.2. Data Summaries

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

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

5.3. Planned Covariates

Covariate analyses will be performed on the primary outcome on the ITT population. The prespecified covariates in this study will be:

Stratification factors

- Presence or absence of HIE
- High or low seizure burden

Baseline values

- Gender (binary)
- Gestational age (continuous)
- birth weight (continuous)
- Apgar scores (continuous)
- Liver function tests (Total Protein, Albumin, Bilirubin, ALP, AST, ALT) (continuous)
- Seizure etiology (binary)
- pre-treatment seizure burden (continuous)

5.4. Subgroup

Subgroup analysis for primary outcome on the ITT population will be performed in a series of predefined strata, which was defined as below. Imputation for these baseline missing covariates will be carried out before categorizing.

Grouping Variable	Subgroups
Gender	Male or Female

Birth weight	Dichotomised at median
Apgar scores	Dichotomised at median
Liver function tests (Total Protein, Albumin, Bilirubin, ALP, AST, ALT)	Dichotomised at median
Seizure etiology	Yes or No
Therapeutic hypothermia	Yes or No
Pre-treatment seizure burden	Dichotomised at median

5.5. Missing data

5.5.1. Baseline covariates

Missing baseline covariates will be imputed using simple imputation methods in the covariate adjusted analysis based on the covariate distributions. For a continuous variable, missing values will be imputed from random values from a normal distribution with mean and SD calculated from the available sample. For a categorical variable, missing values will be imputed from random values from a uniform distribution with probabilities $P1$, $P2$, …, and Pk from the sample. For a count data, missing values will be imputed from random values from a Poisson distribution with λ from the sample. Seed for the imputation is set as 128.

5.5.2. Primary outcome

Randomised subjects with missing responses will be treated as treatment failures

5.5.3. Other Outcomes (e.g. Safety, secondary outcomes etc.)

No imputation will be performed

5.6. Multiplicity

Analysis for secondary outcome and additional analysis for primary outcome are regarded as exploratory in nature, therefore, multiplicity adjustment will not apply to the primary and secondary outcome analyses.

5.7. Interim Analyses

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

5.8. Software

SAS® (version 9.4) will be used to perform all data analyses and generate the data displays or listing.

6. SUBJECT DISPOSITION, BACKGROUND AND DEMOGRAPHIC CHARACTERISTICS

6.1. Participant disposition and Flow chart

A flow chart will be drawn up showing the number of subjects screened, enrolled, randomised, and followed-up in each study arm, and the number contributing to the ITT, PP and safety analysis.

The number screened and not enrolled and the reasons for non-enrolment will be reported by randomized treatment group and for all randomized subjects combined, along with the number of subjects who were lost for follow up (with reasons), and the number who withdrew for safety reasons, and who crossed over between study arms (if happened).

6.2. Demographics and Other Baseline Characteristics

Demographic and baseline characteristics will be summarized by randomized treatment group and for all randomized subjects combined on ITT population. Data will be presented following the reporting convention above for continuous and/or categorical variables.

Any imbalances between treatment groups for the characteristics will be assessed when reviewing the summary tabulations and any differences deemed clinically relevant to the safety or efficacy comparisons may be investigated by controlling for the characteristic in an exploratory way.

6.3. Medical history

Any condition entered on the relevant medical history / current medical conditions CRF will be coded using the MedDRA dictionary. Medical history is collected at Screening visit. The number and percentage of subjects with each medical condition will be provided by treatment group and overall, and system of organ class for the ITT population.

6.4. Treatment exposure

Average dose and dose level will be summarized by visit and overall study and treatment group (mean, standard deviation, median, minimum and maximum). Average dose will be calculated on the safety population.

6.5. Prior and Concomitant medication

The concomitant medication information will be summarized based on the safety population.

Prior and concomitant medications will be assigned a 12-digit code using most recent version of the World Health Organization drug codes. Prior and concomitant medications will be further coded to the appropriate Anatomical-Therapeutic-Chemical (ATC) code indicating therapeutic classification. The number (%) of subjects who use a drug will be summarized overall and by treatment by all 4 ATC class.
Prior and concomitant medications by drug and drug class will be listed.

7. STATISTICAL HYPOTHESIS, MODEL, AND METHOD OF ANALYSIS

The principle of ITT will be the main strategy of the analysis adopted for the primary outcome and the secondary outcomes. Analysis among PP population will be deemed as supportive evidence. The safety analysis set will be used to analyse safety endpoints.

7.1. Primary Outcome Analysis

7.1.1. Primary analysis

The primary outcome in this trial is whether a neonate requires additional seizure treatment after the first dose of phenobarbital during the first 24 hours after treatment, which will be summarized by percent of neonates who do not require additional seizure treatment after the first dose of phenobarbital during the first 24 hours after treatment. The primary analysis will be based on the ITT as defined above.

The primary endpoint will be summarized by number and percent of participants with events by treatment and overall.

The primary hypothesis to be tested is H_0: $\pi_1 - \pi_2 = 0$ versus H_1: $\pi_1 - \pi_2 \neq 0$, where π_1 and π_2 are rates of primary outcome for high dose and low dose group, respectively. The primary efficacy variable will be analyzed using generalized linear model (GLM) with treatment as a study variable, HIE (presence or absence) and seizure burden (high or low) as covariates. Rate difference (RD) together with its 95% confidence interval (CI) will be derived with binomial distribution and identify link function in the GLM model. The estimated risk ratio/relative risk (RR) and its 95% CI will also be provided with binomial distribution and log link function instead.

The above identity-binomial GLM model may not converge when all covariates are introduced into the model simultaneously. To avoid non-convergence issue, we will first calculate a propensity score with treatment as the dependent variable (1 for treatment A and 0 for treatment B) and all covariates listed above as independent variables through a logistic regression model, and then include the calculated propensity score (continuous variable) as a covariate in the identity-binomial GLM model.

7.1.2. Per-protocol analysis of the primary outcome

A supportive analysis of the primary outcome will also be performed using the same primary analysis model for per-protocol populations.

7.1.3. Covariate adjusted analysis of the primary outcome

Adjusted analyses will be carried out on the analysis of the primary endpoint to determine whether the treatment effect estimate, or standard error is affected with the inclusion of additional covariables. The prespecified covariables that will be included in the adjusted analyses are described in the sections ***5.3 planned covariates.***

The GLM model may not converge when all covariates are introduced into the model simultaneously. To avoid non-convergence issue, we will first calculate a propensity score with treatment as the dependent variable (1 for high dose group and 0 for low dose group) and all covariates listed above as independent variables through a logistic regression model, and then include the calculated propensity score (continuous variable) as a covariate in the GLM model.

7.1.4. Subgroup analysis of the primary outcome

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. In principle, there will be no adjustment for multiple comparisons for subgroup analyses and will be performed for the ITT. GLM model with binomial distribution and log link function will be used in subgroup analysis and derived RR and its 95%CI will be reported.

It should be aware that positive findings from these subgroup analyses have to be interpreted with caution since there is a non-negligible chance of false positives.

7.2. Secondary outcome Analysis

All secondary outcomes will be analyzed as a superiority design and two-sided 95% CIs for the treatment differences in these outcomes between two treatment groups will be calculated and presented. Secondary outcome analyses will be based on the ITT population unless specified.

7.2.1. Analysis of binary outcomes

Binary outcome with single measurement will be summarised by number (%) of participants with event by treatment group and analysed in a similar way as the primary endpoint by means of GLM model. The point estimate of RD and its two-sided 95% CI between high dose and low dose will be estimated. If RD cannot be estimated, RR will be estimated**.**

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 estimating equation (GEE) model, in which treatment, time, interaction between treatment and time as fixed effects, HIE (presence or absence) and seizure burden (high or low) as covariates,

and participant as cluster effect. Exchangeable covariance structure will be used. The RR together with their 95%CIs at each time point will be derived.

7.2.2. Analysis of continuous outcomes

The continuous variables such as biochemical markers will be summarised using number of subjects (n), mean, standard deviation (SD), median (IQR), minimum, and maximum by treatment group, and will be analysed by a GLM model with treatment as a study variable, HIE (presence or absence) and seizure burden (high or low) as covariates, and with normal distribution and identity link function. Differences in mean outcomes with their two-sided 95% CIs between two groups will be derived from the GLM model.

8. SAFETY ANALYSES

8.1. Adverse event

Safety analysis will be based on the safety population. Assessment of safety will be based on the incidence of treatment TEAE, TEAEs resulting in discontinuation, and serious TEAEs (SAEs). TEAE summaries will be provided showing the number and percentage of subjects 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.

8.2. Vital Signs

Vital sign data including blood pressure (BP), heart rate, respiratory rate and body temperature will be listed for individual subjects.

Vital sign data, along with changes from baseline will be summarized separately, overall and by treatment using descriptive statistics. For each vital sign variable, baseline value will be calculated as the last value prior to dosing.

8.3. Laboratory Data

Clinical Laboratory evaluation results will be listed for individual subjects. For all laboratory variables, baseline value will be calculated as last laboratory value prior to dosing.

Change from baseline values at each assessment will be calculated as the assessment value minus the baseline value. The quantitative laboratory data, along with changes from baseline will be summarized using descriptive statistics overall and by treatment.

8.4. Physical Examination

A physical examination will be performed at screening and prior to each study period and will be summarized by treatment.

8.5. Dilution and administration procedure of IV Phenobarbital

Dilution and administration procedure of IV Phenobarbital will be listed for individual subjects.

9. EXPLORATORY PHAMACOKINETIC ANALYSIS

Pharmacokinetic analysis will be described in a separate PK analysis plan. Prior to unlocking of the data base a determination will be made if an exploratory pharmacokinetic analysis will be performed based on the total number of plasma samples available.

10. REFERENCES

1. *Gamble, C et al* Guidelines for the Content of Statistical Analysis Plans in Clinical Trials *JAMA. 2017;318(23):2337-23*