

Keck School of Medicine of **USC**

**Alzheimer's Therapeutic Research Institute**

CONFIDENTIAL DOCUMENT

# Statistical Analysis Plan (SAP) VERSION 2

Efficacy of Intravenous Levetiracetam in Neonatal Seizures  
(NEOLEV2)

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## 1 Introduction

This document is the second version of the Statistical Analysis Plan (SAP) describing the planned analyses and reporting for the NEOLEV2 trial. The SAP was updated due to a change in primary outcome from seizure termination at 48 hours after treatment to seizure termination at 24 hours after treatment. This change was approved by the FDA on September 4, 2018.

The planned analyses identified in this SAP, will be included in future manuscripts. Exploratory analyses not necessarily identified in this SAP may be performed to support planned analyses. Any post-hoc or unplanned analyses not specified in this SAP will be clearly identified as such in the manuscripts for publication.

The following documents were reviewed when preparing this SAP:

- NEOLEV2 Clinical Research Plan (*111361 NEOLEV2 Research Plan Clean 20180413.docx*)
- ATRI Statistical Analysis Plan SOP

Readers of this SAP should read the Clinical Research Plan for details on the conduct of this study and the operational aspects of clinical assessments and timing for completing a patient in this study.

## 2 Abbreviations

ABBREVIATION	DEFINITION
AE	Adverse Event
ATRI	Alzheimer's Therapeutic Research Institute
CRF	Case Report Form
DSMC	Data and Safety Monitoring Committee
LEV	Levetiracetam
PB	Phenobarbital
PK	Pharmacokinetic
PP	Per-Protocol
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOP	Standard Operating Procedure

## 3 Study Design/Summary

### 3.1 Overview

*This section is from the NEOLEV2 Clinical Research Plan (111361 NEOLEV2 Research Plan Clean 20180413.docx).*

This study will be a phase II preliminary efficacy, dose escalation and safety study. The study design will be a randomized blinded controlled treatment study. Infants recognized as having neonatal seizures or as being at risk of developing seizures will be recruited and started on continuous video EEG monitoring. The EEG data will then be reviewed continuously for electrographic seizures by study investigators, along with remote continuous EEG monitoring by Corticare technicians. A novel seizure detection algorithm operating in near-real time will be compared to the real-time continuous EEG reading. Once eligibility and consent are confirmed, patients will be randomly assigned to the LEV or control treatment group in a 60:40 allocation ratio, stratified by site.

Blinding will be maintained by appropriate dilution of LEV and Phenobarbital such that the same volume (ml/kg) loads are given to both treatment groups.

Subjects randomized to receive LEV will, at the onset of electrographically confirmed seizure activity, receive an intravenous loading dose of LEV of 40mg/kg given over 15 minutes. If electrographic seizures are confirmed to persist or recur more than

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15 minutes after the first infusion is complete, a further 20mg/kg load of LEV will be administered IV over 15 minutes. Maintenance LEV at 10 mg/kg/dose will be given IV q8 hours and continued for at least 5 days. If seizures persist or recur more than 15 minutes after the second LEV infusion is complete, a PB loading dose of 20 mg/kg will be administered IV over 15 minutes. If seizures persist or recur more than 15 minutes after the first loading dose of PB is complete, a second 20mg/kg load will be administered IV over 15 minutes. This will result in PB being started within 1 hour of the onset of seizures if and when loading with LEV is ineffective. Patients given any PB loading doses will be started on maintenance PB with 1.5 mg/kg/dose given IV q8 hours and continued at least until the end of the study. Subjects randomized to the control group will, at the onset of electrographically confirmed seizure activity, receive an intravenous loading dose of PB of 20 mg/kg given over 15 minutes. If electrographic seizures are confirmed to persist or recur more than 15 minutes after the first infusion is complete, a further 20mg/kg load of PB will be administered IV over 15 minutes. Maintenance PB at 1.5 mg/kg/dose will be given IV q8 hours and continued for at least 5 days. If seizures persist or recur more than 15 minutes following the second PB infusion, a 40 mg/kg load of LEV will be administered IV over 15 minutes. If seizures persist or recur more than 15 minutes after the first loading dose of LEV is complete, a second 20mg/kg load of LEV will be administered IV over 15 minutes. Patients given an LEV loading doses will be started on maintenance LEV with 10 mg/kg/dose given IV q8 hours and continued at least until the end of the study.

If electrographic seizures are still apparent following treatment with both LEV and PB, or if they recur during the 5 days during which the study protocol is active, the patient will be considered to have failed the experimental treatment regime and institutional specific standard seizure management will dictate the ongoing management. Without unblinding, it will be evident that all subjects will have received 40mg/kg Phenobarbital and 60mg/kg of LEV at this stage.

*For study flow, see appendix.*

### 3.2 Specific Aims

*This section is from the NEOLEV2 Clinical Research Plan (111361 NEOLEV2 Research Plan Clean 20180413.docx)*

- Aim 1: To study the preliminary efficacy of intravenous LEV as first line treatment for neonatal seizures. The primary endpoint will be the percentage of neonates whose seizures are terminated with LEV for 48 hours, and do not go on to require a second anticonvulsant agent. A secondary endpoint will be seizure cessation for 1 hour after treatment with LEV.
- Aim 2: Dose escalation: To determine whether there is additional efficacy at higher doses of LEV than 40mg/kg. The percentage of neonates whose seizures stop following 60mg/kg LEV, having not responded to 40mg/kg LEV, will be calculated.
- Aim 3: To study the pharmacokinetics of intravenous LEV at high dosage. The hypothesis is that the pharmacokinetics of high dose intravenous LEV will follow predictions based upon previous lower dosage PK studies.
- Aim 4: To obtain further safety data regarding the use of high dose Levetiracetam in this population.
- Aim 5: **5A:** To prove the feasibility of centralized remote monitoring of continuous EEG monitoring in the NICU via the internet, thereby developing infrastructure vital to further neonatal seizure research.  
**5B:** To test the sensitivity and specificity of a promising neonatal seizure detection algorithm in comparison with readings of each record by 2 electroencephalographers skilled in neonatal EEG analysis.

**ANALYSIS OF AIMS 5A AND 5B WILL NOT BE A PART OF THE PRIMARY ANALYSIS DUE TO MONITORING CHANGES IN THE STUDY.**

### 3.3 Study Population

#### **Inclusion Criteria:**

Term newborns admitted to the UCSD, Children's Hospital and Research Center Oakland, Auckland Hospital, Rady Children's Hospital, Loma Linda University Medical Center or Sharp Mary Birch NICUs with seizures or at risk for seizures:

- Term infants (gestational age greater than or equal to 36 weeks and less than or equal to 44 weeks).
- Postnatal age < 14 days.

- Serum creatinine equal to or less than 1.6 mg/dL at time of enrollment.
- Are still experiencing either clinical or electroencephalographic seizures or are at risk for neonatal seizures.
- For whom parental consent to participate in the study is obtained.

**Exclusion criteria:**

- Biochemical abnormality - hypoglycemia, hypocalcemia - that when treated result in seizure cessation.
- Severe hypoxic ischemic injury likely to result in imminent death.
- Infants with a birth weight less than 2200 g would be excluded the study. This is the new recommended 10th percentile for 36 weeks' gestation. The blood drawn at any one time will be <3 mls/kg which remains within the NIH guidelines for pediatric blood draws.

Patients of all ethnic groups will be included in the study. Based on the patient population our group treats, most patients will be European or Hispanic. Both male and female infants will be recruited to the study.

The only significant exclusions that will be made in recruitment and enrollment will be the exclusion of infants who are judged by the attending neonatologist to be so critically ill that death is imminent and benefit from neonatal intensive care is very unlikely. No rule-based criteria, (using lab or clinical parameters) adequately capture the complete nature of this clinical assessment. In general any child receiving active treatment with head cooling will not be excluded. Mechanical ventilation and/ or the use of inotropic agents to support blood pressure will not be exclusion criteria.

### 3.4 Power and Sample Size Determination

Power calculations were based on a 2-sided chi-square test for detecting a difference between two proportions, assuming a Type 1 error of 0.05. With a sample size of 60 LEV subjects and 40 PB subject and assuming a seizure cessation rate of 50% in the control arm, we have 80% power to detect a seizure cessation rate as low as 22% (an absolute difference in seizure outcome rates of 28% or greater) in the LEV group.

### 3.5 Treatment Allocation

The two interventions are as follows:

- Group 1 (active): Levetiracetam treatment
- Group 2 (control): Standard treatment with Phenobarbital

### 3.6 Modification of Primary Aim

Aim 1 was changed from seizure termination at 48 hours after treatment to seizure termination at 24 hours after treatment. This change was approved by the FDA on September 4, 2018 (see appendix for e-mail confirmation). The updated aim 1 reads as follows:

Aim 1: To study the preliminary efficacy of intravenous LEV as first line treatment for neonatal seizures. The primary endpoint will be the percentage of neonates whose seizures are terminated with LEV for 24 hours, and do not go on to require a second anticonvulsant agent. A secondary endpoint will be seizure cessation for 48 hours and 1 hour after treatment with LEV.

## 4 Study Outcome Variables

### 4.1 Primary Outcome

- Seizure termination at 24 hours after treatment.

### 4.2 Key Secondary Outcome

- Seizure termination at 48 hours after treatment.

### 4.3 Other Secondary Outcomes

- Seizure termination at 1 hour after treatment.
- Dose escalation - Seizure termination at 24 hours following 60mg/kg LEV, having not responded to 40mg/kg.
- Length of seizure freedom.
- Phenobarbital dose

### 4.4 Safety Outcomes

- Rate of Adverse Events (AEs)
- Rate of Serious Adverse Events (SAE)
- Discontinuation Rate
- Laboratory data
- PK analysis of LEV

## 5 Sequence of Planned Analysis

### 5.1 Interim Analysis

AEs and SAEs are being monitored on a regular basis by a Data and Safety Monitoring Committee (DSMC). No interim futility or efficacy analyses are planned.

### 5.2 Final Analyses and Reporting

All final planned analyses identified in the research plan and in this SAP will be performed only after the last patient has completed assessments scheduled for the length of the study period and the database has been cleaned and locked. Any post-hoc exploratory analyses performed to provide support for planned analyses but not identified in this SAP will be documented and clearly identified as unplanned analysis in the manuscript.

## 6 Statistical Methods

### 6.1 Analysis Populations

- Intent-To-Treat [ITT] population: All randomized participants.
- Per-Protocol (PP) population: Excluding babies not following the protocol.
- Subset Populations: Hypoxic-Ischemic Encephalopathy (HIE); Cooled/not cooled

### 6.2 Multiple Comparisons and Multiplicity

Tests of group differences (active LEV compared to the control PB group) for the primary outcome will be conducted at a two-sided alpha level of 0.05. Similarly, other secondary outcomes will also be conducted using a two-sided alpha level of 0.05. No adjustment for multiple comparisons will be used in this study; however, all results will be reported using point estimates with the corresponding 95% confidence intervals.

### 6.3 Covariates

Covariate adjustment will be specified for each analysis in their respective sections in this SAP, as applicable.

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## 6.4 Data Management and Analysis Software

All analyses will be performed using R statistical software (<http://www.r-project.org>). The R version used will be specified in the analysis reports and will be reported in all manuscripts.

# 7 Statistical Analysis

## 7.1 Study Flow

A CONSORT style flow diagram will illustrate patient progression through the trial from enrollment to completion of the final primary outcome assessment. Number (percentage) will be included for neonates enrolled, randomized, study discontinued (with reasons), analyzed, excluded from analysis (with reasons). The diagram will be separated by treatment groups starting from randomization.

## 7.2 Patient characteristics and baseline variables

Baseline demographics and clinical characteristics will be summarized by frequencies and percentages for categorical variables, continuous variables will be summarized by mean and standard deviation as well as quartiles. Group comparisons will be performed using Wilcoxon-rank sum test for continuous variables. Fisher's Exact test will be used for categorical data. The following baseline demographic and clinical characteristics will be presented:

- Demographics
  - Gender
  - Race
  - Ethnicity
  - Family Medical History of Seizures or Neurological Abnormality
  - Pregnancy (Normal/Abnormal)
  - Delivery Situation
  - Mode of Delivery
  - Did Mother Have Anesthesia?
  - Delivery Presentation
  - Birth Weight
  - Head Circumference at Birth
  - Gestational Age at Birth
- Clinical
  - Cord PH
  - APGAR Scores at 1, 5 and 10 min
  - Seizures etiology

## 7.3 Analysis of Primary and Key Secondary Outcomes

### Primary Hypothesis

Hypothesis: Treatment with LEV improves the rate of seizure termination at 24 hours in neonates compared with standard PB treatment.

Analysis Plan: A Fisher's exact test will be used to test the 24 hours seizure termination rate between the two treatment groups. As a secondary analysis, multivariate logistic regression (if there are sufficient number of events), will be performed to study the association between termination rates and treatment. Pre-specified covariates, including seizure etiology (HIE vs Other)

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and hypothermia treatment (yes, no), will be included in the multivariable model as observed confounders.

### Key Secondary Hypothesis

Hypothesis: Treatment with LEV improves the rate of seizure cessation at 48 hours in neonates compared with standard PB treatment.

Analysis Plan: A similar approach as used for the primary outcome will be utilized to analyze the 48 hours seizure cessation rates.

## 7.4 Analysis of Remaining Secondary Outcomes

- Hypothesis 1: Treatment with LEV improves the rate of seizure cessation at 1 hour in neonates compared with standard PB treatment.

Analysis Plan: A similar approach as used for the primary outcome will be utilized to analyze the 1-hour seizure cessation rates.

- Hypothesis 2: Length of seizure freedom will be longer in the LEV group than in the PB group.

Analysis Plan: Actual length of seizure freedom will be summarized by mean and standard deviation as well as quartiles. Comparison between randomization groups will be performed using Wilcoxon-rank sum test.

- Hypothesis 3: Additional LEV treatment in neonates who didn't respond to 40mg/kg LEV will be effective (additional seizure terminations).

Analysis Plan: The percentage of neonates whose seizures stop following 60mg/kg LEV, having not responded to 40mg/kg LEV will be calculated with the corresponding 95% confidence interval.

- Hypothesis 4: LEV treatment will result in a lower dose of phenobarbital being needed as compared to the PB treatment alone.

Analysis Plan: Phenobarbital dose will be summarized by mean and standard deviation as well as quartiles. Comparison between randomization groups will be performed using Wilcoxon-rank sum test.

## 7.5 Analysis of Safety Outcomes

Adverse Event and Serious Adverse Events will be tabulated by randomization group and by drugs received, respectively. Details including seriousness, intensity, causality, withdrawal due to event and outcome will be summarized by frequencies and percentages by randomization group and by drugs received. Fisher's exact tests will be used to compare rates of adverse events, serious adverse events, study discontinuation and deaths between randomization group and by drugs received, respectively.

Findings will be tabulated by randomization group overall and for findings marked as AE and SAE, respectively.

Safety Labs (using DAIDS guidelines) will be presented with shift tables (hour 48 vs baseline) by randomization group and by drugs received, respectively.

## 7.6 Pharmacokinetic Analysis

**These analyses will be performed by Dr. Edmund Capparelli at UC San Diego. The below analysis plan is a preliminary plan as stated in the study research plan, a more detailed analysis plan will be provided by Dr. Capparelli at the time of analysis.**

The actual individual concentration data, collection times and dosing histories will be used to create graphs of plasma LEV concentration vs. time for each infant. Median plasma drug concentration vs. time curves will also be generated. Summary statistics (i.e., n, mean, standard deviation, median, minimum, maximum, and coefficient of variation) will be calculated for

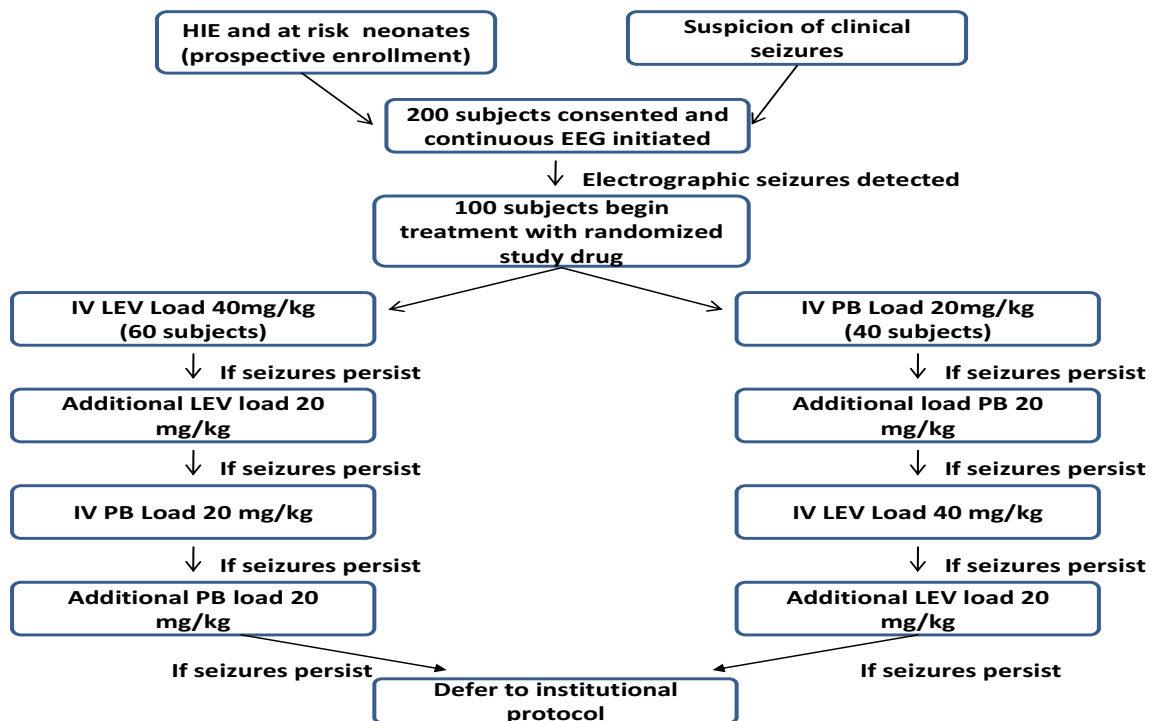
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plasma concentrations for each time point and each dose level. LEV population pharmacokinetic parameters will be determined using the computer program NONMEM (ver. 6 or later). The data will be nested with existing raw infant LEV pharmacokinetic data generated in the preliminary LEV study. Assuming linear pharmacokinetics as suggested by our current pharmacokinetic data, studying 12 subjects at each dosage level will result in 90% confidence intervals for the pharmacokinetic parameters (CL, V and  $t_{1/2}$ ) of  $\pm 15\%$ . The 90% confidence intervals for the exposure parameters ( $C_{max}$ , AUC) will be approximately  $\pm 20\%$ . The pharmacokinetics will be modeled recognizing the two stages of a nonlinear hierarchical model development. The first stage introduces the structural model (e.g. one compartment open model), the population parameters, individual effects and the within-patient variation. The second portion of the model development recognizes that variation between patients in the pharmacokinetic parameters exists and will attempt to determine covariates that may identify different pharmacokinetic subpopulations. A one-compartment model with first order elimination will be utilized. Alternative structural models will be explored if indicated by the data. The impact of clinical factors and demographic factors (including weight, sex, age, race, serum creatinine, hypothermia and dosing regimen) will be assessed as fixed effects in the model. Nested models will be compared graphically and with a likelihood ratio test.

## 8 Appendix

### 8.1 Study Flow Chart



## 8.2 Approval

### APPROVERS

Approved by	Title	Signature	Date
Richard Haas, M.D.	Principal investigator, UCSD	<i>Richard H. Haas</i>	9/14/2018
Rema Raman, PhD	Lead Biostatistician, ATRI	<i>Rema Raman</i>	9/14/2018

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