University of California, San Diego (UCSD) and Children's Hospital and Health Center (CHHC)
IRB Protocol Application
RESEARCH PLAN

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Version date: 4/04

1. PROJECT TITLE:

Efficacy of Intravenous Levetiracetam in the Treatment of Neonatal Seizures:
A Phase 2 Study of Levetiracetam in the Treatment of Neonatal Seizures

2. PRINCIPAL INVESTIGATOR:

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3. FACILITIES:

UCSD Medical Center Neonatal Intensive Care Unit
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Auckland Hospital, Auckland, New Zealand Neonatal Intensive Care Unit
Children's Hospital and Research Center Oakland Neonatal Intensive Care Unit

4. ESTIMATED DURATION OF THE STUDY:

Estimated total duration of the study will be 4 years. At all sites 50 total subjects will be recruited each year of which 25 are estimated to qualify for treatment. The total number of treated subjects at all 5 sites will be 100 over 4 years. Each subject will be treated with the study drug for a total of 5 days.

5. SPECIFIC AIMS:

Specific 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.
Hypothesis: LEV has efficacy for the treatment of neonatal seizures.

Specific 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.

Specific 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.

Specific aim 4:
To obtain further safety data regarding the use of high dose Levetiracetam in this population.

Specific aim 5:
5 A: 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.

Hypothesis: Remote video continuous EEG monitoring is feasible, cost effective, and will allow rapid treatment decisions necessary for future trials of anticonvulsant efficacy in neonates.

5 B: To test the sensitivity and specificity of a promising neonatal seizure detection algorithm in comparison with readings of each record by 2 encephalographers skilled in neonatal EEG analysis.
Hypothesis: A novel seizure detection algorithm will have a high sensitivity and low false positive detection rate, which will allow use in future clinical trials of neonatal seizure management.

6. BACKGROUND AND SIGNIFICANCE:

There are no FDA approved treatments for neonatal seizures (seizures occurring in the first 28 days of life). More effective and less toxic treatments are urgently required. While appropriately holding orphan drug status, neonatal seizures are a common problem within the field of child neurology and cause significant morbidity. Current standard of care treatments Phenobarbital and phenytoin are unsatisfactory[1], each agent is effective in less than 50% of subjects, and both have significant acute and chronic side effects. In animal studies these agents cause accelerated neuronal apoptosis in the neonatal brain. Better treatments could limit brain damage and improve neurodevelopmental outcomes in this high risk population. Levetiracetam (LEV) has great potential as a treatment for neonatal seizures, but is not approved for use in children less than 2 years of age. Given the orphan nature of neonatal seizures and the fact that LEV is out of patent, the pharmaceutical company that manufactures LEV will not seek market approval in this unprofitable age group. LEV has established efficacy and an excellent safety profile in older patients. In animal models this agent does not cause neuronal apoptosis and is neuroprotective. However, the efficacy of LEV has not been systematically studied in neonates and cannot be assumed. Drugs that are effective in terminating seizures in adults may be less effective and have more toxicity in neonates. This study aims to obtain essential data regarding the efficacy and safety of LEV in this vulnerable and under researched population.

Efforts to develop better treatments for neonatal seizures are confounded by the need in any drug trial for systematic continuous EEG monitoring, something that is not yet standard of care because of resource constraints. The proposed project would add to knowledge regarding the efficacy of LEV in neonates, while simultaneously addressing the need to find feasible solutions to the difficulties of providing continuous EEG monitoring through new technology.

Should LEV prove an effective medication in this age group, clinical practice would be dramatically improved. We would have a safe, non-toxic treatment option for neonatal seizures with proven efficacy.
Developing better systems for neonatal seizure detection will facilitate earlier treatment of neonatal seizures and enable further clinical research in this condition.

Prevalence of Neonatal Seizures:
Measures of the incidence of neonatal seizures vary from 1/1000 live births to 3.5/1000 live births. The most recent US study, in Harris County Texas from 1992 to1994 concluded that the incidence was 1.8/1000 live births [2].The National Center for Health Statistics reported in 2009 that there were 4.3 million live births in 2007 [3]. Using the survey data from Harris County, this translates to an incidence of 7740 babies in the US. Estimating the prevalence assuming an average 3-day duration of seizures, 63 neonates experience clinical seizures on any particular day in the US. The true incidence and prevalence figures are in fact likely double these numbers as a number of studies have noted that electrographic seizures without clinical manifestations account for 50% or more of neonatal seizure activity [4, 5], raising the prevalence of neonatal seizures of all types to 126/ day in the US.

Neonatal Seizures cause Brain Injury:
The outcome of neonatal seizures is poor which differs markedly from seizures occurring in older age groups. 20-30% of infants with neonatal seizures die, 20-30% develop epilepsy outside the neonatal period, and 20-

40% develop cerebral palsy and/or mental retardation[6].The most recent prospective long term follow-up of clinical neonatal seizures in the province of Newfoundland reported on all live newborns born between 1990 and 1995. Data was available on 82 of 90 children. In 28 term babies only 45% were normal, 16% died, and 32% were impaired [6]. Whilst much of the mortality and morbidity in neonates with seizures can be attributed to the underlying condition, there is mounting evidence that seizures themselves are harmful, especially in the asphyxiated neonatal brain. Studies in rats show that seizures can damage the developing brain[7, 8]. McBride et al have demonstrated a strong correlation between the amount of electrographic seizure activity and subsequent morbidity and mortality[9]. Preliminary data also demonstrates an improved outcome with prompt and complete cessation of seizures[4] and in a recent European controlled trial, treatment of subclinical seizures showed a trend for reduction in seizure duration which was associated with less brain injury on MRI [10]. Brain MR spectroscopy in neonates suffering seizures secondary to perinatal asphyxia shows brain injury as a consequence of the seizures[11].Recent evidence suggests that neonatal seizures independently contribute to poor outcome at 4 years of age in neonatal hypoxic-ischemic injury[12].

Current AEDs for Neonatal Seizures:

Current treatment relies on medications in use since 1914 (Phenobarbital) and 1938 (phenytoin). These agents are neither effective nor safe. Treatment with Phenobarbital or phenytoin produces cessation of neonatal seizures in less than 50% of infants treated[1]. Acute side effects of phenobarbital and phenytoin include hypotension, suppression of respiratory drive, cardiac arrhythmia and sedation. Chronic exposure to phenobarbital may be associated with decreased cognitive ability [13-15].

Neonatal response to AEDs is different to that of adults:

The neonatal response to AEDs is fundamentally different to that of the adult brain[5, 16, 17]. Neonatal seizures form a unique subset of pediatric seizures for a number of reasons:

a) The developing neonatal brain is different to that of older children and adults. These seizures are occurring at a unique period of vulnerability to the developing brain. GABA is the main inhibitory neurotransmitter in the brain, in the developing brain however, activation of chloride-permeable $GABA_A$-R receptors excite neurons instead of inhibiting them as occurs later in life. This occurs because of high intracellular chloride levels caused by the NKCC1 transporter [16]. Drugs that act via the GABA receptor are therefore rendered less effective in neonates.

In addition, there are differences in cortical versus subcortical GABAergic signalling leading to the phenomenon of uncoupling of clinical and electrographic seizure activity induced by Phenobarbital treatment which is unique to this age group [5, 17]. Phenobarbital, when administered, will act at subcortical sites to eliminate all convulsive movements that give clinical indication of seizure activity, however electrographic monitoring, (if available) demonstrates ongoing cortical seizure activity.

b) There is significant concern that traditional anticonvulsants may be damaging to the neonatal brain. Anticonvulsants can cause 'interference with cell proliferation and migration, axonal arborization, synaptogenesis, synaptic plasticity, and physiological apoptotic cell death'[18].In neonatal rat models traditional anticonvulsants- including phenobarbital and phenytoin and most of the newer agents- trigger apoptotic neuronal cell loss [19]. Levetiracetam does not cause this effect [20].

c) Drug handling in neonates is very different to older populations because of liver and renal immaturity[21].

d) Neonatal seizures have a different set of etiologies when compared to seizures occurring in older children. Fifty percent follow hypoxic ischemic injury in the perinatal period and worsen the prognosis. Other causes include neonatal stroke, brain developmental malformations, perinatal infections (often unique to the newborn), hypoglycemia, hypocalcemia, hypomagnesemia and rare inborn errors of metabolism presenting in the newborn period[22]. These etiologies predispose the neonate to severe seizures; frequently status epilepticus [23], and at the same time an increased susceptibility to brain injury secondary to seizure activity.

Barriers to Detection of Neonatal Seizures:

Because neonatal seizures are very frequently entirely subclinical it is accepted that continuous EEG monitoring is the only way to adequately detect neonatal seizures and the only objective means to judge the

response to AED treatment [5, 24, 25]Murray et al. reported in a video EEG study that only 34% of electrographic seizure events in encephalopathic newborns are clinically identified and only 9% were recognized by nurses at the bedside [26]. Lawrence et al. noted that more than 90% of EEG seizures had no clinical correlate [27].Despite this, continuous EEG monitoring is not standard of care for neonates at risk of seizures in the majority of NICUs in the United States and internationally. This is due largely to the lack of available resources to conduct and review continuous EEG monitoring. Conventional full neonatal montage EEG requires specialist review. In each hospital center there are insufficient neurologists to provide a service of real time review of the data 24 hours a day.

Recent EEG data software developments now allow for remote review of full montage continuous EEG data securely via the internet. This opens the door to the development of a centralized system for review of continuous neonatal EEG data by neurologists.

There would be great value in a reliable automated seizure detection method but such algorithms are not in widespread use for real time neonatal seizure detection. Automated detection of electrographic seizures in the newborn requires different techniques from those employed in adults and must be developed and tested specifically in neonates. Existing algorithms have been shown to have sensitivities of between 43 % to 63% and specificities between 56% to 90%, not high enough for use in a clinical environment [27, 28].

7. PROGRESS REPORT/PRELIMINARY STUDIES:

Preliminary Validation data for Automated seizure detection algorithm

In testing against a large dataset of 114 hours of EEG from 87 neonates, including 389 seizures from 55 seizure-affected neonates, the August 2010 alpha version of Persyst's neonatal seizure detector demonstrated an overall seizure detection sensitivity of 79%, a seizure-affected patient detection rate of 98% (i.e., at least one seizure correctly detected in an infant who is experiencing seizures), and a false positive rate of approximately 1 detection per 11 hours monitoring.

Neonatal Pharmacokinetic Study: A pilot PK, safety and efficacy study of the use of LEV has recently been completed by Dr. Haas' group, funded by the Thrasher foundation.

Study design: The trial was an add-on, open label pharmacokinetic and preliminary safety study. Between August 2007 and February 2009, eligible neonates admitted in 3 participating neonatal intensive care units were recruited to this study. The sites were the University of California San Diego Medical Center, Sharp Mary Birch Hospital San Diego, and Auckland City Hospital, Auckland New Zealand. The institutional review board at each center approved the protocol and informed consent was obtained from the parents in each case. The study was registered with clinical trials registry NCT00461409.

Study entry criteria: Subjects were inpatients in the three participating neonatal intensive care units. To be eligible for the study subjects had to be have a corrected gestational age between 37 weeks and 44 weeks and weight of at least 2440 grams. Subjects had to be experiencing clinical or electrographic seizures which persisted after receiving a 20mg/kg loading dose of Phenobarbital in order to receive the study drug. Subjects were excluded from the study if they had a serum creatinine of greater than 1.2 mg/dL at the time of enrolment, if they were anuric, or if seizures were due to a biochemical abnormality such as hypoglycaemia or hypocalcemia which once rectified resulted in seizure cessation. Patients were also excluded from this study if death of the patient seemed imminent.

Intervention: Patients were recruited in two ways. Patients recognized to be at high risk of developing neonatal seizures, for example neonates with hypoxic ischemic encephalopathy, were recruited prospectively. Other subjects were recruited at the time when they presented with seizure activity. Following recruitment and consent, patients were monitored electrographically to ascertain whether seizure activity occurred, and if so, if it persisted following administration of IV Phenobarbital.

If there was ongoing definite clinical seizure activity or subclinical seizure activity detected by the continuous EEG monitoring 30 minutes after receiving Phenobarbital, an IV LEV loading dose was administered over 15 minutes. LEV maintenance dosing was given starting 12 hours after the initial infusion and continued every 24 hours for a total of 1 week.

The first cohort of babies was treated with a 20mg/kg initial load followed by 5mg/kg/day as a single daily dose. Following planned interim analysis of the first cohort, the dose was escalated. The second cohort received 40 mg/kg as a load followed by 10mg/kg/day as a single daily dose.
(These doses were chosen with the expectation that the dosing regimen in the second cohort would produce steady-state trough concentrations in the range typically seen with therapeutic doses in adults (~35-120μM or 6-20mcg/mL). LEV distribution is characterized by low protein binding and a volume of distribution that approaches total body water (0.7L/kg). Given the high total body water content in infants, it was expected that infant LEV volume of distribution would be slightly larger than in adults. Additionally, based on immature glomerular filtration in neonates and resulting renal function only 20% that of older children, LEV clearance in infants was expected to be between 15-45% of older population with the degree of hydrolysis present in neonates an important unknown variable.
Serial determinations of LEV concentrations were performed to enable pharmacokinetic analyses. Blood samples were collected at 10 time points during the week of treatment to measure peak and trough serum concentrations of LEV and its major metabolite UCB L057. Urine was collected for the first 36 hours after LEV was commenced. Serum and urine LEV and UCB L057 concentrations were measured by LC/MS/MS method [62].

Pharmacokinetic analysis was performed using the computer program NONMEM ver. 6.2. A one-compartment model was utilized and a population pharmacokinetic model was developed that included post-natal age as a covariate on CL. Empiric Bayesian estimates of individual subjects pharmacokinetic parameters were generated using the priors of the population pharmacokinetic model
During the treatment phase of the study each patient was clinically reviewed on a daily basis. Follow-up continued by phone review at 3 days and 6 days after intravenous administration of the study drug was completed. A follow-up visit was conducted 1 to 2 weeks after completion of the treatment phase of the study. Safety monitoring included measurement of CBC, serum creatinine and electrolytes and liver enzymes at baseline, between 48 hours and 72 hours of treatment and at completion of 7 days of treatment.

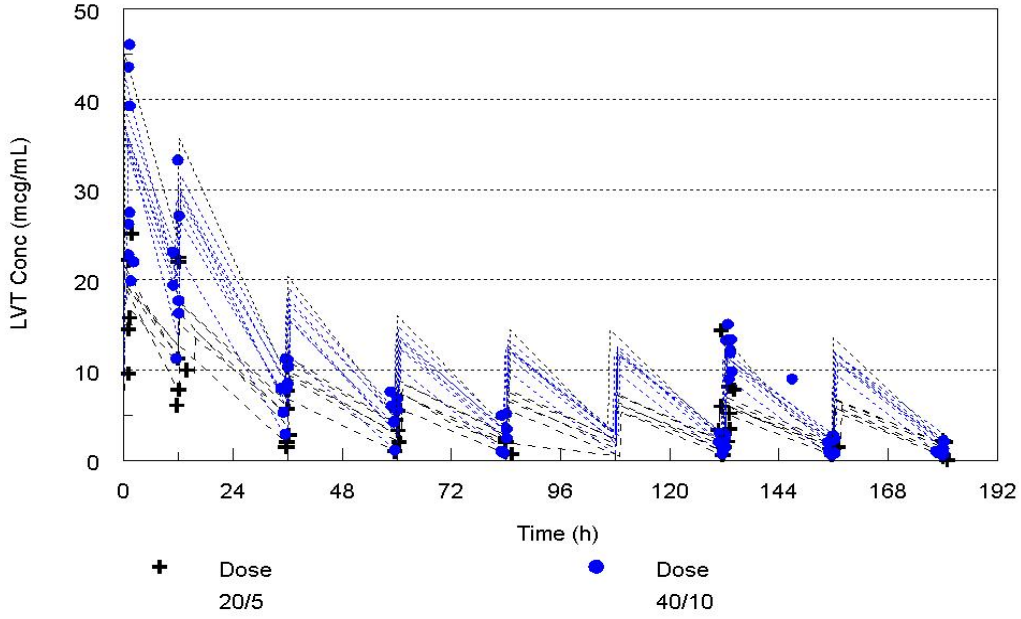
Patient baseline characteristics: Eighteen patients received treatment with intravenous LEV, 6 at the first dosing level and 12 at the higher dose. There were 9 male and 9 female patients. The weight of subjects ranged between 2.5 and 4.7 kg. All subjects had a gestational age between 37 and 41 weeks. In 8 subjects the underlying etiology of seizures was hypoxic ischemic encephalopathy. 5 of these subjects received hypothermia treatment during the study period. 2 subjects were found to have ischemic stroke, 2 subjects had intraventricular hemorrhage.1 subject had a complex brain malformation with polymicrogyria, hypoplastic brainstem and cerebellar vermis with a Dandy Walker malformation. In 4 subjects the etiology of seizures was unknown despite investigation with brain MRI, sepsis screen, lumbar puncture and extensive metabolic investigations.

Table 1

	Mean	SD	Median	Min	Max
Vd (L/kg)	1.01	0.13	0.98	0.81	1.24
CL Day 1 (L/h/kg)	0.042	0.016	0.039	0.023	0.085
CL Last Day (L/h/kg)	0.080	0.021	0.080	0.053	0.142
T1/2 Day 1 (h)	18.5	7.1	15.6	8.8	32.7
T1/2 Last Day (h)	9.1	2.0	9.0	5.3	12.7

Pharmacokinetic results:
The final PK data set contained 149 observations from the 18 subjects. Urine collections were completed in 18 subjects. Table 1 summarizes the PK parameters calculated and Figure 1 shows the raw pharmacokinetic data.

Figure 1.



Adverse events and safety monitoring: Patients were reviewed daily to evaluate for possible adverse clinical events related to LEV treatment. LEV was well tolerated. Only one serious adverse event occurred in this study. One subject with a brain malformation and a high Phenobarbital level required intubation while on treatment with LEV. LEV was not thought to be causal of this event.
There were a few mild adverse events identified that were possibly related to treatment with LEV: mild sedation was reported in 2 subjects. In both cases this resolved spontaneously. Feeding difficulty was documented in 3 subjects. In all cases this difficulty was probably not due to LEV treatment and in all cases feeding difficulty resolved. One subject had mild apnea and bradycardia which resolved. This was probably not related to LEV treatment. Decreased urine output occurred in 1 subject with HIE. This resolved with treatment with frusemide. All blood test abnormalities were reviewed by an independent data safety monitoring board. Mild abnormalities in blood count and serum chemistries were seen, consistent with what would have been expected in this patient population of sick neonates. No serious or consistent treatment- emergent lab abnormalities were observed.
A recent PK study of neonatal LEV treatment by Merhar et al. was based on 3 data point collections over 24 hours in 18 infants ranging from day of life 1 to 32 [67]. These authors reported a half-life median of 8.9 hours (range 3.2-13.3). This finding is similar to our finding of mean t½ of 9.1 (SD 2.0) at 7 days of age [Table 1]. This study adds to the available safety data as no side effects other than sedation in one infant were noted.

Efficacy in seizure cessation- preliminary analysis: Analysis of EEG data from this study demonstrating the effect of LEV on neonatal seizures is underway. As a simple measure of efficacy, 6 of the 18 subjects studied in this trial required no additional AEDs after LEV was commenced. 5 of the responders were amongst

the 12 subjects who received the higher dose of LEV (42%). There was only 1 responder among the 6 subjects receiving the lower LEV dose. (An additional 3 subjects had an initial response to LEV; with temporary cessation of seizure activity as the loading dose was given, but later had recurrence of seizures).
It should be remembered that this was the response rate to LEV used as a *second* line agent in subjects refractory to PB as a first line agent. As such this response rate of 42% seen in the higher dose cohort compares favorably with efficacy data for phenytoin and Phenobarbital when used as second line agents. Painter et al. found that of 4 of 17 (24%) neonates with seizures refractory to Phenobarbital responded to the phenytoin as the next AED, and 5 of 16 (31%) neonates with seizures refractory to phenytoin responded to Phenobarbital as the next AED [63].
Two recent publications document retrospectively the response of neonatal seizures to IV LEV. Abend at al. reported that 23 neonates treated with LEV at a low mean initial dosage of 16 mg/kg had a>50% seizure reduction in 35% of infants with seizure termination in 7 of 23 (30%) [68]. No respiratory or cardiovascular effects were detected. Khan et al. reported that 22 neonates received an LEV load of 10-50 mg/kg and 22 (86% achieved immediate seizure cessation at 1 hour. No serious side effects were evident; one infant became irritable on treatment responding to 50mg of pyridoxine [69].

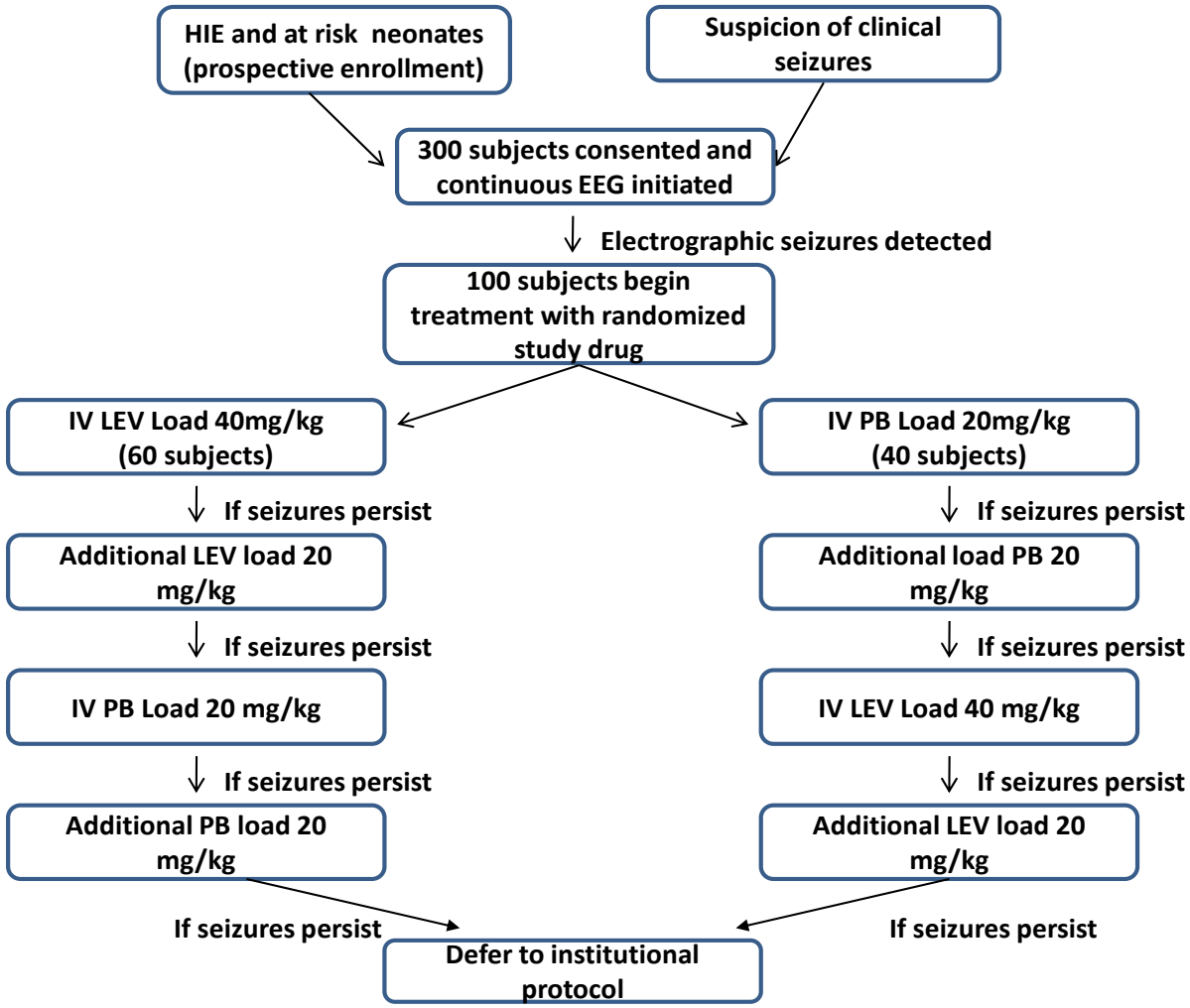
8. RESEARCH DESIGN AND METHODS: (guideline length is 3-6 pages)

Study Design Summary:
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 randomised 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 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

Figure 2 details the study flow.



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.

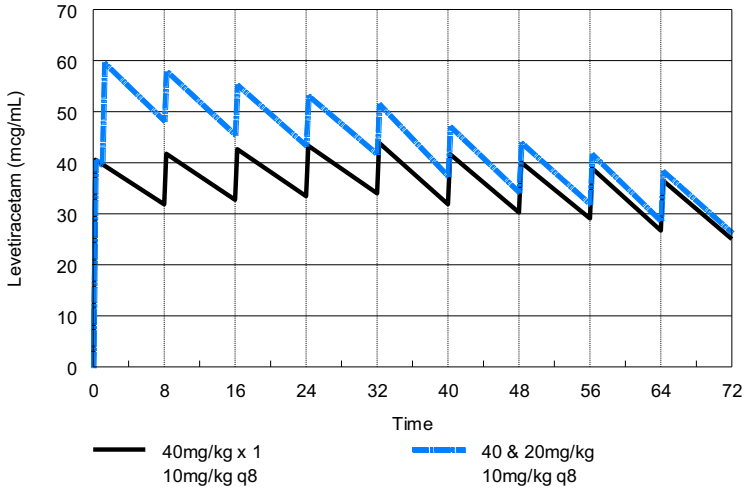


Figure 3 Predicted blood levels at 40 mg/kg and 60 mg/kg loads with 10 mg/kg 8 hourly maintenance

Clinical monitoring and specific laboratory data will be collected at standardized times following study drug administration to determine the pharmacokinetics and safety of LEV on 12 subjects at the 40 mg/kg dose and 12 at the 60 mg/kg dose (see Table 2). The PK studies will be performed only at the San Diego and Auckland sites. Investigators will remain blinded to PB level.

Table 2	CBC diff	Chem Panel, LFTs	LEV level	Phenobarbital Level
Baseline	X	X	X	X
1 hour			X	X
24 hours			X	X
48 hours	X	X	X	X

Study Inclusion and Exclusion criteria: To be eligible for recruitment, subjects will be term infants with corrected gestational age between 36 and 44 weeks, and weight of at least 2.2 kg. Subjects will be excluded if they have received anticonvulsants within 24 hours of enrolling in the study, if serum creatinine is greater than 1.6 mg/dL or if seizures are due to correctable metabolic abnormalities (i.e.hypoglycaemia, hypocalcemia, hyponatremia). Subjects in whom death seems imminent, as assessed by the neonatologist, will also be excluded from the study.

Recruitment: There are 2 groups of babies from which subjects may be recruited-
1. A prospective group, where it can be anticipated that seizures are at high risk of occurring, for example in neonates with HIE. Consent will be obtained prospectively and continuous EEG monitoring will commence.
2. Acutely presenting group, neonates that present with symptoms indicative of or suggestive of seizures. It is recognised that it will not be possible to recruit all patients in this acutely presenting group. Efforts to obtain consent can not be allowed to delay management. However, in many cases there will be opportunity to ethically recruit and consent subjects without delaying care. For example, in cases where clinical seizures are brief and have stopped spontaneously, and in cases where there is suspicion of seizure activity but EEG confirmation is needed before AEDs are indicated.

Expected enrollment: We plan to treat 100 subjects over 4 years and based on our prior PK study experience we anticipate needing to recruit, consent and monitor 300 subjects to yield this number, (a conservative estimate). Randomization will be carried out with a six to four ratio so that 60 subjects will receive treatment with LEV and 40 will receive standard treatment with Phenobarbital treatment. Each site will treat 4 to 6 subjects with seizures each year, recruiting and monitoring up to 13 infants per year in order to yield these numbers. Historical data from the past 3 years in each of four neonatal intensive care units indicate that we will be able to recruit these numbers of patients easily: Rady Children's Hospital have treated on average 39 term infants with seizures per year over the past 3 years, Auckland Hospital NICU have treated on average 12 per year, Oakland Children's Hospital have treated 6-10 per year. In our prior PK study we recruited 36 subjects with neonatal seizures in 18 months, (18 of whom were refractory to PB and therefore received study drug).

Data to be collected:

Clinical Data: Demographic and clinical data will be collected, including birth weight, gestational age at birth, postnatal age, gender, pregnancy history, family medical history, mode of delivery, Apgar scores, and cord blood gas if available. Etiology of seizures will be recorded. Where etiology is unknown, results of neuroimaging, blood, urine metabolic and CSF studies will be reviewed. A record of all medications received by the infant will be kept. Case report forms will be developed to facilitate data entry from all sites into the REDcap data management system available to us through the UCSD Clinical and Translational Institute (CTRI).

Feasibility data: We will record the times of the following events: subject admission to NICU, contact of study staff by treating physicians, subject enrolment, initiation of EEG monitoring, first recognized seizure, and time of study drug administration. Record will be made of whether seizure is first identified by Corticare staff or by onsite study investigator. Record will be systematically collected from each monitored subject of any difficulties with functioning of the remote continuous monitoring infrastructure. All data will be referenced to date/time of birth so that precise age in hours can be determined for age at enrolment, timing of seizures, administration of study drug, and administration of other treatments given to treat seizures.

Safety data: Continuous monitoring of vital signs will occur as part of routine neonatal intensive care. Vital signs will be recorded at baseline, and at 15 minutes after each drug infusion commences. Documentation of presence or absence of adverse event occurring during each treatment phase will be made.
Blood tests of haematological indices, electrolytes, glucose, renal function and liver enzymes will be tested and recorded at baseline, at 24 hours and at 48 hours as part of safety and adverse events monitoring.

Pharmacokinetic data: For subjects at the 3 San Diego sites serum LEV levels will be drawn at baseline, 1 hour after starting the treatment, at 24 and 48 hours.

EEG data monitoring and automated seizure detection: Gold standard 9 channel neonatal montage continuous video-EEG data will be collected for 24 to 48 hours after study drug administration with the length of monitoring time determined by the Principal Investigator for each site. There may be infants who, after 24 hours of remaining seizure-free, are appropriate for discontinuation of monitoring which also may enable them to be safely discharged home. Babies undergoing hypothermia for hypoxic ischemic encephalopathy will have EEG monitoring data captured for 24 hours post start of rewarming as this is a high risk time for seizures. Each site will use available video EEG equipment with Persyst software installed. A research dedicated Cadwell video EEG system is requested under this grant for each site. Following recruitment to monitoring, each site will have their study investigator monitoring the EEG in real time, per the study algorithm. Corticare technicians will provide continuous EEG monitoring by remote access to the acquisition station. If Corticare or the real time onsite observer detects seizure activity the site investigator or local encephalographer will immediately be contacted to review the tracing. Neonatal EEG data obtained from conventional digital EEG machines based at the study sites will be monitored in near- real time using Persyst Insight software running

on the acquisition stations via the site's local area network or remotely via secure Internet access utilizing Citrix server, Microsoft Remote Desktop, or similar institutional remote access capabilities. The EEG data will be continuously analyzed in near- real time using Persyst MagicMarker in order to produce and display EEG data trend graphs. The graphical EEG trends will include Rhythmic Run Detection and Display, amplitude integrated EEG, FFT spectrograms, asymmetry spectrograms, and other trends useful in the rapid recognition of seizures. Following completion of the local study on each subject the full video EEG (totalling 5 Gigabytes of data/ 24 hours) will be archived on the UCSD server for analysis by the encephalographers. Data transfer will be achieved where possible by encrypted internet connections. Alternatively the studies will be mailed on memory sticks or DVDs. Archived video continuous EEG data will be available for trend analysis and seizure detection comparison with the duplicate formal encephalographers reading of the 48 hours of tracing.

Add on Study:
The study population of treated babies with electrographic seizures and the parallel control group at risk for seizures but with none detected forms an excellent cohort for a neurodevelopmental outcome study and the effect of seizures. This is being planned and I am asking the parents in the consent if they are interested in being contacted for a neurobehavioral follow-up study.

Rationale for study design:
Justification for a phase II study: The objectives of this study are to gain preliminary evidence of efficacy of LEV in neonates, to determine if a definitive phase III trial is warranted, and if so, to obtain sample size estimates for that study. In addition further safety data in this population will be aquired.
Planning is underway for a large definitive phase III randomised controlled trial of the leading potential new treatments for neonatal seizures. If LEV is established as efficacious in neonates, a future study comparing it with other agents such as Phenobarbital alone or Phenobarbital in combination with bumetanide or topiramate is likely. Such a study to compare the current standard of care AED treatment with newer agents will require 300-400 treated subjects. Long term follow-up of subjects will be necessary, since neurodevelopmental outcome will be the relevant primary outcome measure.
Prior to a definitive phase III trial of any promising new treatment for neonatal seizures, efficacy in the neonatal age group must be proven. Prospective data on the efficacy LEV in neonatal seizures are not yet sufficient to justify a phase III trial. The precision of the estimated response rate to LEV obtained in this proposed study will be important in the design of a future comparative trial[64].

Justification of study design using LEV as first line treatment:
While it is customary to study new AEDs in subjects who have failed standard treatments, this is especially problematic for the setting of neonatal seizures. On the one hand, by studying efficacy in only those neonates who have failed to respond to a first line agent, a group of refractory neonates has been selected, who may not respond to any AED. On the other hand, neonatal seizures typically resolve spontaneously in a significant proportion of subjects. These factors make interpretation of second line efficacy data difficult, and a poor surrogate for first line treatment efficacy data.

There is sufficient evidence of efficacy and safety to justify study of LEV as a first line agent. LEV has good efficacy in other populations, including in populations experiencing refractory status epilepticus. In neonates there are accumulating reports of its efficacy in cases where seizures are refractory to multiple other agents. Our preliminary analysis of efficacy when LEV is used as a second line agent suggests efficacy similar to that of Phenobarbital and phenytoin when used as second line agents (as described above).

Our study design ensures rapid escalation to treatment with Phenobarbital in subjects who do not respond to LEV. Additionally, because our study will utilize real time monitoring of continuous EEG recordings leading to more rapid detection and treatment of electrographic seizures than is normally standard of care, our study subjects will receive AED treatment in a shorter time frame than is usual.

Plan for Statistical Analysis

General considerations: The general objectives of this study are to gain preliminary evidence of efficacy of LEV in neonates, to determine if a phase III trial is warranted, and if so, and to obtain to obtain sample size estimates for that study. In addition further safety data in this population is required. This study will allow the LEV and PB groups to be compared with respect to (a) preliminary evidence of efficacy, and (b) safety.
All analyses will incorporate the intention-to-treat principle; all randomized participants will be included in the analysis. Analyses for all efficacy outcomes will be guided by descriptive and exploratory analyses. All results will be reported as point estimates (proportions or mean differences across groups, as appropriate) and interval estimates (95% confidence intervals) with two-sided p-values denoting statistical significance. Since this is a phase II preliminary efficacy and safety study, no adjustments for multiple comparisons will be made and a p-value of 0.05 will be considered statistically significant.

Analysis Plan for Aim 1: Evaluating efficacy in seizure cessation
A Fisher's exact test will be used to test the 48 hour seizure cessation rate between the two treatment groups. As a secondary reanalysis, multivariate logistic regression (if there are sufficient number of events), will be performed to study the association between cessation rates and treatment. Any baseline covariates (including stratification factors) that are simultaneously unbalanced between treatment groups at baseline (univariate $p < 0.10$) and associated with the outcome (univariate $p < 0.15$) will be included in the multivariable model as observed confounders. A similar approach will be utilized to analyze the 1-hour seizure cessation rates.
Power Analysis for Aim 1: 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.

Analysis Plan for Aim 2: Dose escalation analysis
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.

Analysis Plan for Aim 3: Pharmacokinetic 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 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 t1/2) of +/- 15%. The 90% confidence intervals for the exposure parameters (Cmax, AUC) will be approximately +/- 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.

Analysis Plan for Aim 4: Safety analysis
A Fisher's exact test will be used to compare rates of adverse events, serious adverse events and study discontinuation rates between the LEV and control groups.

Analysis Plan for Aim 5 A: Feasibility of remote continuous EEG monitoring system

Feasibility will be measured by accuracy and speed of notification of electrographic seizures. We will report the standard metrics of sensitivity and specificity. If Corticare reports a seizure rejected by the study investigator, this will be recorded as a false positive. Conversely seizures recorded by the neurophysiologist but missed by Corticare will be false negatives, and we will determine the rate of false positives and false negative seizure reports by Corticare technologists. We will calculate the specificity and sensitivity of Corticare seizure reporting. For speed of reporting, we will measure the time lapse between the occurrence of seizure and site study investigator notification and the lapse between occurrence of seizure and study drug administration. Descriptive report of technical difficulties experienced with the remote centralized EEG review system will be made.

Analysis plan for Aim 5 B. Analysis of performance of the automated seizure detection algorithm

In addition to standard visual EEG review by the study's electroencephalographers, the early detection of seizures will be assisted by automated seizure detection notifications generated by the computerized algorithm. These notifications, and images of their associated EEG tracing and graphical trends, can be automatically broadcast via email to specifically designated study personnel for verification of the nature of the detected event. The performance of the seizure detection algorithm will be assessed by comparing its output to the final EEG markings of two neonatal electroencephalographers. The following seizure detection algorithm performance parameters will be calculated: overall seizure detection sensitivity, false positive rate, and seizure-affected patient detection rate (the percentage of patients with seizures in whom at least one seizure is correctly identified).

9. HUMAN SUBJECTS:

The trial will be a Phase II trial to test the efficacy, pharmacokinetic and safety of intravenous Levetiracetam (LEV) as first line treatment for neonatal seizures with greater than minimal risk.

Human Subjects Involvement and Characteristics

Subject characteristics: This trial will be conducted in term-age newborns with seizures who are admitted to the neonatal intensive care units (NICUs) at one of five participating hospitals, namely Rady Children's Hospital University of California, San Diego, UCSD Medical Center, Sharp Mary Birch Hospital San Diego, Children's Hospital and Research Center Oakland, and Auckland Hospital. The subjects will have a corrected gestational age at birth of 36-44 weeks and a postnatal age of < 14 days. Subjects are expected to be critically ill newborns, reflecting the underlying etiology of the seizures as well as the physiologic effects of seizures per se. Approximately 50% of neonatal seizures are caused by hypoxic ischemic encephalopathy, (HIE). These infants have sustained global hypoxic ischemic injury with consequent renal, hepatic, gastrointestinal, respiratory and cardiac sequelae. Other common etiologies of neonatal seizures include intracranial hemorrhage, infection and developmental defects, which may also be associated with impaired function of other organs. Therefore seizures are frequently associated with high mortality and morbidity. 20-30% of infants with neonatal seizures die, 20-30% develop epilepsy outside the neonatal period, and 20-40% develop cerebral palsy and/or mental retardation[6]

Rational for enrolling newborns as subjects: There are several important factors contributing to the rationale for involving term newborns in this research. The drug to be studied has been extensively studied in adults and older children and shown to be safe and effective. It cannot be assumed however that this will mean that the drug is safe or effective in neonates without study in this population. The neonatal response to AED's is fundamentally different to that of the mature brain. Agents that have good efficacy and safety in the mature brain, (Phenytoin and Phenobarbital) are much less effective and have increased toxicity in the immature brain.

There is an urgent need for safe and effective treatments in this neonatal population. Currently used treatments are poorly effective and have significant side effects, none are FDA approved. The incidence of seizures is higher in newborns (2-3.5 per 1000 live births) than at any later age. Neonatal seizures are often

refractory to treatment and lead to frequent and serious long-term consequences in survivors, such as significant cognitive and motor disabilities and later epilepsy.

Collaborating sites and study investigators: This trial will be a collaborative effort among Pediatric Neurology, Epilepsy/ Neurophysiology and Neonatology at the five sites. Together the study investigators have extensive experience in clinical trials in newborns and expertise in the, diagnosis, monitoring and treatment of neonatal seizures. The investigators have recently successfully completed a pharmacokinetic study of the study drug in neonates. The PI will oversee all aspects of the trial and will be the site PI coordinating conduct of the study at Rady Children's, UCSD Medical Center NICU and Sharp Mary Birch San Diego NICU. Gail Reiner DNP, FNP based at UCSD will be the study coordinator assisted by research coordinators Lilly Lee, B.A., Kimberly Thomas, M.S., and Mallory Boutin, MPH. Dr Mary Harbert will be a study co-investigator at the San Diego sites. Dr. Kuperman will be the site PI supervising conduct of the study at the Oakland Children's Hospital NICU. Dr Sharpe will be the site PI supervising conduct of the study at the Auckland Hospital NICU. Drs. Honold and Lane neonatologists at the Rady Children's UCSD NICU and will be the neonatologists contributing to coordination of the study at that site. Durand will be the primary neonatologist at the Oakland Children's Hospital NICU and Dr .Battin will be the participating neonatologist at the Auckland Hospital site. Drs. Garey and Rasmussen will be the neonatologists at UCSD Medical Center and Sharp Mary Birch Hospital respectively. Drs. Wang, Nespeca and Kuperman have expertise and certification in neurophysiology and neonatal seizures in particular, and will be the study investigators supervising analysis of EEG data. In addition, at each site we have collaborating neurophysiologists: Dr. Davis at the Auckland site, and Dr. Kuperman at the Oakland site. Dr. Edmund Caparelli is an internationally recognized expert in pediatric pharmacology and will be a consultant regarding those aspects of the study. Dr Rema Raman, biostatistician at University of California, San Diego has advised on study design and will continue to provide statistical support. Corticare Corporation will provide continuous remote EEG monitoring under HIPPA regulations and Persyst Corporation will assist in EEG analysis and seizure detection using their commercial Magic Marker software which is installed on all EEG machines in use in the study. This software with it's trending capability is already in daily use at the study sites.

<u>Sources of Material</u>
Data that will be collected specifically for the purposes of this trial will include clinical data regarding the subjects' medical history and hospital course including clinical characteristics such as weight, medications administered, blood pressure measurements, and fluid input/output. Most clinical data will be obtained from the subjects' medical records, but there may be some data obtained by interviewing the parent(s).

Much of the laboratory data that will be recorded in the database will be laboratory data obtained in newborns as part of standard clinical care of newborns with seizures, such as serum blood gas measurements, serum electrolytes, levels of AEDs administered and liver function tests. Some laboratory tests such as albumin and serum bicarbonate may not be collected at both time points for standard care for some subjects and thus will be obtained for purposes of the trial. Serum LEV levels will be collected only for purposes of this trial.

EEG data will be read and used for clinical purposes at each site with the assistance of the remote centralized continuous monitoring system. For the purposes of the study itself EEG data collected at each site will be de-identified before analysis by the study neurophysiologists who will be blinded to the subjects' group assignment or demographic information.

Study staff at each site will have access to individually identifiable information about newborns enrolled at that site. Data will be collected by each site's study investigators and the research coordinator and recorded onto case report forms (CRFs) by the research coordinator, and data from the CRFs will subsequently be entered into the database by the research coordinator. The CRFs and the electronic database will only have de-identified information to preserve confidentiality of patients' protected health information. Files that link the study identification number for each subject to the subject's personal information (such as name, medical

record number, parents' names) will be kept at each site and accessible only by the site PI and research coordinator.

10. RECRUITMENT AND SCREENING:

300 subjects will be recruited in total from the 5 NICU's over 4 years. A higher than anticipated screening to treatment ratio of approximately 3:1 monitored to treated neonates has occurred in the initial 85 neonates recruited to this study. The originally anticipated 2:1 EEG monitoring: treatment ratio is likely lower due to the beneficial effects of hypothermia treatment many neonates receive as a usual standard of care. Hypothermia treatment may be reducing the incidence of seizures in this population. This change in ratio may also be a reflection of a benefit of this study in that behavior in a few neonates which may have previously been clinically judged to reflect seizure activity, because of continuous EEG monitoring viewed by EEG technicians in this study also reviewed by a neurologist, have been correctly diagnosed as myoclonic jerks rather than seizures. This has thereby prevented some neonates from receiving medication for seizure treatment because their behavior is not confirmed by their EEG as seizure activity. For these reasons an increased number of total subjects will be needed in this study in order for 100 subjects to be treated.

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

- Term infants (gestational age greater than or equal to 36 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.

These patients will be a very sick population, reflecting the underlying etiology of the seizures as well as the physiologic effects of seizures per se. Approximately 50% of neonatal seizures are caused by hypoxic ischemic encephalopathy, (HIE). These infants have sustained global hypoxic ischemic injury with consequent renal, hepatic, gastrointestinal, respiratory and cardiac sequelae. Other common etiologies of

neonatal seizures include intracranial hemorrhage, infection and developmental defects, which may also be associated with impaired function of other organs. Therefore seizures are frequently associated with high mortality and morbidity. In studies of term infants with neonatal seizures, it has generally been found that between 20 to 30% died, 20 to 30% developed post-neonatal epilepsy and between 20 and 40% developed cerebral palsy[1,2].

Commonly, it is expected that a new drug or drug formulation would be studied extensively in healthy adult populations prior to its use in studies in vulnerable newborns. The basic assumption underlying this common practice is that standard management is adequate. This is not the case in the treatment of neonatal seizures. Agents that have good efficacy and safety in the mature brain, (Phenytoin and Phenobarbital) are much less effective and have increased toxicity in the immature brain. The dire need for better treatments for neonatal seizures, combined with the existing data that suggest that this new agent may well be safer than traditional agents and that it may also have good efficacy is justification for studying the drug in this population.

Infants admitted to the 5 participating NICU's will be recruited in 3 separate situations:

A. Infants with HIE identified prospectively as being at high risk for developing seizures,
B. Infants admitted to NICU with pathologies other than HIE that also place the infant of high risk to develop seizures.
C. Infants with seizures as first indication of illness, transferred to the NICU for treatment. Initially etiology may not be clear but may include infection, stroke, hemorrhage, brain malformation and idiopathic seizures.

Partial HIPAA waiver will need to be granted for the recruitment procedure, specifically the waiver of consent for the transfer from the clinical team to the study team of clinical information needed to determine patient eligibility for the study.

In groups A + B it will be possible to prospectively obtain consent for participation in the trial at the time when the infant is admitted to the unit. Neonatal staff (Admitting neonatal fellow or resident) will identify eligible subjects in group A or B briefly discuss the study with patients' families and obtain permission to contact study staff. Study staff will screen the patient for inclusion and exclusion criteria as listed above and obtain consent from parents for participation in the study in the event that seizures should occur

Group C:

Frequently seizures are the first indication that a neonate is unwell. These occur in the regular postnatal nursery. Once the infant is transferred to the NICU and stabilized cardiovascularly, and if seizures are ongoing and hypoglycemia and hypocalcemia have been excluded as cause for the seizure, a continuous EEG monitor will be placed, (this is not yet standard of care in the majority of NICU's in the United States but is an option available in many centers).
While the EEG leads are being placed intensive efforts will be made by neonatal and study staff at this stage to obtain parental consent for participation in the study. If consent can be obtained the patient will be enrolled in study and a randomized to a treatment arm during the EEG set up. If electrographic seizures are confirmed, IV treatment with study drug according to the protocol will commence.

11. INFORMED CONSENT:

1. The admitting resident or fellow who identifies seizures or high risk of seizures developing in the future will request the parents' permission for the study staff to approach them to discuss their possible participation.

It will be made clear to the parents that participation is voluntary and that their child will receive standard care if they do not wish to participate. Furthermore it will be made clear that the study staff is separate from the infant's own primary physician and nursing group.

2. If permission is granted the study staff will be contacted and will review the clinical details to ensure inclusion and exclusion criteria are met.

3. Study staff will approach the parent with study information to obtain consent in person.

Parents not available in person, (in situations for example where the infant has been transferred from a remote hospital), will be faxed a consent form for review and signature. Study staff will be available by phone to the parents, should they have any questions regarding the faxed consent.

If the follow-on study question is left unanswered, the study team may use an IRB approved phone script to contact the family in order to complete the question. From recent review post recruitment closure, the follow-on study question was missed in approximately 10% of the consents. A waiver of documentation will be used for contacting the families, and all responses will be documented in a separate study source document."

12. ALTERNATIVES TO STUDY PARTICIPATION:

If parents do not wish to participate in the study they will not be coerced to do so and will be reassured that their infant will continue to receive the normal standard of care. Study staff is separate from the primary physicians and nurses involved in the infants' care, which should help to free the parents from feelings of undue pressure to participate.

13. POTENTIAL RISKS:

The potential risks to the subjects include 1) adverse drug reaction risk secondary to LEV, 2) risks of blood drawing, 3) risk that LEV is a less efficacious drug and that consequently study subject will have a longer duration of seizures before adequate treatment is given 4) risks regarding confidentiality.

Potential risks related to LEV

LEV has been used for many years as an anticonvulsant in older children and adults and has a very favorable safety profile. LEV toxicity data show a very wide safety margin, with a TD50/ED50 ratio of >148 in rodents[37] .No deaths, organ failure or other irreversible toxicity were seen after long-term oral treatment up to doses of 1,800 mg/kg/d in the rat, 960 mg/kg/d in the mouse and 1,200 mg/kg/d in the dog.[38] Animal fetal toxicity data are reassuring[38]. Data from the UK Epilepsy and pregnancy register[65] documents that no fetal malformations occurred in 39 pregnancies where levetiracetam was used as monotherapy. Where levetiracetam was used with other anticonvulsants 3 major malformations occurred in 78 pregnancies.

Clinical experience with LEV use in adults and in children has found it to be well tolerated and safe[29, 30, 33, 39-42] including 3 recent reports of use at high dosage [43-45] . In humans 2 cases of reversible thrombocytopenia have been reported. Tan et al have reported a case in which levetiracetam was implicated as a possible cause of liver failure[66]. No other significant hematological, hepatic or renal toxicities have been reported. In children and adults the most common side effects are somnolence and behavioral side effects. Glauser et al[50] report 23% of children treated with levetiracetam experienced somnolence, compared to 11% of placebo patients however this side effect rarely requires cessation of treatment. Behavioral side effects associated with treatment included agitation, anxiety, apathy, depersonalization, depression, emotional lability, hostility and hyperkinesias. A total of 37.6% of children in this study reported some behavioral side effect, compared with 18.6% of placebo patients. These behavioral abnormalities were significant enough to prompt

discontinuation of treatment or dose reduction in 10.9% of levetiracetam treated patients compared with 6.2% of placebo patients.

In our recently completed pharmacokinetic and preliminary safety study of LEV in neonates, the drug was well tolerated. Only one serious adverse event occurred in this study. One subject with a brain malformation and a high Phenobarbital level required intubation while on treatment with LEV. LEV was not thought to be causal of this event. There were a few mild adverse events identified that were possibly related to treatment with LEV: mild sedation was reported in 2 subjects. In both cases this resolved spontaneously. Feeding difficulty was documented in 3 subjects. In all cases this difficulty was probably not due to LEV treatment and in all cases feeding difficulty resolved. One subject had mild apnea and bradycardia which resolved. This was probably not related to LEV treatment. Decreased urine output occurred in 1 subject with HIE. This resolved with treatment with frusemide. All blood test abnormalities were reviewed by an independent data safety monitoring board. Mild abnormalities in blood count and serum chemistries were seen, consistent with what would have been expected in this patient population of sick neonates. No serious or consistent treatment- emergent lab abnormalities were observed.
Two recent publications of LEV use in neonates reported only sedation and irritability as side effects .

It therefore appears that levetiracetam is a very safe drug with a wide therapeutic range. However, any adverse drug reaction is possible.

Potential risks of blood drawing: blood loss and pain
Blood draws will be required in a subset of patients for measuring the pharmacokinetics of LEV. For some patients there may also be additional blood drawn for safety studies, although in most patients these laboratory tests would be obtained as standard care because of their underlying illness (i.e., systemic asphyxia). Furthermore, additional blood tests required for the study may not require additional blood draws, because laboratory tests such as liver function tests can be run on serum drawn for other purposes, such as serum electrolytes. The required volume of each blood sample for LEV levels will be 0.5 mL. Drawing a total blood volume of 2 ml/kg (ca. 3% of blood volume) over 24 hours is considered acceptable. For a 2.2-kg term newborn, this would amount to 4.4 ml of blood/ 24 hours. This protocol requires 4 samples for LEV analysis, over 48 hours for a total volume of 2 ml. For additional blood draws of laboratory tests which may not otherwise be drawn for clinical purposes, the total volume of blood will be 2-3 ml. Thus the total amount of blood drawn for research purposes will be ≤5 ml over 48 hours. It is unlikely that this small increase in the amount of blood drawn would result in significant anemia. Most blood draws will be via indwelling vessel catheter in place as part of routine care. This will render blood sampling painless and free of any risk beyond that usually associated with the care of a critically ill newborn. Blood sampling towards the end of the 1-week treatment period may require additional venipunctures or heel stick blood draws. All efforts will be made to reduce infants discomfort by warming the heel adequately prior to blood draw, administering oral glucose during blood draw and feeding infant after blood draw.

Risk of less effective drug. There is a potential risk that LEV is a less efficacious drug than standard treatments, placing study subjects at risk of a longer duration of seizures before effective treatment is given if randomized to receive LEV. This will be minimized as described in the following section.
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Potential risks regarding confidentiality. There is a potential risk for breach of confidentiality, which will be minimized by the procedures described in the following section.

14. RISK MANAGEMENT PROCEDURES:

Adequacy of Protections against Risk

Recruitment and Informed Consent

Research conducted in this project will be reviewed and approved by the appropriate Institutional Review Board (IRB) for each site. Only trained study investigators listed on the IRB protocol will obtain written informed consent using the locally approved consent form.

Recruitment procedure: Newborns will be recruited from the five neonatal intensive care units (NICUs) once the IRB protocol has been approved at all the five institutions. We will provide in-servicing to NICU staff in each unit and post flyers in the NICUs to alert neonatology staff to the study's purpose, inclusion criteria, and the pager number needed to contact the study staff regarding potential subjects. We anticipate that we will recruit subjects in two groups. The first group will be subjects in whom it can be anticipated that seizures are at high risk of occurring, for example in neonates with hypoxic ischemic encephalopathy. These subjects will be identified by the neonatology staff who will then contact the investigator on call for the research study. The neonatologist will first mention the study to the parents and then ask whether the parents will give permission for the study investigators to talk with them about the study in detail. Only parents who consent to hearing about the research study will be approached regarding enrollment of their newborn.
In the second acutely presenting group, neonates present with symptoms indicative of or suggestive of seizures without prior warning. It is recognised that it will not be possible to recruit all patients in this group. Efforts to obtain consent will not be allowed to delay management. However, in many cases there will be opportunity to ethically recruit and consent subjects without delaying care. For example, in cases where clinical seizures are brief and have stopped spontaneously, and in cases where there is suspicion of seizure activity but EEG confirmation is needed before AEDs are indicated.

Consent procedure: The PI and other selected study investigators (neurology and neonatology attendings and fellows) will obtain consent. All study investigators who will be obtaining consent will receive training in how to determine eligibility of subjects and how to discuss the research study and consent process with parents of a critically ill newborn. It is anticipated that some mothers may be hospitalized at a referring hospital where the baby was born; in that case the consent form will be faxed to that hospital for the parent(s) to review. If consent is obtained in part by telephone with either or both parents, the telephone consent will be witnessed by one of the medical personnel at the NICU where the newborn is being enrolled. The child's assent will not be obtained as only newborns will be enrolled. Parents will be reassured that participation in this study is completely voluntary and that a refusal to provide consent will not affect their child's clinical care. Parents will be informed that they can ask questions of study staff at any time during the study and contact information for the study staff will be provided on the consent form. The study investigator who obtains consent will ask the parent(s) questions to determine whether they have understood the informed consent process and the requirements of the study.

Data and Safety Monitoring Plan (DSMB)

The population being studied is a very sick pediatric population. A high rate of morbidity and mortality is expected in this patient group. Therefore a DSMB will be used to monitor adverse events occurring during the trial, to try to carefully determine whether the study drug is implicated in the outcome.
The study will be conducted in compliance with the IRBs of the participating hospitals and with FDA regulations, since Dr. Haas holds an IND (#109622) from the FDA for the use of LEV to treat neonatal seizures and an Orphan Drug Designation for the treatment of neonatal seizures with levetiracetam (#10-3033).

The FDA will appoint a Data Safety and Monitoring Board (DSMB). The DSMB will have the opportunity to request changes in protocol design and conduct and to design the Data Safety Monitoring Plan for the trial. The DSMB will define how frequently it wants to meet, and the exact nature of the information it wants to review, in adherence to FDA specific guidelines for DSMBs. We expect that the DSMB will request to monitor adverse events, patient screening, study accrual, eligibility violations, and any protocol violations. The DSMB will report their conclusions and recommendations to the sponsor (Dr. Haas).

We have assembled a highly qualified group who have agreed to serve on our DSMB pending approval from the FDA. The suggested DSMB participants include James Cloyd PhD (Univ of Michigan) Director, Center

for Orphan Drug Research ,Lawrence C. Weaver Endowed Chair-Orphan Drug Development is an expert in the clinical pharmacology antiepileptic drugs and medications used to treat rare pediatric neurological disorders; Our statistician is Sonya Jain PhD Associate Professor of Biostatistics & Bioinformatics at UCSD in the Department of Family & Preventive Medicine; Professor Terrie Inder MD (Washington University Saint Louis) is a pediatric neurologist and neonatologist and an international authority on neonatal clinical trials. These DSMB members were chosen because (apart from our UCSD Statistician) they are external to the trial sites and have experience in conducting clinical trials in newborns or drug trials in childhood epilepsy. The current plan for reporting adverse events is proposed:

Adverse event Reporting: Newborns seizures who will be enrolled in this study are at high risk for several serious medical complications and death related to the underlying etiology of their seizures. Life support is withdrawn in a significant minority of severely asphyxiated newborns because of the high probability of severe neurologic disability. It is very unlikely that LEV will result in death or any serious adverse event. The following adverse events not described in the investigator's brochure for LEV will be considered expected adverse events in this population:

Clinical or electrographic seizures
EEG abnormalities (seizures or background abnormalities)
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 LEV, 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 the sponsor 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 the sponsor to the local IRBs for determining continuing review, and to the FDA for annual IND safety reports.

Any adverse events that are deemed by the investigator to be unexpected, possibly related to LEV, and severe in nature will be reported to the sponsor within 24 hours of the event. These unanticipated adverse events will also be reported by the PI to the local IRB in accordance with the local IRB policies. The sponsor will be responsible for reporting the unanticipated adverse events to the DSMB chair 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 subjects. If the information changes the known risk to subjects, the DSMB's report regarding the change in risk will be released to all participating investigators by the sponsor. The DSMB may request changes to the DSMP.

Mechanisms to minimize potential risks to privacy and confidentiality of data

Privacy Provisions: The treating neonatology staff or pediatric neurologist will first approach a newborn's parents to ask permission for the study investigator to discuss the study with the family. Only if and after parents agree to this step will a study investigator then talk with the parents and review the subject's medical record. Once informed consent is obtained, the parents may need to answer a few questions about the maternal and family health history, but study staff will obtain most of the data from the subjects' medical records.

Confidentiality Provisions: The physicians and nurses caring for each newborn will need to know that the newborn is enrolled in this study but will not have access to data collected only for research purposes.

Medical information collected during this study will become part of the subject's hospital record, if the information is determined to be pertinent to the subject's medical care or is a usual part of the subject's care (e.g., results of tests ordered by treating clinicians that are also recorded for research purposes). Medical records are available to other health care professionals at the hospital and may be reviewed by hospital staff in their course of carrying out their responsibilities. However, they are required to maintain confidentiality in accordance with applicable laws and hospital policies. Information contained in a medical record may not be given to anyone unaffiliated with the hospital in a way that could identify the subject or parent without written consent, except as required or permitted by law. Information collected during the study that does not become part of a subject's medical record will be stored in separate research files maintained by the investigators. These research records will not be made available to any individuals who are not part of the research team except upon a parent's request or as required by law. If a subject is withdrawn from the research study, information that has already been collected will become part of the research database.

Only the research coordinator, neurology fellow and site PI at each NICU will have access to individually identifiable information about newborns enrolled at that site. Data will be collected by the study investigators and research coordinator and recorded onto case report forms (CRFs) by the research coordinator. The CRFs and the electronic database containing the research data will contain only de-identified information to preserve confidentiality of patients' protected health information. Only the study identification number will identify the subjects on the CRFs and in the electronic database. Files that link the study identification number for each subject to the subject's personal information (such as name, medical record number, parents' names) will be kept at each study site and accessible only by the site PI and research coordinator. The research database containing the subjects' study ID numbers and research data will be stored on a single computer, protected with a password, and will be backed up on the network in a private folder accessible only to study staff.

<u>Mechanism to minimize potential risks of adverse drug reaction related to LEV</u>
As described above LEV is a very safe drug with a wide therapeutic range. However, any adverse drug reaction is possible. Careful monitoring for adverse drug reactions will be maintained throughout the treatment period. Neonates will be monitored continuously during the study period for heart rate, respiratory rate and blood pressure. Serum chemistries and CBC will be performed at baseline and after 48 hours of treatment with study drug. Daily physical examinations will be performed while in-house and record made of the occurrence of hypotonia, sedation, poor feeding, irritability or infection. Since all subjects will be closely monitored by clinical, laboratory and EEG data, it is expected that adverse events can be anticipated and managed rapidly and effectively by the treating clinicians.
If there are serious adverse events, these events will be reported to the IRB within 24 hours of knowledge of the event, and will be reported to the DSMB in the timeframe that they require.

<u>Mechanism proposed to minimize the patient's potential risks involving blood drawing</u>
Blood drawing will be done by clinical staff who are trained and experienced in drawing blood from newborns. A clinically indicated indwelling arterial or venous catheter will be present in most study subjects, so that in most cases there will be no discomfort to the newborns from needle sticks for research-only blood draws. Sterile techniques will be used at all times to minimize the chance of infection, thereby minimizing potential risks related to the collection of blood samples. It is possible that in some subjects indwelling arterial or venous catheter access will not be maintained throughout the study period and may require additional venipunctures or heel stick blood draws. All efforts will be made to reduce infants discomfort by warming the heel adequately prior to blood draw, administering oral glucose during blood draw and feeding infant after blood draw.

<u>Mechanism proposed to minimize patients' risk of time delay to effective AED therapy</u>
Our study design ensures rapid escalation to treatment with Phenobarbital in subjects who do not respond to LEV. Additionally, because our study will utilize real time monitoring of continuous EEG recordings leading to more rapid detection and treatment of electrographic seizures than is normally standard of care, our study subjects will receive AED treatment in a shorter time frame than is usual. LEV has good efficacy in other

populations, including in populations experiencing refractory status epilepticus. In neonates there are accumulating reports of its efficacy in cases where seizures are refractory to multiple other agents. Our preliminary analysis of efficacy when LEV is used as a second line agent suggests efficacy similar to that of Phenobarbital and phenytoin when used as second line agents. Therefore we believe that the risk that the overall risk to patients is low in this regard

Specific Measures to reduce potential risks listed in (13)

1.Measures to reduce pain from venipunctures

For the first days of the study all patients will have indwelling vessel catheter in place as part of their routine care. This will render blood sampling painless and free of any risk beyond that usually associated with the care of a critically ill newborn. Blood sampling towards the end of the 1-week treatment period may require additional venipunctures or heel stick blood draws. All efforts will be made to reduce infants discomfort by warming the heel adequately prior to blood draw, administering oral glucose during blood draw and feeding infant after blood draw.

2. Measures to reduce risk secondary to blood loss from venipunctures
The total blood drawn over 2 days for the study will be 44 to 5 ml (4 x 0.5 ml specimens for levetiracetam levels, and at the same lab draw as the last levetiracetam level an additional 1.0 ml for chemistry CBC and phenobarbital level). By excluding infants with birth weights less than 2440g we will be able to ensure that this is a safe percentage (≤3%) of the infants total blood volume.

3. Adverse drug reaction

As above levetiracetam is apparently a very safe drug with a wide therapeutic range. Adverse drug reactions to orally administrated levetiracetam in adults and older children have been exceedingly rare. Use of the IV formulation in animal studies and a PK study in adults has so far not been associated with serious adverse drug reactions. However, any adverse drug reaction is possible including serious anaphylactic reaction and death. Careful monitoring for adverse drug reactions will be maintained throughout the treatment period:

Levetiracetam has been well tolerated in other age groups and found to be without significant risks. Adverse events have mainly consisted of CNS effects such as somnolence, asthenia, coordination difficulty and behavioral symptoms. Other than 2 cases of reversible thrombocytopenia associated with levetiracetam use, no unexpected toxicities or serious laboratory abnormalities have been reported.

Neonates will be monitored continuously during the study period for heart rate, respiratory rate and blood pressure.
Serum chemistries, and CBC will be performed at baseline and at end of treatment.
Daily physical examinations will be performed while in-house and record made of the occurrence of hypotonia, sedation, poor feeding, irritability or infection.
Following the last dose of study drug, a phone review of the same symptoms will be conducted every 3 days and a visit and physical examination will be conducted one week after the last dose of study drug. A home visit or clinic visit can be conducted if the subject is discharged.

4. Mechanism proposed to minimize patients' risk of time delay to effective AED therapy

Our study design ensures rapid escalation to treatment with Phenobarbital in subjects who do not respond to LEV. Additionally, because our study will utilize real time monitoring of continuous EEG recordings leading to more rapid detection and treatment of electrographic seizures than is normally standard of care, our study

subjects will receive AED treatment in a shorter time frame than is usual. LEV has good efficacy in other populations, including in populations experiencing refractory status epilepticus. In neonates there are accumulating reports of its efficacy in cases where seizures are refractory to multiple other agents. Our preliminary analysis of efficacy when LEV is used as a second line agent suggests efficacy similar to that of Phenobarbital and phenytoin when used as second line agents. Therefore we believe that the overall risk to patients is lower than the usual clinical care risk when using our treatment protocol. The continuous EEG monitoring that will be used for the study will mean that these determinations of the efficacy of medications will be possible sooner than is possible with routine management.

5. Measures to safeguard confidentiality of data.

Personal identifiers such as name, date of birth and medical record number will be eliminated from each case as it is entered in the database. A single copy of the list linking medical record number to study number will be kept locked and will be available only to study investigators. Data will be entered on single secure database, accessible only by study investigators. The code will be destroyed at the end of the study.

15. POTENTIAL BENEFITS:

The main benefit of the study will be to society and future neonates with seizures, in the knowledge that will be gained from the study. If LEV efficacy, safety and pharmacokinetics can be established in neonates this will be of great benefit to all neonates with seizures. Developing better infrastructures to increase the feasibility of continuous monitoring and the early detection of neonatal seizures will also significantly improve the management of this condition.

Several potential benefits to enrollment in this study compare favorably with the low risks associated with participation in the study. One principal benefit of this study is that early and more intensive “real time” EEG monitoring will likely lead to more rapid diagnosis and treatment of neonatal seizures than in the current standard of care. Subjects enrolled in both treatment and control groups will have the potential benefit of this early EEG monitoring.

Although there are potential risks associated with administration of LEV, the overall risk of adverse events are low. All subjects will be carefully monitored with respect to clinical, laboratory and EEG data in a more systematic fashion than is typical in the care of such newborns, thus any adverse event will be recognized quickly. Our study design ensures rapid escalation to standard treatment with Phenobarbital in subjects who do not respond to LEV. Additionally, because our study will utilize real time monitoring of continuous EEG recordings leading to more rapid detection and treatment of electrographic seizures than is normally standard of care, it is likely our study subjects will receive AED treatment in a shorter time frame than is usual.

Benefits to the individual participant:

Potential Benefits of the Proposed Research to Human Subjects and Others

There may be no benefit to the individual participant. A potential benefit of this study is that earlier placement of EEG leads and greater than routine monitoring of the prolonged EEG recording, clinical and laboratory data will improve the overall management of the subjects’ seizures and other clinical conditions, compared with standard management of these patients. In particular, there will likely be more rapid diagnosis and management of seizures than in the standard care of newborns with seizures, because of the early, continuous EEG monitoring analyzed in near-real time. This relates in part to the lack of availability of rapid placement of EEG electrodes and less vigilant or less frequent/prolonged monitoring of EEG data for recurrent seizures in the standard clinical care of newborns. EEG monitors cannot usually be placed rapidly because of the lack of availabile EEG technologists to place EEG leads on an urgent basis, especially at night or on weekends. In standard practice, continuous EEG monitoring is often not started until seizures are

clearly shown to be refractory, which may not be recognized when most or all of the seizures are electrographic only (subclinical). EEG monitoring for clinical purposes is often intermittent rather than continuous and prolonged over two days.

Another potential benefit of the study to the participant is that LEV may be a more effective antiepileptic medication than the standard treatments. It is also a potential benefit to the participant that LEV may be a safer drug associated with fewer side effects than the standard treatments.

Benefits to society:

Importance of the Knowledge to be gained

There are no FDA approved treatments for neonatal seizures. More effective and less toxic treatments are urgently required. Neonatal seizures are a common problem and cause significant morbidity. Current standard of care treatments Phenobarbital and phenytoin are unsatisfactory[1], each agent is effective in less than 50% of subjects, and both have significant acute and chronic side effects. In animal studies these agents cause accelerated neuronal apoptosis in the neonatal brain. Better treatments could limit brain damage and improve neurodevelopmental outcomes in this high risk population. LEV has great potential as a treatment for neonatal, hhowever; the efficacy of LEV has not been systematically studied in neonates and cannot be assumed. Drugs that are effective in terminating seizures in adults may be less effective and have more toxicity in neonates.

Efforts to develop better treatments for neonatal seizures are confounded by the need in any drug trial for systematic continuous EEG monitoring, something that is not yet standard of care because of resource constraints. The proposed project would address the need to find feasible solutions to the difficulties of providing continuous EEG monitoring through new technology.

Should LEV prove an effective medication in this age group, clinical practice would be dramatically improved. We would have a safe, non-toxic treatment option for neonatal seizures with proven efficacy.

Developing better systems for neonatal seizure detection will facilitate earlier treatment of neonatal seizures and enable further clinical research in this condition.

If levetiracetam efficacy, pharmacokinetics and safety can be established in neonates with seizures this will be of great benefit to all neonates with seizures.

This study will be registered in ClinicalTrials.gov. website as required by the FDA.

16. RISK/BENEFIT RATIO:

No drug study in neonates is without risk, however IV levetiracetam appears to be a very safe drug. There is a dire need for better anti-epileptic agents for neonates and there are substantial risks associated with the use of standard therapies. The potential benefit to all neonates with seizures outweighs the risks that would be involved in this study. There is considerable potential that the risk/benefit ratio is favorable in the study subjects themselves.

17. EXPENSE TO SUBJECT:

None.

Cost of levetiracetam, all blood and urine specimens drawn for the purposes of the study and EEG monitoring for the purposes of the study will be paid for by the study.

18. COMPENSATION FOR PARTICIPATION:

None.

19. PRIVILEGES/CERTIFICATIONS/LICENSES AND ROLES OF THE RESEARCH TEAM:

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21. INDUSTRY-SPONSORED OR COLLABORATING STUDIES:

Not applicable.

22. OTHER FUNDING SUPPORT FOR THIS STUDY:

FDA Orphan Products Development Grant
Application FD-R-4147-01 Richard Haas MD Principal Investigator

23. BIOLOGICAL MATERIALS TRANSFER AGREEMENT

A materials transfer agreement is on file with UCSD.

24. INVESTIGATIONAL DRUG FACT SHEET:

See Attached – Package Insert for Keppra

25. IMPACT ON STAFF:

Neonatal residents, fellows and attendings will be asked to identify patients at risk, briefly discuss the study with patients families and request permission to notify study staff.
Respiratory technicians and nurses or EEG technicians together with study investigators will be required to apply continuous EEG monitors, depending on the procedures in place at each study site.
If blood collection is to be done via indwelling catheters, the infant's primary nurse will be required to draw these labs.
Nursing staff will be asked to administer the IV medication.
Investigational pharmacy will prepare all doses of medication.
Study staff will conduct all other duties, including the labeling and sending of specimens for collection.
The procedures to be done by non-study staff require minimal additional time and are not expected to adversely impact the staff.

26. CONFLICT OF INTEREST (COI)

None

27. SUPPLEMENTAL INSTRUCTIONS FOR CANCER-RELATED STUDIES

NA

28. PROCEDURES FOR SURROGATE CONSENT AND/OR DECISIONAL CAPACITY ASSESSMENT

NA

29. HIPAA AND CONFIDENTIALITY

Application for partial waiver of HIPAA authorization is requested for the recruitment process in order to allow communication between clinicians and investigators. Specifically the waiver of consent for the transfer from the clinical team to the study team of clinical information needed to determine patient eligibility for the study is required. This was granted in our currently active IRB levetiracetam protocol.

Protected health information will be entered into the database with the consent of the subject's parents.
In addition to the pharmacokinetic, seizure cessation and safety data for each patient, data entered will include demographic data and clinical baseline data, such as gestational age, mode of delivery, postnatal age at presentation, presumed etiology but personal identifiers such as name, date of birth and medical record

number will be eliminated from each case as it is entered in the database Personally identifiable data will not be publicly disclosed. Single copy of list linking medical record number to study number will be kept locked and available only to study investigators. Data will be entered on single secure database, accessible only by study investigators.

Study records will not be released without separate consent, except as required by law.
Code linking medical record to study number in the database will be destroyed once the study is published.