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Levetiracetam efficacy and safety as first-line treatment of neonatal seizures occuring in hypoxic-ischemic encephalopathy context : LEVNEONAT 1- Phase II trial

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ABSTRACT

Introduction: Therapeutic schedule for treating neonatal seizures remains elusive. Lonely, the first-line treatment by phenobarbital is widely admitted. This situation is due to the lack of well conducted trials concerning anti-epileptic drugs (AED) during the neonatal period. Levetiracetam is an emerging and promising AED. The aim of this phase II trial is to estimate the optimal dose of levetiracetam as a first-line AED to treat seizures in newborns suffering from hypoxic-ischemic encephalopathy (HIE). **Methods and analysis:** LEVNEONAT-1 is an open and sequential dose-finding study with 1 loading dose of 30, 40, 50 and 60 mg/kg and 8 quarter-loading maintenance doses for a 3-day treatment. The optimal dose will be the one estimated to be associated with a toxicity not exceeding 10% and an efficacy higher than 60%. Efficacy has been defined by a seizure burden reduction of 80% after the loading dose. A 2-patient cohort will be necessary at each dose level to consider an upper dose level assignment with a dynamic consideration of each participant data. The maximal sample size expected is 50 participants with a minimum of 24 patients or less in case of high rate of toxicity. Patients will be recruited in 5 French neonatal intensive care units from October 2017 for 2 years. In parallel, the levetiracetam pharmacokinetic will be measured at 5 time-points. **Ethics:** Levetiracetam cannot be infused prior of obtaining the written parental or authorized guardians consent. Ethics approval for this study have been obtained from regional ethical committee under the reference 2016-R25 (November 9 2016) and the French drug safety agency under the reference 160652A-31(October 5 2016). **Registration details:** Eudra CT identifier, 2014-000791-26. Clinical Trail.gov identifier, NCT02229123.

ARTICLE SUMMARY

Article Focus

- The principal aim of LEVNEONAT-1 is to determine the levetiracetam optimal dose defined as the highest efficient dose under toxicity restrictions for treating neonatal seizures.
- LEVNEONAT-1 is an open-label, sequential dose-finding study with 4 increasing dose levels of levetiracetam.

Strenghts and limitation of study

- For the first time, levetiracetam will be used as the first-line treatment of neonatal seizures and not as an add-on therapy.
- Statistical model is designed for a rare clinical situation with a sequential adaptive method updating in real time the dose allocation for the next patient on the basis of all available data from previous participants.
- The targeted population, i.e. the newborn less than 3 days of life, is particularly sensitive and the written consent of both parents is required before the levetiracetam administration.

INTRODUCTION

Neonatal seizures concerns about 1 to 5 per 1000 live births [1]. Hypoxic-ischemic encephalopathy (HIE) represents the first etiology of neonatal seizures [1,2]. In 80% of cases, seizures associated with HIE ocurred in the first 3 days of life [2]. Seizure treatment during the neonatal period raises a dilemma concerning the developing brain facing deleterious effects of seizure burden to the potential toxicity of conventional anti-epileptic drugs (AED). Indeed, Van Rooig et al. demonstrated that the seizure duration were correlated to brain lesions on MRI [3]. Therefore, the World Health Organisation recommended in 2011 to start a anticonvulsivant treatment as soon as clinically apparent seizures lasted more than 3 minutes or in case of brief repeated seizures. This recommendation was supported with a strong strenght but no gradation was attributable due to the lack of scientific evidence [4]. Further, conventional AEDs as phenobarbital (PHB) are employed in an off-label manner in neonates. Epidemiologic studies showed that PHB is widely used as the first-line treatment all over the world with a lack of consensus concerning the subsequent add-on lines including phenytoin (PHT), lidocaïne, midazolam and others benzodiazepins [5]. Surprisingly, a meta-analysis from the Cochrane Data Base concluded in 2004 that « there is little evidence from randomised controlled trials to support the use of any of the anticonvulsants currently used in the neonatal period ». This was recently reinforced by a systematic review published in 2015 assuming that « there is an urgent need for more evidence-based studies to guide neonatal seizure management » [5,6]. This vague position resulted from the lack of well conducted trial and from few available data on efficacy and safety of AED use during the neonatal period. Only one randomized controlled trial was performed on AED efficacy in neonates. In this trial, PHB as PHT led to seizure cessation only in 44% of cases in monotherapy and in 60% of cases in association. The seizure burden intensity seemed to be inversely related to the therapeutic success [7]. This restricted efficacy could be explained by the signalling pathway

of PHB on GABA receptors which are paradoxically excitatory in the immature brain before swithching later on to their inhibitory function [8,9]. Further, recurrent seizures induced an intracellular chloride accumulation reinforcing excitatory function of GABA receptors and , then, leading to PHB inefficiency to treat intense seizure burden during the neonatal period [10]. Moreover, some concerns about the safety use of PHB exist. Experimental data showed that PHB increased in a dose-dependant manner neuronal apoptosis in immature brain [11]. Therefore, a need of new efficient and safe AED for newborns has emerged. Levetiracetam (LEV) could be the good candidate to fullfill these criteria. First, LEV exhibit an original way of action by reducing, through the SV2a protein, the glutamate release by presynaptic neuron and, then, regulating the intracellular calcium of postsynaptic neuron through the NMDA and AMPA receptors [12]. Second, LEV seems to be free of toxicity on neonatal brain. Indeed, experimental data demonstrated that LEV did not induce neuronal apoptosis in neonatal brain [13,14]. Further, an observational study showed that LEV cumulative dose received during the neonatal period was not associated with the probability to develop a cerebral palsy later on [15]. Third, an intravenous galenic form of LEV is available allowing the treatment of non-fed newborns. Fourth, an off-label use of LEV as second-line treatment after PHB is now widely observed for neonatal seizures with various dose regimens and administration schedules [16,17]. Fifth, until now, the treatment of approximatively 445 newborns by LEV have been reported with few side-effects including one case of anaphylactic shock and rare sleepy state fostered by a simultaneous PHB treatment [18–22]. In this context, it becomes very important to determine the most effective and safest dose of LEV in neonates following a rigorous and prospective methodology. In this study, a phase II trial have been designed to achieve the ideal LEV loading and maintenance doses in newborns suffering from HIE. An original approach have been chosen by using LEV as the first-line treatment.

METHODS AND ANALYSIS

Study Settings

Patient recruitement will be performed in 5 french Neonatal Intensive Care Units (NICU) (Angers, Lille, Reims, Rennes and Tours) from October 2017 for 2 years. The coordinating site for this study is the Universitary Hospital Center of Tours (France).

Participants

Eligible patients are term newborns with hypoxic ischemic encephalopathy (HIE) and aged less than 72 hours (Figure 1). Briefly, 3 criteria have been chosen: i) birth term above 36 gestational weeks and birth weight above 1800 grams, ii) pernatal asphyxia defined as apgar score equal or under 5 at 5 min and/or resuscitation required at birth and/or metabolic acidosis on ombilic arterial blood gas or until 1 hour after birth (pH < 7.1, Excess Base ≥ 16 mmol/L or lactate ≥ 11), and iii) neurologic impairment in the first 6 hours of life including consciousness, tone, sucking, archaic reflexes and/or pupillar alterations.

Inclusion should be considered when clinical signs and/or an EEG pattern compatible with seizures occur and when a monitoring with a continuous 8-electrode EEG recording is possible. A seizure lasting more than 3 minutes or more than 2 seizures lasting more than 20 seconds on a 1 hour-period on standard EEG recording confirm inclusion criteria. Finally, the written consent of both parents or authorized guardians and a subscription to social security health insurance are required to complete inclusion.

Exclusion criteria concern patients already treated with an AED except a midazolam bolus required for intubation, patients suffering from seizures due to a treatable metabolic aetiology as hypoglycemia and hypocalcemia, patients with a severe renal failure consisting in a serum creatinine above 150 µmol/L, patients with evident signs of genetic or congenital malformation or infectious embryofoetopathy and patients already recruted in another interventional research trial.

Intervention

Therapeutic schedule consists in a loading dose (T0) followed by 8 maintenance doses every 8 hours resultin in a 3 day- treatment period (Figure 2). Eight-hour interval between doses was chosen based on the LEV pharmacokinetics obtained from 18 newborns showing a shorter half-life of 8.9 hours compared to older patients [20]. Four increasing loading doses were chosen: i) 30 mg/kg; ii) 40 mg/kg; iii) 50 mg/kg; iv) 60 mg/kg. Each maintenance dose corresponds to the loading dose quarter (7.5, 10, 12.5 and 15 mg/kg, respectively). LEV will be administered intravenously over 15 min at a final concentration of 5 mg/mL through a central or peripheral line whatever the LEV quantity to infuse is.

Principal Aim

The principal aim is to achieve the most efficient dose regimen under toxicity restrictions of LEV for neonates taking into account efficacy, toxicity and pharmacokinetics, respectively.

Efficacy criteria

Efficacy has been defined as an 80% reduction of seizure burden on EEG recording between the period just before the LEV loading dose (from 20 min to 3 hours) and the 3 hour time-interval from 1 hour 15 min ($T1^{1/4}$) to 4 hours 15 min ($T4^{1/4}$) after the starting of loading dose infusion (T0) (Figure 2). Seizure burden corresponds to the cumulative time of ictal electric activity on the EEG analysed time lap. A first analysis of EEG recording will be performed locally in each investigator center and will be reported in e-CRF format on the 6^{th} day following T0. A second blinded and centralized analysis is planned later on, every 6 months. If there is more than 10% difference between EEG interpreters or an opposite conclusion, a third EEG analysis will be performed. A subsequent correction of efficacy criteria in the statistical model can be performed (whenever it is identified). Efficacy criteria will not be taken into account in the dose allocation process in case of a second AED requirement before $T4^{1/4}$ or an unexpected event in LEV preparation or infusion leading to an unknown injected dose.

Toxicity criteria and safety monitoring

Toxicity will be assessed according to both modalities: i) Short-term toxicity; ii) Long-term toxicity (Figure 2). Short-term toxicity has been designed to trigger quickly a decreasing dose allocation to the next potential participant through a e-CRF alert. Short-term toxicity focuses on 4 adverse events potentially attributable to LEV: i) Severe apnoea leading to mechanical ventilation during the 4-hour period following the LEV infusion [18–21]; ii) Anaphylactic shock occurring during the 30 minutes following the LEV infusion [22]; iii) Toxic epidermic necrosis; iv) Stevens-Jonhson Syndrome. Investigator have to declare the occurrence of one of this adverse event without delay to the pharmacovigilance unit and in e-CRF. At day 6, if none of these adverse events have been observed, investigator ticks the no box corresponding to each effect in the e-CRF and, therefore, short-term toxicity will be considered as negative. Long-term toxicity includes all the adverse events observed and declared to the pharmacovigilance unit up to the hospital discharge or the 30th day of life at the latest. A short-term toxicity alert or any adverse event considered as suspected unexpected serious adverse reaction (SUSAR) will trigger as soon as possible the meeting of the scientific committee composed by a pharmacist (CM) and a neonatologist (GF) and the LEV treatment will be discontinued. If no severe and/or unexpected adverse reaction are declared, a systematic meeting of the scientific committee will be planned during the 10 days following the participant discharge or the participant's 30th day of life at the latest. Scientific committee will decide on imputability (not related/possible/probable) and acceptability of each declared adverse event according to the severity at acute phase, the quality of recovery (partial or complete) with potential subsequent disability and the frequency of occurrence. At the end, a single adverse event considered as imputable to LEV and inacceptable will lead to declare toxicity as positive into the statistical model. Requirement of another AED will be also included into the statistical model as well as the delay between T0 and treatment beginning to

the extent that it could alter proper LEV efficacy and toxicity. An independent data monitoring committee (DMC) have been set-up including a neonatologist, a neuropeadiatrician and a pharmacologist. A DMC opinion on the trial continuation will be solicited every 6 patients or in emergency at the request of the scientific committee.

Secondary objectives

Pharmacokinetics of Levetiracetam: blood samples

LEV pharmacokinetics (PK) in participant blood will be measured at 5 time-points at 30 min, 4 hours and 7 hours after the end of LEV loading dose infusion, respectively and at 1 to 3 hours and 12 hours to 18 hours after the last LEV maintenance dose, respectively (Figure 2). Each PK sample requires 500 µL of blood, i.e. 2.5 mL in total. The pharmacokinetics of LEV in the population of the study will be investigated by a population approach [23]. The mean values of the PK parameters (elimination clearance, central and peripheral distribution volumes, distribution clearance) and their respective interindividual variability will be estimated. Possible relationships between covariates (birth bodyweight, gestational age) and the interindividual variability of the PK parameters will be investigated. Individual PK parameters will be estimated and used to calculated the maximum concentration and the AUC corresponding to the loading dose, after the first maintenance dose, and the cumulative AUC of the entire treatment. Possible relationships between these PK parameters and the efficacy and safety criteria will be investigated, and these pharmacokinetic/pharmacodynamic relationships will be used to determine the optimal dosing regimen.

Seizure recurrence from $T4^{1/4}$ to day 6

Clinical and/or electric seizures occurrence and frequency during LEV treatment (i.e. from $T4^{1/4}$ to T72) and until the complete LEV elimination (i.e. day 6) will be reported in e-CRF as well as concomitant AED treatment. An EEG recording lasting 1 hour will be performed on day 1, 2, 3 and 6 after LEV treatment beginning.

Pre-treatment Seizure Burden and LEV Efficacy

PB and PHT efficacy on complete seizure control have been directly linked to the pre-treatment burden seizure intensity [7]. Then, to explore this association with LEV, a new analysis will be performed retrospectively by adjusting the efficacy criteria to the seizure burden on the pre-treatment EEG. Two subgroups will be considered according to the seizure burden (SB) intensity on the pre-treatment EEG, i.e equal or above to 50% of the EEG recording duration (high SB group) and strictly under 50% of it (low SB group), respectively. LEV efficacy will be considered positive when a SB reduction of 50% will be observed on the post-treatment EEG recording in the high SB group whereas the reduction of 80% will be still valid for the low SB group.

Patient follow-up

The participant follow-up will last up to the hospital discharge or otherwise, at the latest, the 30th day of life. A assessment have been planned consisting in repeated clinical examinations, hemodynamic monitoring, brain imaging and auditory and electroencephalographic recordings (Figure 2). Clinical examinations will be performed at day 0, 1, 2, 3 and 6 through the Thompson score [24] which measure the neurological distress depth. In addition, a Amiel-tison scoring [25] focusing on neurological status of the newborn is planned at the hospital discharge or, at the latest, the 30th day of life. Arterial pressure and heart rate will be measured just before each LEV injection and every 5 minutes for 15 min then every 15 min for 45 minutes after the LEV injection. Apnea, bradycardia under 80 beats per minute and oxygen saturation drop below 85% will be reported. Electroencephalographic recordings will be required, at least, one hour per day during the LEV treatment and a last 1 hour- recording at day 6. Brain MRI will be performed between the 4th to the 8th day of life. A auditory evoked potential measurement will be exigible too before hospital discharge.

Others AED requirement

If a persistence or a recurrence of seizures is observed after the LEV loading dose, investigator is completely free to start an other anti-epileptic treatment. Drug name, administered dose, therapeutic schedule and treatment duration will be reported in e-CRF. If an other AED is required during the 4 hours following LEV loading dose end, efficacy data will not be included in the statistical model.

LEV treatment stop rules

LEV treatment will have to be discontinued if : i) A short-term toxicity or a SUSAR occurs ; ii) Serum creatinine raises above 150 µmol/L in the 7 to 36 hour-interval following the LEV loading dose ; iii) A complete unknown LEV loading dose has been infused due to a hazard event; iv) a mistaken maintenance dose above 60 mg/kg has been infused; v) A limitation of intensive cares begin before the 3rd day of LEV treatment ; vi) At least one of the 2 parents or authorized guardians withdraw their consent.

Statistical model and dose allocation

LEVNEONAT-1 is an open-label, sequential dose-finding study with 4 increasing dose levels. The short term, long term toxicity and the efficacy endpoints were modelled under Bayesian inference. The optimal dose of LEV was defined as the highest efficient dose under toxicity restrictions. Before the beginning of the trial the investigators have chosen efficacy and toxicity thresholds associated with the desirable optimal dose. Indeed, the optimal dose should not be associated with less than 60% of efficacy probability and not more than 10% of short term and long term toxicity probabilities. After the inclusion of successive cohort of 2 patients, the endpoint observations are binarized as; efficacy (yes/no), short-term toxicity (yes/no, if yes when), long-term toxicity (yes/no), other AED use (yes/no and if yes, when) and the number of infused maintenance doses with timing (Figure 1). A statistical model was designed specifically for this trial as no other dose-allocation method was available for this indication. It is a sequential adaptive method as it uses all of the available information before

trial onset and all the data from the trial that have been accumulated each new cohort inclusion. On the basis of updated data, probabilities of efficacy, short term and long term toxicities are re-estimated after each cohort. The dose allocated to each further cohort was the estimated optimal dose known so far. The first cohort of patients will receive the lowest dose level and doses will be increased one by one according to the model estimates (no dose skipping will be allowed if the dose was not yet evaluated). Moreover, as long term toxicity will be long to be observed a time to event approach will be considered to avoid stopping inclusions between two successive cohorts.

When a short-toxicity alert occurs, a reduction of current loading dose allocated to the lower level is planned until the scientific committee's conclusion concerning LEV imputability or not. The maximal sample size expected is 50 participants with a minimum of 24 patients unless safety stopping criteria are fulfilled. Furthermore, the trial will be stopped prematurely if all doses do not reach efficacy threshold or the lowest dose exceed toxicity threshold. Exclusion of a patient from the dose-finding allocation design will be considered when efficacy criteria cannot be reliable because either the real loading dose infused was completely ignored by investigator due to a hazard event or another AED has been required before the post-injection EEG recording end (i.e. $T4^{1/4}$).

Trial interruption criteria

Three criteria have been identified: i) a high probability of wrong dose range (either for efficacy or for toxicity) will lead to a temporary interruption of the trial. After the IMC consulting, a new range of doses could be proposed, ii) new valid information are published during the course of LEVNEONAT-1 answering to the principal aim and making this trial outdated, iii) the scientific committee can decide to stop the trial at any time if a unacceptable toxicity is assigned to LEV.

Patient and Public Involvement

Patients and or public were not involve in the design of the study.

ETHICS

LEV cannot be infused prior of obtaining the written parental or authorized guardians consent. One of parents or authorized guardians can retrieve their consent at any time leading to the interruption of the newborn participation to LEVNEONAT-1. Nevertheless, safety monitoring will be performed to assure adequate treatment of potential LEV side effect but it will not be recorded in the database. An authorization from parents or authorized guardians will be necessary to use the data obtained before the agreement retrieval.

Ethics approval for this study (version 4, 06-06-2017) have been obtained from regional ethical committee (CPP Ouest 1) under the reference 2016-R25 on the November 9th 2016. The French drug safety agency (Agence Nationale de la Sécurité du Médicament) approved LEVNEONAT-1 (version 4, 06-06-2017) under the reference 160652A-31 on the October 5th 2016.

This trial has been registered on EudraCT (February 20 2014) and on Clinical Trial.gov (September 1 2014). The Eudra CT reference is 2014-000791-26. The Clinical Trail.gov reference is NCT02229123.

DATA QUALITY

A agent will be assigned by the sponsor , i.e. Universitary Hospital Center of Tours, for meeting investigators and local research teams regularly according to the inclusion dynamic. These on-site visits aim to check the regular filling of consent form, the protocol respect and the accuracy of recorded data from source documents. An audit trigger by the French drug Safety Agency could be possible at any time of the trial course. Data management have been validated through the MR-001 reference methodology.

DISSEMINATION

Any modifications to the protocol which may impact on the conduct of the study or may affect patient safety will lead to a substantive protocol amendment and will be reviewed by regional Ethics Committee and the French Drug Safety agency. This substantive changes will be communicated to relevant stakeholders (trial registries, regulatory agencies, investigators). Results of LEVNEONAT-1 study will be published in peer-reviewed journal following the Uniform Requirements For Manuscripts Submitted to Bio-medical Journals (htttp://www.icmje.org/). Publications will be distributed to investigating centers and to all relevant person or organization. The LEVNEONAT-1 study will also be presented at relevant national and international medical and scientific meetings related to both aspects: i) Methodology and biostatistics; ii) Brain development and seizure treatment during the neonatal period. At the end of the study, a summary of results will be produced for non-medical public and will be given to participant's parents on demand.

TIME-LINE

Investigating centers have been open successively from the 21th of September 2017 to the 20th of October 2017. Patient recruitment is effective from the 20th of October 2017 for 2 years.

DISCUSSION

Seizure management at the neonatal period remains elusive and PHB is not completely efficient to stop critical activity and not really safe for the immature brain. LEV could be promising and more suitable in this condition. However, although LEV is widely used in neonatal care units worldwide, no dose regimen has been clearly established.

LEVNEONAT-1 is particularly original by using LEV as the first-line treatment and not in add-on after PHB resulting in purer efficacy and safety data and opening the possibility of a new therapeutic schedule in neonates. The other original characteristic of LEVNEONAT-1 is

the design of statistical model allowing a restricted sample size to determine optimal LEV dose in neonates by integrating dynamically data of each participant.

The first LEVNEONAT-1 weakness is the targeted population, i.e. the newborn less than 3 days of life suffering from a HIE complicated with seizures. Indeed, seizure incidence in the HIE context vary according to studies from 29% to 65% of cases [26–28]. Surprisingly, this discrepancy could be due, in part, to the care-giver ability to recognize clinical sign of seizure and ictal activity on aEEG recording [29–31]. In parallel, the relative emergency to obtain parent consent in this stressful context remains a sensitive point for investigator. However, the median time of the first seizures reported in literature was around 9 to 13 hours of life [26,32] leaving a potential interval for reflexion to the parents. The second critical point is the opportunity to monitor the newborn by a standard EEG as soon as seizures are identified with various logistical problems according to each investigator centers including a variable delay or, worst, the inability to implement standard EEG monitoring out of the working hours.

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AUTHORS CONTRIBUTION

GF, ES are implicated in the study design included aims, assessment criteria and intervention schedule ; SZ, MU have been implicated in the assessment criteria and the trial design, they have developped the dose-finding model; CM, GF assume pharmacovigilance procedure ; EB deals with legal aspect and authorizations ; JL, AR, ET designed e-CRF and assume the logistic organisation necessary to record on live data in the statistical model and to deal with e-CRF alerts ; VJ designed pharmacokinetics time-points and perform the LEV pharmacokinetics measurements.

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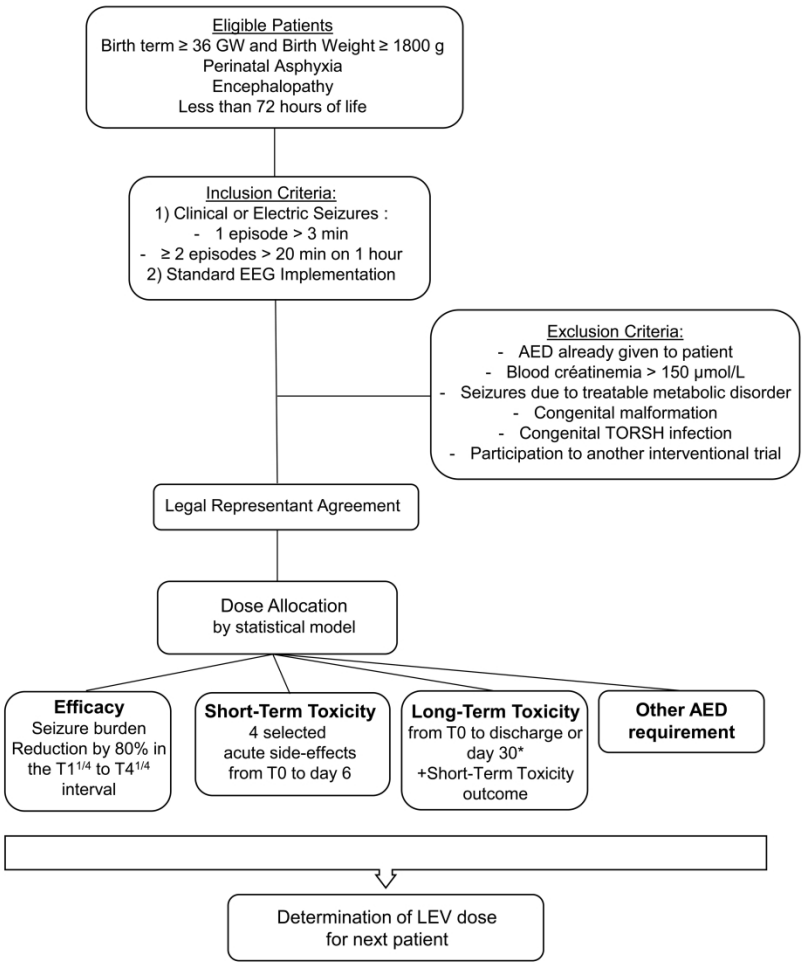
COMPETING INTEREST STATEMENT

The authors declare they have no competing interest.

FIGURE LEGEND

Figure 1: LEVNEONAT-1 Study Flowchart. GW: Gestational Weeks; EEG: Electroencephalogram; AED: Anti-Epileptic Drug; T0: Levetiracetam Loading Dose Infusion Start; $T1^{1/4}$: 1 hour and 15 min from the levetiracetam loading dose infusion start; $T4^{1/4}$: 4 hours and 15 min from the levetiracetam loading dose infusion start; LEV: Levetiracetam

Figure 2: LEVNEONAT-1 Experimental Schedule and Time-Line. LEV: Levetiracetam; PK: Pharmacokinetic; EFF: Efficacy; TOX: Toxicities; AED: Anti-Epileptic Drug; EEG: Electroencephalogram; MRI: Magnetic Resonance imaging; AEP: Auditory Evoked Potentials.



Favrais G et al., Figure 1

Figure 1: LEVNEONAT-1 Study Flowchart. GW: Gestational Weeks; EEG: Electroencephalogram; AED: Anti-Epileptic Drug; T0: Levetiracetam Loading Dose Infusion Start; T11/4: 1 hour and 15 min from the levetiracetam loading dose infusion start; T41/4: 4 hours and 15 min from the levetiracetam loading dose infusion start; LEV: Levetiracetam

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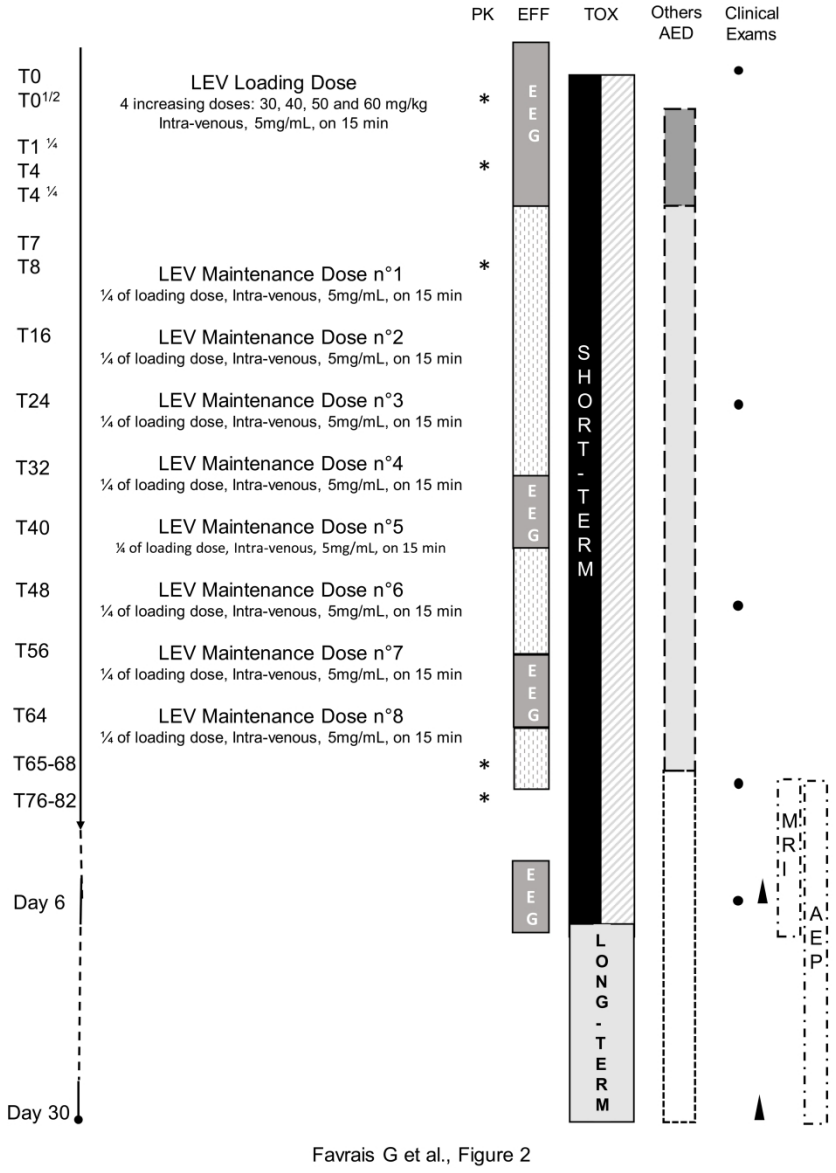

Favrais G et al., Figure 2

Figure 2: LEVNEONAT-1 Experimental Schedule and Time-Line. LEV: Levetiracetam; PK: Pharmacokinetic; EFF: Efficacy; TOX: Toxicities; AED: Anti-Epileptic Drug; EEG: Electroencephalogram; MRI: Magnetic Resonance imaging; AEP: Auditory Evoked Potentials.

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Reporting checklist for protocol of a clinical trial.

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		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	3, 13
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	14
Funding	#4	Sources and types of financial, material, and other support	19
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1, 18
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	1

sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	18
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	9
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	NA
Objectives	#7	Specific objectives or hypotheses	6, 8, 10
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8

Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	12
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	NA
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8,11
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	11, Fig 2
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	13
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	11
Allocation concealment	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed	11

mechanism		envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	NA
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	14
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11

Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	9
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	11
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	13
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	14
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	20
Data access	#29	Statement of who will have access to the final trial dataset,	14

		and disclosure of contractual agreements that limit such access for investigators	
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	14
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	14
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

BMJ Open

A multi-center, single group, open study, levetiracetam optimal dose-finding as first-line treatment for neonatal seizures occurring in hypoxic-ischemic encephalopathy context (LEVNEONAT-1): study protocol of a phase II trial

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Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Neurology, Research methods, Evidence based practice
Keywords:	levetiracetam, newborn, seizure, hypoxic-ischemic encephalopathy, phase II trial

A multi-center, single group, open study, levetiracetam optimal dose-finding as first-line treatment for neonatal seizures occurring in hypoxic-ischemic encephalopathy context (LEVNEONAT-1): study protocol of a phase II trial

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Keywords : levetiracetam, newborn, seizure, hypoxic-ischemic encephalopathy, phase II trial

ABSTRACT

INTRODUCTION: Therapeutic schedules for treating neonatal seizures remain elusive. First-line treatment by phenobarbital is widely supported but without strong scientific evidence. Levetiracetam is an emerging and promising anti-epileptic drug (AED). The aim of this phase II trial is to determine the benefits of levetiracetam by applying a strict methodology and estimate the optimal dose of levetiracetam as a first-line AED to treat seizures in newborns suffering from hypoxic-ischemic encephalopathy.

METHODS AND ANALYSIS: LEVNEONAT-1 is an open and sequential levetiracetam dose-finding study. The optimal dose is that which is estimated to be associated with a toxicity not exceeding 10% and an efficacy higher than 60%. Efficacy is defined by a seizure burden reduction of 80% after the loading dose. Four increasing dose regimens will be assessed including one loading dose of 30, 40, 50 or 60 mg/kg followed by eight maintenance doses (i.e., loading dose quarter) injected every eight hours. A two-patient cohort will be necessary at each dose level to consider an upper dose level assignment. The maximal sample size expected is 50 participants with a minimum of 24 patients or fewer in the case of a high rate of toxicity. Patients will be recruited in five French neonatal intensive care units beginning in October 2017 and continuing for two years. In parallel, the levetiracetam pharmacokinetic will be measured at five times (i.e, 30 minutes, four and seven hours from the loading dose and one to three hours and 12 hours to 18 hours from the last maintenance dose).

ETHICS AND DISSEMINATION: Ethics approval has been obtained from the regional ethical committee (2016-R25) and the French Drug Safety Agency (160652A-31). Results will be published in a peer-reviewed journal. Results will also be presented at medical meetings.

TRIAL REGISTRATION NUMBER: Eudra CT, 2014-000791-26. ClinicalTrial.gov, NCT02229123, Pre-results.

Strengths and limitations of this study

- For the first time, levetiracetam will be used as the first-line treatment for neonatal seizures rather than as an add-on therapy.
- The statistical model is designed for a rare clinical situation with a sequential adaptive method which updates in real time the dose allocation for the next patient based on all available data from previous participants.
- The design performances were assessed through extensive simulation studies. On average, the proposed design prompts recommendations of the correct dose at about 60% of efficacy for a sample size of 30, increasing to over 80% in many scenarios for a sample size of 50. Moreover, this method maintains an acceptable number of neonates with toxicities. In case of promissing efficacy results, a randomized study should be performed further to confirm it.
- The targeted population (i.e., the newborn less than 3 days of life) is particularly vulnerable, and the ethical authority requires the written consent of both parents before the levetiracetam administration. This could be a critical point for inclusion since seizures can occur early after birth and therefore require an anti-epileptic drug (AED) treatment in emergency. Both parents should then be informed soon after birth even prioir to seizure occurrence, and the reflection period of participants'guardians could be brief.
- Continuous EEG monitoring is scheduled during the three day-treatment by levetiracetam and an one-hour recording on day six. Unfortunately, the restricted availability of EEG device in each investigating center led after the cessation of seizure burden to a minimal requirement of an one- hour EEG recording on day one, two, three and six.

INTRODUCTION

Neonatal seizures occur in approximately one to five per 1000 live births [1]. Hypoxic-ischemic encephalopathy (HIE) represents the first etiology of neonatal seizures [1,2]. In 80% of cases, seizures associated with HIE occurred in the first three days of life [2]. Seizure treatment during the neonatal period creates issues concerning the developing brain facing deleterious effects of seizure burden to the potential toxicity of conventional anti-epileptic drugs (AED). Indeed, Van Rooig et al. demonstrated that the seizure duration was correlated with brain lesions through MRI [3]. Therefore, the World Health Organisation recommended in 2011 that anticonvulsivant treatment should be initiated immediately when clinically apparent seizures have lasted for longer than three minutes or in the case of brief repeated seizures. This recommendation was supported strongly but no gradation was attributable due to the lack of scientific evidence [4]. Furthermore, conventional AEDs such as phenobarbital (PHB) are employed in an off-label manner in neonates. Epidemiologic studies have illustrated that PHB is widely used as the first-line treatment across the world with a lack of consensus concerning subsequent add-on lines, including phenytoin (PHT), lidocaïne, midazolam and others benzodiazepins [5]. Surprisingly, a meta-analysis from the Cochrane Data Base concluded in 2004 that 'there is little evidence from randomised controlled trials to support the use of any of the anticonvulsants currently used in the neonatal period'. This was recently reinforced by a systematic review published in 2015, which presumed that 'there is an urgent need for more evidence-based studies to guide neonatal seizure management' [5,6]. This vague position resulted from the lack of well-conducted trials and from the limited available data regarding efficacy and safety of AED use during the neonatal period. Only one randomised controlled trial was performed which concerned AED efficacy in neonates. In this trial, PHB as PHT precipitated seizure cessation in merely 44% of cases in monotherapy and in 60% of cases in association. The seizure burden intensity appeared to be inversely related

to therapeutic success [7]. This restricted efficacy may be explained by the signalling pathway of PHB on GABA receptors, which are paradoxically excitatory in the immature brain before transitioning to their inhibitory function [8,9]. Furthermore, recurrent seizures induced an intracellular chloride accumulation, reinforcing excitatory function of GABA receptors and then creating PHB inefficiency in treating intense seizure burden during the neonatal period [10]. Moreover, some concerns about the safety of PHB exist. Experimental data has demonstrated that PHB increased in a dose-dependent manner neuronal apoptosis in the immature brain [11]. Therefore, a necessity for new efficient and safe AED for newborns has emerged. Levetiracetam (LEV) may be the appropriate candidate to fulfil these criteria. First, LEV exhibits an original means of action by reducing, through the SV2a protein, the glutamate release by presynaptic neuron and then regulating the intracellular calcium of postsynaptic neurons through NMDA and AMPA receptors [12]. Second, LEV appears to be free of toxicity in relation to the neonatal brain. Indeed, experimental data has demonstrated that LEV did not induce neuronal apoptosis in the neonatal brain [13,14]. Furthermore, an observational study illustrated that LEV cumulative doses received during the neonatal period were not associated with the probability of subsequently developing a cerebral palsy [15]. Third, an intravenous galenic form of LEV is available, allowing the treatment of non-fed newborns. Fourth, an off-label use of LEV as second-line treatment after PHB has now been widely observed [16,17]. Retrospective studies reported levetiracetam use in neonates with various dose regimens and administration schedules such as increasing doses until seizure cessation, similar doses twice a day, or a loading dose with subsequent maintenance doses. Loading doses infused to neonates varied from 10 mg/kg to 60 mg/kg [17–25]. The maximal amount of LEV infused in a newborn was 150 mg/kg within a 24-hour period [17]. Fifth, until now, the treatment of approximatively 445 newborns through LEV has been reported with limited side effects including one case of anaphylactic shock and a rare sleepy state fostered

by a simultaneous PHB treatment [19,22,23,26,27]. Similarly, LEV efficacy for significantly reducing or eliminating neonatal seizures has been recently estimated at 77% of cases in first-line treatment and 66% of cases in add-on therapy in a cohort of 102 patients from five retrospective studies [28]. In this context, it is highly important to determine the benefits of levetiracetam for treating neonatal seizures and to determine the most effective and safest dose of LEV in neonates following a rigorous and prospective methodology. In this study, a phase II trial has been designed to achieve the ideal LEV loading and maintenance doses in newborns suffering from HIE. An original approach has been adopted by using LEV as the first-line treatment.

METHODS AND ANALYSIS

Study Settings

Patient recruitement will be performed in five French Neonatal Intensive Care Units (NICU) (Angers, Lille, Rouen, Rennes, and Tours) beginning in October 2017 and continuing for two years. These centres have been selected for their expertise in managing neonatal seizures. The coordinating site for this study is the University Hospital Center of Tours (France). Protocol have been written according to the SPIRIT reporting guidelines [29].

Participants

Eligible patients are term newborns with hypoxic ischemic encephalopathy (HIE) who are less than 72 hours old (Figure 1). Briefly, three criteria have been selected: i) birth term above 36 gestational weeks and birth weight above 1800 grams, ii) perinatal asphyxia, defined as Apgar Score equal to or below five at five minutes, or resuscitation required at birth, or metabolic acidosis on umbilical arterial blood gas or until one hour after birth ($pH < 7.1$, Excess Base ≥ 16 mmol/L or lactate ≥ 11), and iii) neurologic impairment in the first six hours of life, including consciousness, tone, sucking, archaic reflexes, or pupillary alterations.

Brain cooling implementation is delegated entirely to the discretion of investigators according to the French guidelines [30]. Therapeutic hypothermia implementation will be reported in the e-CRF form as well as the rewarming time. Since seizures generally occur during moderate and severe HIE requiring therapeutic hypothermia, it is expected that the majority of participants are on brain cooling. Therapeutic hypothermia is regarded as a variable in pharmacokinetic analysis.

Inclusion should be considered when clinical signs or an EEG pattern compatible with seizures will be recognised by investigator and when monitoring with a continuous 8-electrode EEG recording is possible. A seizure lasting more than three minutes or more than two seizures lasting more than 20 seconds within a one-hour period on a standard EEG recording fulfils inclusion criteria. Critical-activity recognition on EEG recording is based on the investigator‘s experience. A specific training for recognizing critical activity on standard EEG has been provided to investigators in each centres. An inclusion could be diagnosed incorrectly by a retrospective interpretation of a EEG recording by a local electrophysiologist planned as soon as possible (cf. *Efficacy Criteria*). A second interpretation by an independent and blind reader will be organised in a brief delay to assess the lack of critical activity on the EEG recording. If the lack of critical activity on the EEG is confirmed, the patient will not be included in the statistical model, and the experimental treatment will be immediately disrupted, but pharmacokinetic and safety analysis will be performed. Finally, the written consent of both parents or authorised guardians and a subscription to social security health insurance are required to complete inclusion.

Exclusion criteria concern patients already treated with an AED aside from a midazolam bolus required for intubation, patients suffering from seizures due to a treatable metabolic aetiology such as hypoglycemia and hypocalcemia, patients with severe renal failure associated with serum creatinine above 150 µmol/L, patients with evident signs of genetic or

congenital malformations or infectious embryofoetopathy and patients who have already been recruited in another interventional research trial.

Intervention

A therapeutic schedule consists of a loading dose (T0) followed by eight maintenance doses every eight hours resulting in a three-day treatment period (Figure 2). Eight-hour intervals between doses were implemented based on the LEV pharmacokinetics obtained from 18 newborns, demonstrating a shorter half-life of 8.9 hours relative to older patients [26]. Four increasing loading doses were selected: i) 30 mg/kg; ii) 40 mg/kg; iii) 50 mg/kg; iv) 60 mg/kg. Each maintenance dose corresponds with the loading dose quarter (7.5, 10, 12.5 and 15 mg/kg, respectively). Levetiracetam (levetiracetam Mylan®, 100 mg/ml) will be diluted in 5% glucose solution to a final concentration of 5 mg/mL. LEV will be administered intravenously over a fixed time of 15 minutes through a central or peripheral line.

Principal Aim

The principal aim is to achieve the most efficient dose regimen under toxicity restrictions of LEV for neonates while accounting for efficacy, toxicity, and pharmacokinetics, respectively.

Efficacy criteria

Efficacy has been defined as an 80% reduction of seizure burden in a EEG recording between the period immediately before the LEV loading dose (from 20 minutes to three hours) and the three-hour time interval from one hour and 15 minutes ($T1^{1/4}$) to four hours and 15 minutes ($T4^{1/4}$) after the beginning of loading dose infusion (T0) (Figure 2). Seizure burden corresponds with the cumulative time of ictal electric activity on the EEG- analysed time lap. A first analysis of EEG recording will be performed locally in each investigator center and will be reported in e-CRF format on the sixth day following T0. A second blinded and centralised analysis is scheduled to occur subsequently, every six months. If there is more than a 10% difference between EEG interpreters or an opposite conclusion, a third EEG

analysis will be performed. A subsequent correction of efficacy criteria in the statistical model can be performed (whenever it is identified). Efficacy criteria will not be accounted for in the dose allocation process in case of a second AED requirement before T4[1/4] or an unexpected event in LEV preparation or infusion precipitating an unknown dose injection.

Toxicity criteria and safety monitoring

Toxicity will be assessed according to both modalities: i) Short-term toxicity; ii) Long-term toxicity (Figure 2). Short-term toxicity has been designed to rapidly trigger a decreasing dose allocation to the next potential participant through an e-CRF alert. Short-term toxicity focuses on four adverse events potentially attributable to LEV: i) Severe apnea which leads to mechanical ventilation during the four-hour period following the LEV infusion [19,22,23,26]; ii) Anaphylactic shock occurring during the 30 minutes following the LEV infusion [27]; iii) Toxic epidermic necrosis; iv) Stevens-Johnson Syndrome. Investigators must declare the occurrence of one of these adverse events immediately to the pharmacovigilance unit and in e-CRF. On day six, if none of these adverse events have been observed, the investigator ticks the 'no' box which corresponds to each effect in the e-CRF and short-term toxicity will therefore be regarded as negative. Long-term toxicity encompasses all of the adverse events observed and declared to the pharmacovigilance unit up to the hospital discharge, or the 30th day of life at the latest. A short-term toxicity alert or any serious unexpected suspected adverse reaction (SUSAR) will immediately trigger the meeting of the scientific committee, which consists of a pharmacist (CM) and a neonatologist (GF), and the LEV treatment will be discontinued. If no severe or unexpected adverse reactions are declared, a systematic meeting of the scientific committee will be planned during the 10 days following the participant discharge or the participant's 30th day of life at the latest. The scientific committee will then determine imputability (unrelated/possible/probable) and acceptability of each declared adverse event based on the severity at the acute phase, the quality of recovery (partial or

complete) with potential subsequent disability, and the frequency of occurrence. Ultimately, a single adverse event regarded as imputable to LEV and inacceptable precipitates a declaration of toxicity as positive in the statistical model. The requirement of another AED will also be included in the statistical model as well as the delay between T0 and treatment beginning to the extent that it could alter proper LEV efficacy and toxicity.

Secondary objectives

Pharmacokinetics of levetiracetam: blood samples

LEV pharmacokinetics (PK) in participant blood will be measured at five times at 30 minutes, four hours, and seven hours after the end of LEV loading dose infusion, respectively, and at one to three hours and 12 hours to 18 hours after the last LEV maintenance dose, respectively (Figure 2). Each PK sample requires 500 µL of total blood (i.e., 2.5 mL in total). After centrifugation, plasma will be harvested, and samples will be frozen at -20 or -80 degrees before sending for measurement (VJ). The pharmacokinetics of LEV in the population of the study will be investigated through a population approach [31]. The mean values of the PK parameters (elimination clearance, central and peripheral distribution volumes, distribution clearance) and their respective interindividual variability will be estimated. Possible relationships between covariates (birth bodyweight, gestational age, therapeutic hypothermia) and the interindividual variability of the PK parameters will be investigated. Individual PK parameters will be estimated and used to calculated the maximum concentration and the AUC corresponding with the loading dose, after the first maintenance dose, and the cumulative AUC of the entire treatment. Potential relationships between these PK parameters and the efficacy and safety criteria will be investigated, and these pharmacokinetic/pharmacodynamic relationships will be used to determine the optimal dosing regimen.

Seizure recurrence from $T4^{1/4}$ to day 6

Clinical or electric seizures occurrence and frequency during LEV treatment (i.e., from T4[1/4] to T72) and until complete LEV elimination (i.e., day six) will be reported in e-CRF in addition to concomitant AED treatment. The complete and definitive cessation of seizures will be notified in e-CRF. Continuous EEG monitoring is scheduled during the three- day treatment by LEV as well as an one-hour recording on day six. Unfortunately, the restricted availability of EEG devices in each investigating center after the cessation of seizure burden prompted a minimal requirement of an one-hour EEG recording on day one, two, three and six after the LEV treatment initiation. Then, the detection of seizure recurrence and the duration of EEG monitoring is then under the responsibility of each investigator.

Pre-treatment seizure burden and LEV efficacy

PB and PHT efficacy in relation to complete seizure control have been directly associated with the pre-treatment burden seizure intensity [7]. Therefore, to explore this association with LEV, a new analysis will be performed retrospectively by adjusting the efficacy criteria to the seizure burden on the pre-treatment EEG. Two subgroups will be considered based on the seizure burden (SB) intensity on the pre-treatment EEG which is equal to or above 50% of the EEG recording duration (high SB group) and strictly under 50% of it (low SB group), respectively. LEV efficacy will be deemed positive when a SB reduction of 50% is observed on the post-treatment EEG recording in the high SB group, whereas the reduction of 80% will still be valid for the low SB group.

Patient follow-up

The participant follow-up will continue until hospital discharge or until the 30th day of life. An assessment has been planned which consists of repeated clinical examinations, hemodynamic monitoring, brain-imaging, and auditory and electroencephalographic recordings (Figure 2). Clinical examinations will be performed at day one, two, three and six through the Thompson score, [32] which measure the neurological distress depth. In addition,

an Amiel-Tison scoring, [33] focusing on neurological status of the newborn, is planned to occur upon hospital discharge or on the 30th day of life. Arterial pressure and heart rate will be measured immediately before each LEV injection and every five minutes for 15 minutes, and then every 15 minutes for 45 minutes after the LEV injection. Apnea, bradycardia under 80 beats per minute, and oxygen saturation drops below 85% will be reported. Brain MRI will be performed between the fourth to the eighth day of life. An auditory evoked potential measurement will also be required before hospital discharge.

Others AED requirements

If persistence or recurrence of seizures is observed after the LEV loading dose, investigators are completely free to initiate another anti-epileptic treatment. Drug name, administered dose, therapeutic schedule, and treatment duration will be reported in the e-CRF. If another AED is required during the four hours following LEV loading dose end, efficacy data will not be included in the statistical model.

LEV treatment cessation rules

LEV treatment will have to be discontinued in any of the following cases : i) a short-term toxicity or a SUSAR occurs ; ii) Serum creatinine raises above 150 µmol/L in the seven- to 36- hour interval following the LEV loading dose ; iii) a complete unknown LEV loading dose has been infused due to a hazardous event; iv) a mistaken maintenance dose above 60 mg/kg has been infused; v) a limitation of intensive cares begins before the third day of LEV treatment ; vi) at least one of the two parents or authorised guardians withdraws his consent.

Statistical model and dose allocation

LEVNEONAT-1 is an open-label, single group, sequential dose-finding study with four increasing dosage levels. The short-term, long-term toxicity, and the efficacy endpoints were modelled under Bayesian inference. The optimal dose of LEV was defined as the highest efficient dose under toxicity restrictions. Before the beginning of the trial, investigators have

selected efficacy and toxicity thresholds associated with the desirable optimal dose. The optimal dose should not indeed be associated with less than 60% of efficacy probability and no more than 10% of short-term and long-term toxicity probabilities. After the inclusion of successive cohorts of two patients, the endpoint observations are binarised as the following: efficacy (yes or no), short-term toxicity (yes or no, if yes when), long-term toxicity (yes or no), other AED use (yes or no and if yes, when) and the number of infused maintenance doses with timing (Figure 1). A statistical model was designed specifically for this trial, since no other dose-allocation method was available for this indication. It is a sequential adaptive method since it incorporates all of the available informations before trial onset and all of the data from the trial which have been accumulated for each new cohort inclusion. Based on updated data, probabilities of efficacy, short-term and long-term toxicities are re-estimated after each cohort. The dose allocated to each further cohort is the estimated optimal dose known thus far. The first cohort of patients will receive the lowest dose level, and doses will be increased one-by-one based on the model estimates (no dose-skipping will be allowed if the dose was not yet evaluated). Moreover, since long-term toxicity will be long to be observed, a time to event approach will be considered to avoid ceasing inclusions between two successive cohorts.

When a short-toxicity alert occurs, a reduction of current loading dose allocated to the lower level is planned until the scientific committee's conclusion concerning LEV imputability or lack thereof. The maximal sample size is expected to be 50 participants with a minimum of 24 patients. However, the trial will be terminated prematurely if all doses do not reach the efficacy threshold or the lowest dose exceed toxicity threshold. When a patient is eligible, the current dose regimen is available on the trial sponsor's web site. This dose will be renewed in real time according to the previous participants'data.

Independent data-monitoring committee

An independent data-monitoring committee (DMC) have been established, which includes a neonatologist, a neuropeadiatrician and a pharmacologist. A DMC opinion concerning the trial continuation will be solicited every six patients or in the case of an emergency upon the request of the scientific committee.

Trial interruption criteria

Three criteria have been identified: i) a high probability of incorrect dose range (either for efficacy or for toxicity) will cause a temporary interruption of the trial. After the IMC consultation, a new range of doses could be proposed, ii) new valid information are published during the course of LEVNEONAT-1 which address the principal aim and render this trial outdated, iii) the scientific committee can decide to terminate the trial at any time if an unacceptable toxicity is assigned to LEV.

Patient and public involvement

Patients and the public were not involved in the design of the study.

ETHICS

LEV cannot be infused prior to obtaining the written parental or authorised guardians consent. One of two parents or authorized guardians can withdraw their consent at any time prompting the interruption of the newborn participation to LEVNEONAT-1. Nevertheless, safety monitoring will be performed to ensure adequate treatment of potential LEV side effects but it will not be recorded in the database. An authorisation from parents or authorised guardians will be necessary to use the data obtained before the agreement withdrawal.

Ethical approval for this study (version 4, 06-06-2017) have been obtained from regional ethical committee (CPP Ouest 1) under the reference 2016-R25 on the 9th of November 2016. The French drug safety agency (Agence Nationale de la Sécurité du Médicament) approved LEVNEONAT-1 (version 4, 06-06-2017) under the reference 160652A-31 on the 5th of October 2016.

This trial has been registered on EudraCT (20 February, 2014) and on Clinical Trial.gov (1 September, 2014). The Eudra CT reference is 2014-000791-26 and the Clinical Trail.gov reference is NCT02229123. Trial registration data are reported in Table 1.

DATA QUALITY

Standardised LEV prescriptions have been designed in an Excel format. These documents have been joined to the allocation-dose web site. Therefore, investigator will only fill the allocated dose and the participant's birth weight to obtain personalised LEV prescriptions (dilution, quantity, infusion speed, monitoring). For each included participant, all data will be anonymised under a specifc code (centre city and number of inclusion) and a personalised filed (e-CRF) will be created on the trial sponsor's web site. Data will be reported in the participant's e-CRF accessible with a personal code (lead investigator). Lead investigators will only have access to the e-CRF of their own centre. Only coordinating investigator (GF), scientific committee members (GF, CM) and data manager (EB) have access to all participants'data. Data will be checked by faxing original paper documents (drug prescriptions, vital-sign monitorings, biological measurements, EEG interpretations) to the trial sponsor. An agent (EB) will be assigned by the sponsor (i.e., Universitary Hospital Center of Tours) for meeting lead investigators and local research teams regularly, based on the inclusion dynamic. These on-site visits aim to monitor the regular filing of consent forms, the compliance with the protocol and the accuracy of recorded data from source documents. An audit trigger by the French Drug Safety Agency could be possible at any time of the trial course. Data management have been validated through the MR-001 reference methodology.

DISSEMINATION

Any modifications to the protocol which may impact the conduct of the study or affect patient safety will precipitate a substantive protocol amendment and will be reviewed by the regional Ethics Committee and the French Drug Safety Agency. These substantive changes will be

communicated to relevant stakeholders (trial registries, regulatory agencies, investigators). Results of the LEVNEONAT-1 study will be published in a peer-reviewed journal following the Uniform Requirements For Manuscripts Submitted to Bio-medical Journals (htttp://www.icmje.org/). Publications will be distributed to investigation centers and to all relevant persons or organisations. The LEVNEONAT-1 study will also be presented at relevant national and international medical and scientific meetings related to both elements: i) methodology and biostatistics; ii) brain development and seizure treatment during the neonatal period. At the end of the study, a summary of results will be produced for the non-medical public and will be provided to participants' parents on demand.

TIME-LINE

Investigation centers have been open from the 21th of September, 2017 to the 20th of October, 2017. Patient recruitment is effective from the 20th of October 2017 for two years. The first enrolment occured on February 2018.

DISCUSSION

Seizure management at the neonatal period remains elusive, and PHB is not completely efficient in terminating critical activity and is not truly safe for the immature brain. LEV may be promising and more suitable in this condition. However, although LEV is widely used in neonatal care units worldwide, no dose regimen has been clearly established.

LEVNEONAT-1 is particularly original by using LEV as the first-line treatment and not in add-ons after PHB, resulting in purer efficacy and safety data and allowing the possibility of a new therapeutic schedule in neonates. The other original characteristic of LEVNEONAT-1 is the design of a statistical model allowing a restricted sample size to determine optimal LEV dose in neonates by integrating data in real time of each participant.

The first LEVNEONAT-1 weakness is the targeted population, which consists of newborns with fewer less than three days of life who suffer from a HIE complicated with seizures.

Indeed, seizure incidence in the HIE context varies according to studies, from 29% to 65% of cases [34–36]. Surprisingly, this discrepancy may be partially due to the caregiver's ability to recognise clinical signs of seizure and ictal activity on an EEG recording [37–39]. In parallel, the relative urgency to obtain parental consent in this stressful context remains a sensitive issue for investigators. However, the median time of the first seizures reported in the literature was around nine to 13 hours of life [34,40], allowing time for reflection to the parents. The second critical point is the opportunity to monitor the newborns through a standard EEG as soon as seizures are identified with various logistical problems according to each investigation center including a variable delay or the inability to implement standard EEG monitoring outside of working hours.

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AUTHORS CONTRIBUTION

GF and ES are implicated in the study design including aims, assessment criteria and intervention schedule ; SZ and MU provided statistical expertise in trial design, they have developped the dose-finding model, and they are conducting the statistical analysis; CM and GF assume the pharmacovigilance procedure ; EB handles legal aspects and authorisations, and helped with implementation ; VJ designed pharmacokinetic analysis and performed the LEV pharmacokinetic measurements. All authors contributed to refinement of the study protocol and approved the final manuscript.

FUNDING STATEMENT

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COMPETING INTEREST STATEMENT

The authors declare that they have no competing interest.

PROTOCOL VERSION

Issue date: 6 Jun 2017

Protocol Amendment number: 04

Authors: GF, ES, SZ, CM and EB

Revision chronology

2016-Jul-1 Original

2016-Nov-3 Amendment N°1

At the request of French Drug Safety Agency, precisions about the procedure triggered by the occurrence of a side effect and the allocation-dose process have been added to the protocol.

2017-May-26 Amendment N°2

At the request of French Drug Safety Agency, the upper dose regimen including a loading dose at 60 mg/kg have been withdrawn considering the lack of significant data justifying this dosage in newborns.

2017-Jun-6 Amendment N°3

The upper dose level including a loading dose at 60 mg/kg have been validated by the French Drug Safety Agency in light of Venkatesan et al. [17].

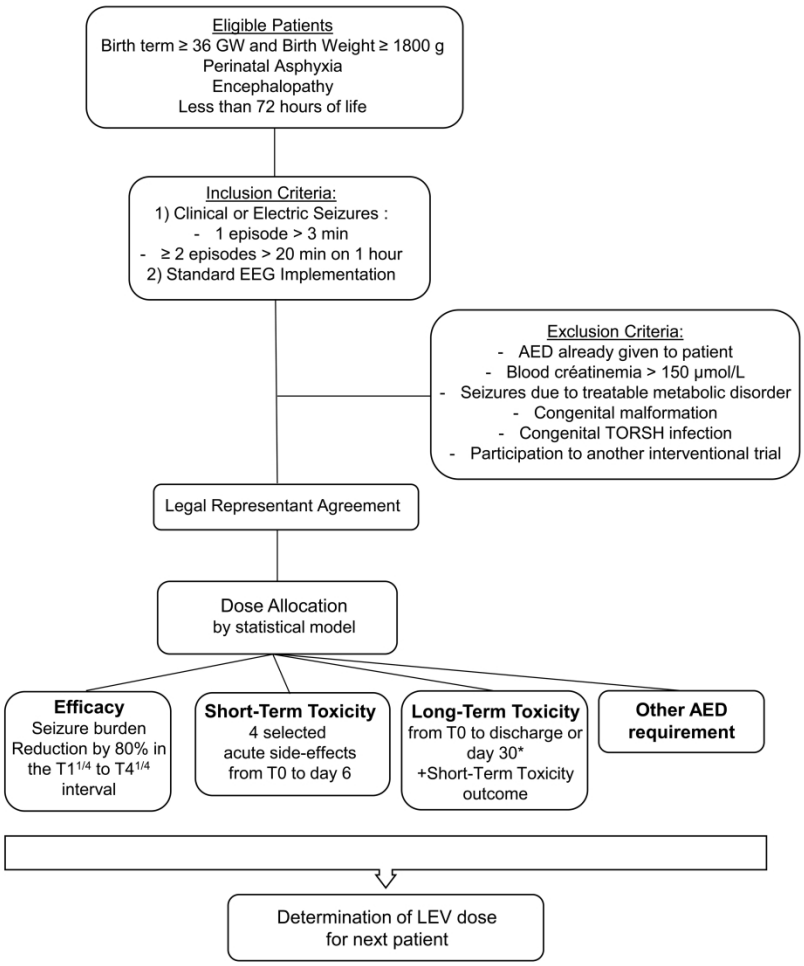
Table 1 : LEVNEONAT-1 trial registration data

Data category	Information
Primary registry and trial identifying number	ClinicalTrial.gov NCT02229123
Date of registration in primary registry	1 September, 2014
Secondary identifying numbers	EudraCT 2014-000791-26
Source of monetary or material support	French Ministry of Health
Primary sponsor	French Ministry of Health
Secondary sponsor	European Union's Seventh Framework Programme for research
Contact for public queries	GF [email address]
Contact for scientific queries	GF
Public title	Levetiracetam efficacy and safety as first-line treatment of neonatal seizures occuring in hypoxic-ischemic encephalopathy context
Scientific title	A multi-center, single group, open study, levetiracetam optimal dose-finding as first-line treatment for neonatal seizures occurring in hypoxic-ischemic encephalopathy context (LEVNEONAT-1): a phase II trial
Country of recruitement	France
Health condition(s) or problem(s) studied	Anti-epileptic drug, neonatal seizures
Intervention	Experimental drug : levetiracetam
Key inclusion and exclusion criteria	Age eligible for study :Newborns born after 36 gestational weeks and weighting more than 1800 g at birth
	Inclusion criteria : perinatal asphyxia signs , abnormal neurological examination on the first six hours of life ; clinical or electrical seizures occurring before 72 hours of life ; 8-electrode standard EEG available
	Exclusion criteria : newborns already treated with an anti-epileptic drug, seizures secondary to treatable metabolic abnormalities (i.e, hypoglycemia, hypocalcemia), serum creatine concentration above 150 µmol/l ; congenital malformation or genetic syndrome, proven infectious embryofoetopathy, participation to another interventional trial
Study type	Interventional
	Allocation : single arm, open study, four increasing dose regimens, two-patient cohort per dose level
	Primary purpose : optimal-dose finding, efficacy and safety
	Phase II
Date of first enrolment	February 2018
Target sample size	50
Recruitment status	Recruiting
Primary outcomes	Efficacy : seizure-burden reduction of 80% after loading dose on EEG recording
	Safety : short-term and long-term toxicities
Key secondary outcomes	Pharmacokinetic analysis through 5 times (i.e, 30 minutes, four hours and seven hours from the loading dose and one to three hours and 12 hours to 18 hours from the last maintenance dose)
	Seizure recurrence
	Pre-treatment seizure burden and levetiracetam efficacy

FIGURE LEGEND

Figure 1: LEVNEONAT-1 Study Flowchart. GW: Gestational Weeks; EEG: Electroencephalogram; AED: Anti-Epileptic Drug; T0: Levetiracetam Loading Dose Infusion Start; $T1^{1/4}$: one hour and 15 minutes from the levetiracetam loading dose infusion start; $T4^{1/4}$: four hours and 15 minutes from the levetiracetam loading dose infusion start; LEV: Levetiracetam

Figure 2: LEVNEONAT-1 Experimental Schedule and Time-Line. LEV: Levetiracetam; PK: Pharmacokinetic; EFF: Efficacy; TOX: Toxicities; AED: Anti-Epileptic Drug; EEG: Electroencephalogram; MRI: Magnetic Resonance Imaging; AEP: Auditory Evoked Potentials.



Favrais G et al., Figure 1

Figure 1: LEVNEONAT-1 Study Flowchart. GW: Gestational Weeks; EEG: Electroencephalogram; AED: Anti-Epileptic Drug; T0: Levetiracetam Loading Dose Infusion Start; T11/4: 1 hour and 15 min from the levetiracetam loading dose infusion start; T41/4: 4 hours and 15 min from the levetiracetam loading dose infusion start; LEV: Levetiracetam

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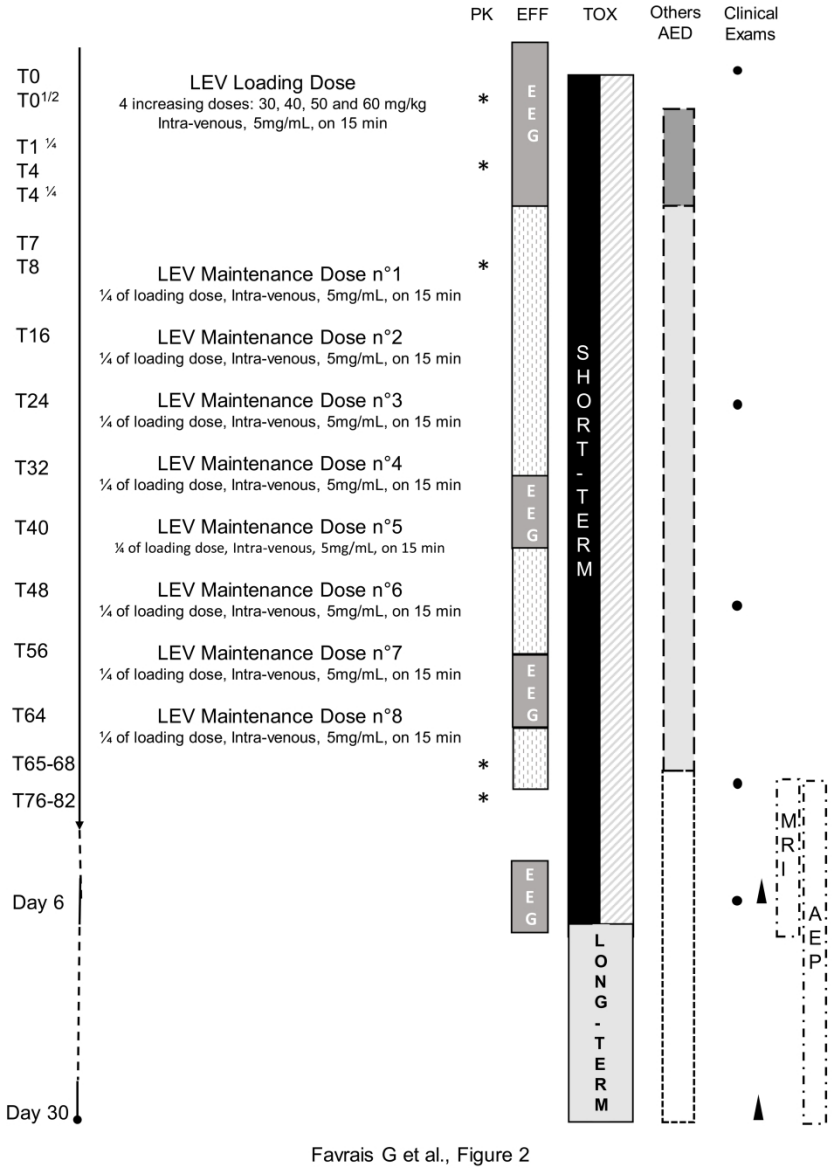

Favrais G et al., Figure 2

Figure 2: LEVNEONAT-1 Experimental Schedule and Time-Line. LEV: Levetiracetam; PK: Pharmacokinetic; EFF: Efficacy; TOX: Toxicities; AED: Anti-Epileptic Drug; EEG: Electroencephalogram; MRI: Magnetic Resonance imaging; AEP: Auditory Evoked Potentials.

297x420mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

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		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	**1**
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	**4, 16**
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	**16 and 24, Table 1**
Protocol version	#3	Date and version identifier	**22 and 23**
Funding	#4	Sources and types of financial, material, and other support	**22**
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	**1, 22**

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	**2**
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	**22**
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	**15-17**
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	**6-8**
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	**NA** **Single arm**
Objectives	#7	Specific objectives or hypotheses	**10-13**
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	**14 and 15**
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	**8**
Eligibility criteria	#10	Inclusion and exclusion criteria for participants.	**8 and 9**

		If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	**9 and 10**
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	**14**
Interventions: adherence	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	**NA**
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	**14**
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	**10-14**
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	**13 and 14, Fig. 1 and 2**
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	**5, 14 and 15**

Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	**5,8,18 and 19**
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	**NA** **Open study, single arm**
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	**NA** **Open study, single arm**
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	**NA** **Open study, single arm**
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	**NA** **Open study**
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	**NA** **Open study**
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	**16 and 17**

Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	**NA** **Short follow-up**
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	**17**
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	**14 and 15**
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	**13** **Retrospective subgroup analysis: Seizure burden intensity effect on LEV efficacy**
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	**NA** **Single arm**
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	**15**
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have	**15 and 16**

		access to these interim results and make the final decision to terminate the trial	
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	**11 and 12**
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	**17** **Not scheduled but possible through the French Drug Safety Agency**
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	**16**
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	**17, 22 and 23**
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	**5, 16, 18 and 19**
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	**NA**
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	**16 and 17**
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	**22**
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for	**16 and 17**

		investigators	
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	**NA**
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	**17 and 18**
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	**NA** **Pre-result step**
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	**NA**
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	**Not joined** **Available on demand (in French)**
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	**12**

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Levetiracetam optimal dose-finding as first-line treatment for neonatal seizures occurring in the context of hypoxic-ischaemic encephalopathy (LEVNEONAT-1): study protocol of a phase II trial

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Levetiracetam optimal dose-finding as first-line treatment for neonatal seizures occurring in the context of hypoxic-ischaemic encephalopathy (LEVNEONAT-1): study protocol of a phase II trial

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Keywords: levetiracetam, newborn, seizure, hypoxic-ischaemic encephalopathy, phase II trial

ABSTRACT

INTRODUCTION: Therapeutic schedules for treating neonatal seizures remain elusive. First-line treatment with phenobarbital is widely supported but without strong scientific evidence. Levetiracetam is an emerging and promising anti-epileptic drug (AED). The aim of this phase II trial is to determine the benefits of levetiracetam by applying a strict methodology and to estimate the optimal dose of levetiracetam as a first-line AED to treat seizures in newborns suffering from hypoxic-ischaemic encephalopathy.

METHODS AND ANALYSIS: LEVNEONAT-1 is an open and sequential levetiracetam dose-finding study. The optimal dose is that which is estimated to be associated with a toxicity not exceeding 10% and an efficacy higher than 60%. Efficacy is defined by a seizure burden reduction of 80% after the loading dose. Four increasing dose regimens will be assessed including one loading dose of 30, 40, 50 or 60 mg/kg followed by eight maintenance doses (i.e., a quarter of the loading dose) injected every eight hours. A two-patient cohort will be necessary at each dose level to consider an upper dose level assignment. The maximal sample size expected is 50 participants with a minimum of 24 patients or fewer in the case of a high rate of toxicity. Patients will be recruited in five neonatal intensive care units beginning in October 2017 and continuing for two years. In parallel, the levetiracetam pharmacokinetics will be measured five times (i.e., 30 minutes and four and seven hours after the loading dose and one to three hours and 12 hours to 18 hours after the last maintenance dose).

ETHICS AND DISSEMINATION: Ethics approval has been obtained from the regional ethical committee (2016-R25) and the French Drug Safety Agency (160652A-31). The results will be published in a peer-reviewed journal. The results will also be presented at medical meetings.

TRIAL REGISTRATION NUMBER: Eudra CT, 2014-000791-26.

ClinicalTrial.gov, NCT02229123, Pre-results.

Strengths and limitations of this study

- For the first time, levetiracetam will be used as first-line treatment for neonatal seizures rather than as an add-on therapy.
- The statistical model is designed for a rare clinical situation with a sequential adaptive method, which updates in real time the dose allocation for the next patient based on all available data from previous participants.
- The design performances were assessed through extensive simulation studies. On average, the proposed design prompts recommendations of the correct dose at approximately 60% of efficacy for a sample size of 30, increasing to over 80% in many scenarios for a sample size of 50. Moreover, this method maintains an acceptable number of neonates with toxicities. In the case of promising efficacy results, a randomised study should be performed further to confirm the findings.
- The targeted population (i.e., newborns less than 3 days old) is particularly vulnerable, and the ethical authority requires the written consent of both parents before levetiracetam administration. This fact could be a critical point for inclusion because seizures can occur early after birth and therefore require anti-epileptic drug (AED) treatment in emergency. Both parents should then be informed soon after birth even prior to seizure occurrence, and the reflection period of participants' guardians could be brief.
- Continuous EEG monitoring is scheduled during the three day-treatment with levetiracetam and a one-hour recording on day six. Unfortunately, the restricted availability of EEG devices in each investigating centre led after the cessation of seizure burden to a minimal requirement of a one-hour EEG recording on days one, two, three and six.

INTRODUCTION

Neonatal seizures occur in approximately one to five per 1000 live births [1]. Hypoxic-ischaemic encephalopathy (HIE) represents the first aetiology of neonatal seizures [1,2]. In 80% of cases, seizures associated with HIE occur in the first three days of life [2]. Seizure treatment during the neonatal period creates issues concerning the developing brain facing deleterious effects of seizure burden to the potential toxicity of conventional anti-epileptic drugs (AED). Van Rooig et al. demonstrated that the seizure duration was correlated with brain lesions through MRI [3]. Therefore, the World Health Organisation recommended in 2011 that anticonvulsant treatment should be initiated immediately when clinically apparent seizures have lasted for longer than three minutes or in the case of brief repeated seizures. This recommendation was supported strongly but no gradation was attributable due to the lack of scientific evidence [4]. Furthermore, conventional AEDs such as phenobarbital (PHB) are employed in an off-label manner in neonates. Epidemiologic studies have illustrated that PHB is widely used as the first-line treatment across the world with a lack of consensus concerning subsequent add-on lines of treatment, including phenytoin (PHT), lidocaine, midazolam and other benzodiazepines [5]. A meta-analysis from the Cochrane Database concluded in 2004 that 'there is little evidence from randomised controlled trials to support the use of any of the anticonvulsants currently used in the neonatal period'. This statement was recently reinforced by a systematic review published in 2015, which presumed that 'there is an urgent need for more evidence-based studies to guide neonatal seizure management' [5,6]. This vague position resulted from the lack of well-conducted trials and from the limited available data regarding the efficacy and safety of AED use during the neonatal period. Only one randomised controlled trial was performed focusing on AED efficacy in neonates. In this trial, PHB as PHT precipitated seizure cessation in only 44% of cases in monotherapy. The seizure burden intensity appeared to be inversely related to therapeutic success [7]. This restricted efficacy

might be explained by the signalling pathway of PHB on GABA receptors, which are paradoxically excitatory in the immature brain before transitioning to their inhibitory function [8,9]. Furthermore, recurrent seizures induced an intracellular chloride accumulation, reinforcing the excitatory function of GABA receptors and then creating PHB inefficiency in treating intense seizure burden during the neonatal period [10]. Moreover, some concerns about the safety of PHB exist. Experimental data have demonstrated that PHB increased in a dose-dependent manner neuronal apoptosis in the immature brain [11]. Therefore, a necessity for new efficient and safe AEDs for newborns has emerged. Levetiracetam (LEV) might be the appropriate candidate to fulfil these criteria. First, LEV exhibits an original means of action by reducing, through the SV2a protein, glutamate release by presynaptic neurons and then regulating the intracellular calcium of postsynaptic neurons through NMDA and AMPA receptors [12]. Second, LEV appears to be free of toxicity in relation to the neonatal brain. Experimental data have demonstrated that LEV did not induce neuronal apoptosis in the neonatal brain [13,14]. Furthermore, an observational study illustrated that LEV cumulative doses received during the neonatal period were not associated with the probability of subsequently developing a cerebral palsy [15]. Third, an intravenous galenic form of LEV is available, allowing for the treatment of non-fed newborns. Fourth, an off-label use of LEV as second-line treatment after PHB has now been widely observed [16,17]. Retrospective studies reported levetiracetam use in neonates with various dose regimens and administration schedules such as increasing doses until seizure cessation, similar doses twice a day, or a loading dose with subsequent maintenance doses. Loading doses infused to neonates varied from 10 mg/kg to 60 mg/kg [17–25]. The maximal amount of LEV infused in a newborn was 150 mg/kg within a 24-hour period [17]. Fifth, to date, the treatment of approximatively 445 newborns through LEV has been reported with limited side effects including one case of anaphylactic shock and a rare sleepy state fostered by a simultaneous PHB treatment [19,22,23,26,27]. Similarly, LEV

efficacy for significantly reducing or eliminating neonatal seizures has been recently estimated at 77% of cases in first-line treatment and 66% of cases in add-on therapy in a cohort of 102 patients from five retrospective studies [28]. In this context, it is highly important to determine the benefits of levetiracetam for treating neonatal seizures and to determine the most effective and safest dose of LEV in neonates following a rigorous and prospective methodology. In this study, a phase II trial has been designed to identify the ideal LEV loading and maintenance doses in newborns suffering from HIE. An original approach has been adopted by using LEV as the first-line treatment.

METHODS AND ANALYSIS

Study Settings

Patient recruitment will be performed in five French Neonatal Intensive Care Units (NICU) (Angers, Lille, Rouen, Rennes, and Tours) beginning in October 2017 and continuing for two years. These centres have been selected for their expertise in managing neonatal seizures. The coordinating site for this study is the University Hospital Center of Tours (France). The protocol has been written according to the SPIRIT reporting guidelines [29].

Participants

Eligible patients are term newborns with hypoxic ischaemic encephalopathy (HIE) who are less than 72 hours old (Figure 1). Briefly, three inclusion criteria have been selected: i) birth term above 36 gestational weeks and birth weight above 1800 grams; ii) perinatal asphyxia, defined as Apgar score equal to or below five at five minutes, resuscitation required at birth, or metabolic acidosis on umbilical arterial blood gas or until one hour after birth (pH < 7.1, Excess Base ≥ 16 mmol/L or lactate ≥ 11); and iii) neurologic impairment in the first six hours of life, including consciousness, tone, sucking, archaic reflexes, or pupillary alterations.

Brain cooling implementation is delegated entirely to the discretion of investigators according to the French guidelines [30]. Therapeutic hypothermia implementation and the rewarming time

will be reported in the e-CRF form. Since seizures generally occur during moderate and severe HIE requiring therapeutic hypothermia, it is expected that most participants are on brain cooling. Therapeutic hypothermia is regarded as a variable in the pharmacokinetic analysis.

Inclusion should be considered when clinical signs or an EEG pattern compatible with seizures are recognised by the investigator and when monitoring with a continuous 8-electrode EEG recording is possible. A seizure lasting more than three minutes or more than two seizures lasting more than 20 seconds within a one-hour period on a standard EEG recording fulfils the inclusion criteria. Critical-activity recognition on EEG recording is based on the investigator's experience. A specific training for recognising critical activity on standard EEG has been provided to the investigators in each centre. An inclusion could be diagnosed incorrectly by a retrospective interpretation of an EEG recording by a local electrophysiologist planned as soon as possible (cf. *Efficacy Criteria*). A second interpretation by an independent and blind reader will be organised in a brief delay to assess the lack of critical activity on the EEG recording. If the lack of critical activity on the EEG is confirmed, the patient will not be included in the statistical model, and the experimental treatment will be immediately disrupted, but pharmacokinetic and safety analysis will be performed. Finally, written consent of both parents or authorised guardians and a subscription to social security health insurance are required to complete inclusion.

Exclusion criteria concern patients already treated with an AED aside from a midazolam bolus required for intubation, patients suffering from seizures due to a treatable metabolic aetiology such as hypoglycemia and hypocalcemia, patients with severe renal failure associated with serum creatinine above 150 µmol/L, patients with evident signs of genetic or congenital malformations or infectious embryofoetopathy or patients who have already been recruited in another interventional research trial.

Intervention

A therapeutic schedule consists of a loading dose (T0) followed by eight maintenance doses every eight hours resulting in a three-day treatment period (Figure 2). Eight-hour intervals between doses were implemented based on the LEV pharmacokinetics obtained from 18 newborns, demonstrating a shorter half-life of 8.9 hours relative to older patients [26]. Four increasing loading doses were selected as follows: i) 30 mg/kg; ii) 40 mg/kg; iii) 50 mg/kg; and iv) 60 mg/kg. Each maintenance dose corresponds with a quarter of the loading dose (7.5, 10, 12.5 and 15 mg/kg, respectively). Levetiracetam (levetiracetam Mylan®, 100 mg/ml) will be diluted in a 5% glucose solution to a final concentration of 5 mg/mL. LEV will be administered intravenously over a fixed time of 15 minutes through a central or peripheral line.

Principal Aim

The principal aim is to determine the most efficient dose regimen under toxicity restrictions of LEV for neonates while accounting for efficacy, toxicity, and pharmacokinetics.

Efficacy criteria

Efficacy has been defined as an 80% reduction of seizure burden in an EEG recording between the period immediately before the LEV loading dose (from 20 minutes to three hours) and the three-hour time interval from one hour and 15 minutes ($T1^{1/4}$) to four hours and 15 minutes ($T4^{1/4}$) after the beginning of loading dose infusion (T0) (Figure 2). Seizure burden corresponds with the cumulative time of ictal electric activity on the EEG- analysed time lap. A first analysis of the EEG recording will be performed locally at each investigator centre and will be reported in e-CRF format on the sixth day following T0. A second blinded and centralised analysis is scheduled to occur subsequently, every six months. If there is more than a 10% difference between EEG interpreters or an opposite conclusion, a third EEG analysis will be performed. A subsequent correction of efficacy criteria in the statistical model can be performed (whenever it is identified). Efficacy criteria will not be accounted for in the dose allocation process in case

of a second AED requirement before T4[1/4] or an unexpected event in LEV preparation or infusion precipitating an unknown dose injection.

Toxicity criteria and safety monitoring

Toxicity will be assessed according to both of the following modalities: i) short-term toxicity; and ii) long-term toxicity (Figure 2). Short-term toxicity has been designed to rapidly trigger a decreasing dose allocation for the next potential participant through an e-CRF alert. Short-term toxicity focuses on four adverse events potentially attributable to LEV: i) severe apnoea that leads to mechanical ventilation during the four-hour period following the LEV infusion [19,22,23,26]; ii) anaphylactic shock occurring during the 30 minutes following the LEV infusion [27]; iii) toxic epidermic necrosis; and iv) Stevens-Johnson syndrome. Investigators must declare the occurrence of one of these adverse events immediately to the pharmacovigilance unit and in the e-CRF. On day six, if none of these adverse events have been observed, the investigator ticks the 'no' box that corresponds to each effect in the e-CRF and short-term toxicity will therefore be regarded as negative. Long-term toxicity encompasses all the adverse events observed and declared to the pharmacovigilance unit up to hospital discharge, or the 30th day of life at the latest. A short-term toxicity alert or any serious unexpected suspected adverse reaction (SUSAR) will immediately trigger a meeting of the scientific committee, which consists of a pharmacist (CM) and a neonatologist (GF), and the LEV treatment will be discontinued. If no severe or unexpected adverse reactions are declared, a systematic meeting of the scientific committee will be planned during the 10 days following the participant discharge or the participant's 30th day of life at the latest. The scientific committee will then determine the imputability (unrelated/possible/probable) and acceptability of each declared adverse event based on the severity at the acute phase, the quality of recovery (partial or complete) with potential subsequent disability, and the frequency of occurrence. Ultimately, a single adverse event regarded as imputable to LEV and inacceptable precipitates

a declaration of toxicity as positive in the statistical model. The requirement of another AED will also be included in the statistical model as well as the delay between T0 and treatment beginning to the extent that it could alter proper LEV efficacy and toxicity.

Secondary objectives

Pharmacokinetics of levetiracetam: blood samples

LEV pharmacokinetics (PK) in participant's blood will be measured five times at 30 minutes, four hours, and seven hours after the end of LEV loading dose infusion and at one to three hours and 12 hours to 18 hours after the last LEV maintenance dose (Figure 2). Each PK sample requires 500 µL of total blood (i.e., 2.5 mL in total). After centrifugation, the plasma will be harvested, and samples will be frozen at -20 or -80 degrees before sending for measurement (VJ). The pharmacokinetics of LEV in the population of the study will be investigated through a population approach [31]. The mean values of the PK parameters (elimination clearance, central and peripheral distribution volumes, distribution clearance) and their respective interindividual variability will be estimated. Possible relationships between the covariates (birth bodyweight, gestational age, therapeutic hypothermia) and the interindividual variability of the PK parameters will be investigated. Individual PK parameters will be estimated and used to calculated the maximum concentration and the area under curve (AUC) corresponding with the loading dose, after the first maintenance dose, and the cumulative AUC of the entire treatment. Potential relationships between these PK parameters and the efficacy and safety criteria will be investigated, and these pharmacokinetic/pharmacodynamic relationships will be used to determine the optimal dosing regimen.

Seizure recurrence from $T4^{1/4}$ to day 6

Clinical or electric seizures occurrence and frequency during LEV treatment (i.e., from $T4^{1/4}$ to T72) and until complete LEV elimination (i.e., day six) will be reported in the e-CRF in addition to concomitant AED treatment. The complete and definitive cessation of seizures will be

recorded in the e-CRF. Continuous EEG monitoring is scheduled during the three-day treatment by LEV as well as a one-hour recording on day six. Unfortunately, the restricted availability of EEG devices in each investigating centre after the cessation of seizure burden prompted a minimal requirement of a one-hour EEG recording on day one, two, three and six after the LEV treatment initiation. Then, the detection of seizure recurrence and the duration of EEG monitoring are then under the responsibility of each investigator.

Pre-treatment seizure burden and LEV efficacy

PB and PHT efficacy in relation to complete seizure control have been directly associated with the pretreatment burden seizure intensity [7]. Therefore, to explore this association with LEV, a new analysis will be performed retrospectively by adjusting the efficacy criteria to the seizure burden on the pretreatment EEG. Two subgroups will be considered based on the seizure burden (SB) intensity on the pretreatment EEG equal to or above 50% of the EEG recording duration (high SB group) and strictly under 50% of the duration (low SB group). LEV efficacy will be deemed positive when a SB reduction of 50% is observed on the post-treatment EEG recording in the high SB group, whereas a reduction of 80% will still be valid for the low SB group.

Patient follow-up

The participant follow-up will continue until hospital discharge or until the 30th day of life. An assessment has been planned that consists of repeated clinical examinations, hemodynamic monitoring, brain-imaging, and auditory and electroencephalographic recordings (Figure 2). Clinical examinations will be performed at days one, two, three and six through the Thompson score, [32] which measures the neurological distress depth. In addition, Amiel-Tison scoring, [33] focusing on neurological status of the newborn, is planned to occur upon hospital discharge or on the 30th day of life. Arterial pressure and heart rate will be measured immediately before each LEV injection and every five minutes for 15 minutes, and then every 15 minutes for 45 minutes after the LEV injection. Apnoea, bradycardia under 80 beats per minute, and oxygen

saturation drops below 85% will be reported. Brain MRI will be performed between the fourth and eighth day of life. An auditory evoked potential measurement will also be required before hospital discharge.

Other AED requirements

If the persistence or recurrence of seizures is observed after the LEV loading dose, the investigators are completely free to initiate another anti-epileptic treatment. The drug name, administered dose, therapeutic schedule, and treatment duration will be reported in the e-CRF. If another AED is required during the four hours following the LEV loading dose ends, the efficacy data will not be included in the statistical model.

LEV treatment cessation rules

LEV treatment will have to be discontinued in any of the following cases : i) a short-term toxicity or a SUSAR occurs ; ii) serum creatinine raises above 150 µmol/L in the seven- to 36-hour interval following the LEV loading dose ; iii) a complete unknown LEV loading dose was infused due to a hazardous event; iv) a mistaken maintenance dose above 60 mg/kg was infused; v) a limitation of intensive cares begins before the third day of LEV treatment ; or vi) at least one of the two parents or authorised guardians withdraws his consent.

Statistical model and dose allocation

LEVNEONAT-1 is an open-label, single group, sequential dose-finding study with four increasing dosage levels. The short-term toxicity, long-term toxicity, and the efficacy endpoints were modelled under Bayesian inference. The optimal dose of LEV was defined as the highest efficient dose under the toxicity restrictions. Before the beginning of the trial, efficacy and toxicity thresholds associated with the desirable optimal dose have been selected. The optimal dose should not be associated with less than 60% of efficacy probability and no more than 10% of short-term and long-term toxicity probabilities. After the inclusion of successive cohorts of two patients, the endpoint observations are binarised as follows: efficacy (yes or no), short-term

toxicity (yes or no and, if yes, when), long-term toxicity (yes or no), other AED use (yes or no and if yes, when) and the number of infused maintenance doses with timing (Figure 1). A statistical model was designed specifically for this trial, because no other dose-allocation method was available for this indication. The model is a sequential adaptive method since it incorporates all the available information before the trial onset and all the data from the trial that have been accumulated for each new cohort inclusion. Based on updated data, probabilities of efficacy, short-term and long-term toxicities are re-estimated after each cohort. The dose allocated to each further cohort is the estimated as the optimal dose known thus far. The first cohort of patients will receive the lowest dose level, and doses will be increased one-by-one based on the model estimates (no dose-skipping will be allowed if the dose was not yet evaluated). Moreover, since long-term toxicity will take an extended time to be observed, a time to event approach will be considered to avoid ceasing inclusions between two successive cohorts.

When a short-term toxicity alert occurs, a reduction of the current loading dose allocated to the lower level is planned until the scientific committee's conclusion concerning LEV imputability or lack thereof. The maximal sample size is expected to be 50 participants with a minimum of 24 patients. However, the trial will be terminated prematurely if all doses do not reach the efficacy threshold or the lowest dose exceeds the toxicity threshold. When a patient is eligible, the current dose regimen is available on the trial sponsor's web site. This dose will be renewed in real time according to the previous participants' data.

Independent data-monitoring committee

An independent data-monitoring committee (DMC) has been established, which includes a neonatologist, a neuropaediatrician and a pharmacologist. A DMC opinion concerning the trial continuation will be solicited every six patients or in the case of an emergency upon the request of the scientific committee.

Trial interruption criteria

Three criteria have been identified for trial interruption: i) a high probability of incorrect dose range (either for efficacy or for toxicity) will cause a temporary interruption of the trial. After the IMC consultation, a new range of doses could be proposed; ii) new valid information is published during LEVNEONAT-1, which addresses the principal aim and render this trial outdated; or iii) the scientific committee can decide to terminate the trial at any time if an unacceptable toxicity is assigned to LEV.

Patient and public involvement

Patients and the public were not involved in the design of the study.

ETHICS

LEV cannot be infused prior to obtaining the written parental or authorised guardian's consent. One of two parents or authorized guardians can withdraw their consent at any time, prompting the interruption of the newborn participation in LEVNEONAT-1. Safety monitoring would still be performed to ensure adequate treatment of potential LEV side effects but it will not be recorded in the database. An authorisation from parents or authorised guardians will be necessary to use the data obtained before the agreement withdrawal.

Ethical approval for this study (version 4, 06-06-2017) has been obtained from the regional ethical committee (CPP Ouest 1) under the reference 2016-R25 on the 9th of November 2016. The French drug safety agency (Agence Nationale de la Sécurité du Médicament) approved LEVNEONAT-1 (version 4, 06-06-2017) under the reference 160652A-31 on the 5th of October 2016.

This trial has been registered on EudraCT (20 February 2014) and on Clinical Trial.gov (1 September 2014). The Eudra CT reference is 2014-000791-26 and the Clinical Trail.gov reference is NCT02229123. Trial registration data are reported in Table 1.

DATA QUALITY

Standardised LEV prescriptions have been designed in an Excel format. These documents have been joined to the allocation-dose web site. Therefore, the investigator will only fill in the allocated dose and the participant's birth weight to obtain personalised LEV prescriptions (dilution, quantity, infusion speed, monitoring). For each included participant, all data will be anonymised under a specific code (centre city and number of inclusion), and a personalised file (e-CRF) will be created on the trial sponsor's web site. Data will be reported in the participant's e-CRF accessible with a personal code (lead investigator). Lead investigators will only have access to the e-CRFs of their own centre. Only the coordinating investigator (GF), scientific committee members (GF, CM) and data manager (EB) have access to all participants' data. Data will be checked by faxing original paper documents (drug prescriptions, vital-sign monitorings, biological measurements, EEG interpretations) to the trial sponsor. An agent (EB) will be assigned by the sponsor (i.e., Universitary Hospital Center of Tours) for meeting the lead investigators and local research teams regularly, based on the inclusion dynamic. These on-site visits aim to monitor the regular filing of consent forms, the compliance with the protocol and the accuracy of the recorded data from source documents. An audit trigger by the French Drug Safety Agency could be possible at any time during the trial course. Data management have been validated through the MR-001 reference methodology.

DISSEMINATION

Any modifications to the protocol that might impact the conduct of the study or affect patient safety will precipitate a substantive protocol amendment and will be reviewed by the regional Ethics Committee and the French Drug Safety Agency. These substantive changes will be communicated to relevant stakeholders (trial registries, regulatory agencies, investigators). The results of the LEVNEONAT-1 study will be published in a peer-reviewed journal following the Uniform Requirements for Manuscripts Submitted to Bio-medical Journals (htttp://www.icmje.org/). Publications will be distributed to investigational centres and to all

relevant persons or organisations. The LEVNEONAT-1 study will also be presented at relevant national and international medical and scientific meetings related to both of the following elements: i) methodology and biostatistics and ii) brain development and seizure treatment during the neonatal period. At the end of the study, a summary of the results will be produced for the non-medical public and will be provided to the participants' parents on demand.

TIME-LINE

Investigational centres were open from the 21st of September 2017 to the 20th of October 2017. Patient recruitment was effective from the 20th of October 2017 for two years. The first enrolment occurred in February 2018.

DISCUSSION

Seizure management during the neonatal period remains elusive, and PHB is not completely efficient in terminating critical activity and is not truly safe for the immature brain. LEV might be promising and more suitable in this condition. However, although LEV is widely used in neonatal care units worldwide, no dose regimen has been clearly established.

LEVNEONAT-1 is a particularly original study using LEV as the first-line treatment and not as add-on treatment after PHB, resulting in purer efficacy and safety data and allowing the possibility of a new therapeutic schedule in neonates. The other original characteristic of LEVNEONAT-1 is the design of a statistical model allowing for a restricted sample size to determine the optimal LEV dose in neonates by integrating data in real time of each participant.

The first LEVNEONAT-1 weakness is the targeted population, which consists of newborns with less than three days of life who suffer from HIE complicated with seizures. The seizure incidence in the HIE context varies according to studies from 29% to 65% of cases [34–36]. This discrepancy might be partially due to the caregiver's ability to recognise clinical signs of seizure and ictal activity on an EEG recording [37–39]. In parallel, the relative urgency to obtain parental consent in this stressful context remains a sensitive issue for investigators. However,

the median time of the first seizures reported in the literature was around nine to 13 hours of life [34,40], allowing for time for reflection to the parents. The second critical point is the opportunity to monitor the newborns through a standard EEG as soon as seizures are identified with various logistical problems according to each investigational centre, including a variable delay or the inability to implement standard EEG monitoring outside of working hours.

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AUTHORS CONTRIBUTION

GF and ES were responsible for the study design including aims, assessment criteria and intervention schedule; SZ and MU provided statistical expertise in trial design, developed the dose-finding model, and conducted the statistical analysis; CM and GF assumed the pharmacovigilance procedure ; EB handled the legal aspects and authorisations and helped with implementation; and VJ designed the pharmacokinetic analysis and performed the LEV pharmacokinetic measurements. All authors contributed to the refinement of the study protocol and approved the final manuscript.

FUNDING STATEMENT

This work was supported by a grant from the French Ministry of Health (LEVNEONAT-1, 2013, PHRCI 2013 API13/T/065). This funding source had no role in the design of the study and will not have any role during the study execution, analyses, interpretation of the data, or decision to submit results. No financial support from industry took part in the LEVNEONAT-1 funding. Levetiracetam will not be provided by industry.

SZ and MU were supported by the InSPiRe (innovative methodology for small populations research) project, which was funded by the European Union's Seventh Framework Programme for research, technological development, and demonstration, under grant agreement number FP HEALTH 2013-602144

COMPETING INTEREST STATEMENT

The authors declare that they have no competing interests.

PROTOCOL VERSION

Issue date: 6 Jun 2017

Protocol Amendment number: 04

Authors: GF, ES, SZ, CM and EB

Revision chronology

2016-Jul-1 Original

2016-Nov-3 Amendment N°1

At the request of French Drug Safety Agency, details about the procedure triggered by the occurrence of a side effect and the allocation-dose process have been added to the protocol.

2017-May-26 Amendment N°2

At the request of French Drug Safety Agency, the upper dose regimen including a loading dose of 60 mg/kg was withdrawn considering the lack of significant data justifying this dosage in newborns.

2017-Jun-6 Amendment N°3

The upper dose level including a loading dose of 60 mg/kg was validated by the French Drug Safety Agency in light of Venkatesan et al. [17].

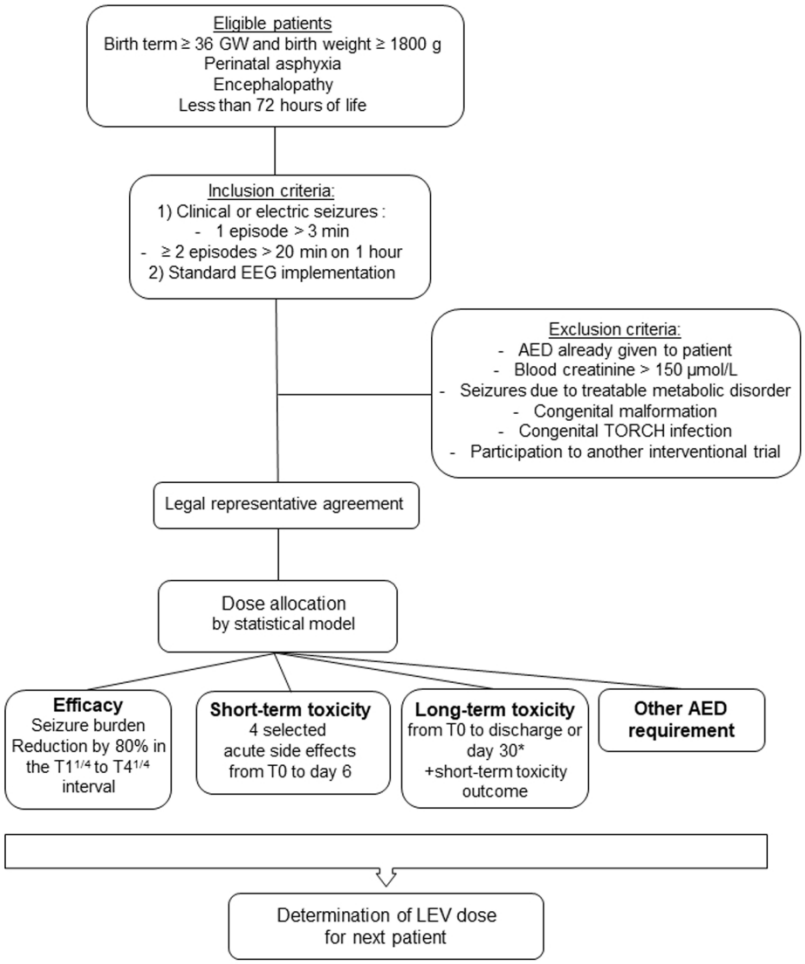
Table 1: LEVNEONAT-1 trial registration data

Data category	Information
Primary registry and trial identifying number	ClinicalTrial.gov NCT02229123
Date of registration in primary registry	1 September, 2014
Secondary identifying numbers	EudraCT 2014-000791-26
Source of monetary or material support	French Ministry of Health
Primary sponsor	French Ministry of Health
Secondary sponsor	European Union's Seventh Framework Programme for research
Contact for public queries	GF [email address]
Contact for scientific queries	GF
Public title	Levetiracetam efficacy and safety as first-line treatment of neonatal seizures occurring in hypoxic-ischaemic encephalopathy context
Scientific title	Levetiracetam optimal dose-finding as first-line treatment for neonatal seizures occurring in the context of hypoxic-ischaemic encephalopathy (LEVNEONAT-1): study protocol of a phase II trial
Country of recruitment	France
Health condition(s) or problem(s) studied	Anti-epileptic drug, neonatal seizures
Intervention	Experimental drug: levetiracetam
Key inclusion and exclusion criteria	Age eligible for study: newborns born after 36 gestational weeks and weighting more than 1800 g at birth
	Inclusion criteria: perinatal asphyxia signs, abnormal neurological examination on the first six hours of life; clinical or electrical seizures occurring before 72 hours of life; 8-electrode standard EEG available
	Exclusion criteria: newborns already treated with an anti-epileptic drug, seizures secondary to treatable metabolic abnormalities (i.e., hypoglycemia, hypocalcemia), serum creatine concentration above 150 µmol/l ; congenital malformation or genetic syndrome, proven infectious embryofoetopathy, participation to another interventional trial
Study type	Interventional
	Allocation: single arm, open study, four increasing dose regimens, two-patient cohort per dose level
	Primary purpose: optimal-dose finding, efficacy and safety
	Phase II
Date of first enrolment	February 2018
Target sample size	50
Recruitment status	Recruiting
Primary outcomes	Efficacy: seizure-burden reduction of 80% after loading dose on EEG recording
	Safety: short-term and long-term toxicities
Key secondary outcomes	Pharmacokinetic analysis through 5 times (i.e., 30 minutes, four hours and seven hours from the loading dose and one to three hours and 12 hours to 18 hours from the last maintenance dose)
	Seizure recurrence
	Pre-treatment seizure burden and levetiracetam efficacy

FIGURE LEGENDS

Figure 1: LEVNEONAT-1 Study Flowchart. GW: Gestational Weeks; EEG: Electroencephalogram; AED: Anti-Epileptic Drug; T0: levetiracetam loading dose infusion start; $T1^{1/4}$: one hour and 15 minutes after the levetiracetam loading dose infusion start; $T4^{1/4}$: four hours and 15 minutes after the levetiracetam loading dose infusion start; LEV: Levetiracetam

Figure 2: LEVNEONAT-1 experimental schedule and time-line. LEV: Levetiracetam; PK: Pharmacokinetic; EFF: Efficacy; TOX: Toxicities; AED: Anti-Epileptic Drug; EEG: Electroencephalogram; MRI: Magnetic Resonance Imaging; AEP: Auditory Evoked Potentials.



Favrais G et al., Figure 1

Figure 1: LEVNEONAT-1 Study Flowchart. GW: Gestational Weeks; EEG: Electroencephalogram; AED: Anti-Epileptic Drug; T0: levetiracetam loading dose infusion start; T11/4: one hour and 15 minutes after the levetiracetam loading dose infusion start; T41/4: four hours and 15 minutes after the levetiracetam loading dose infusion start; LEV: Levetiracetam

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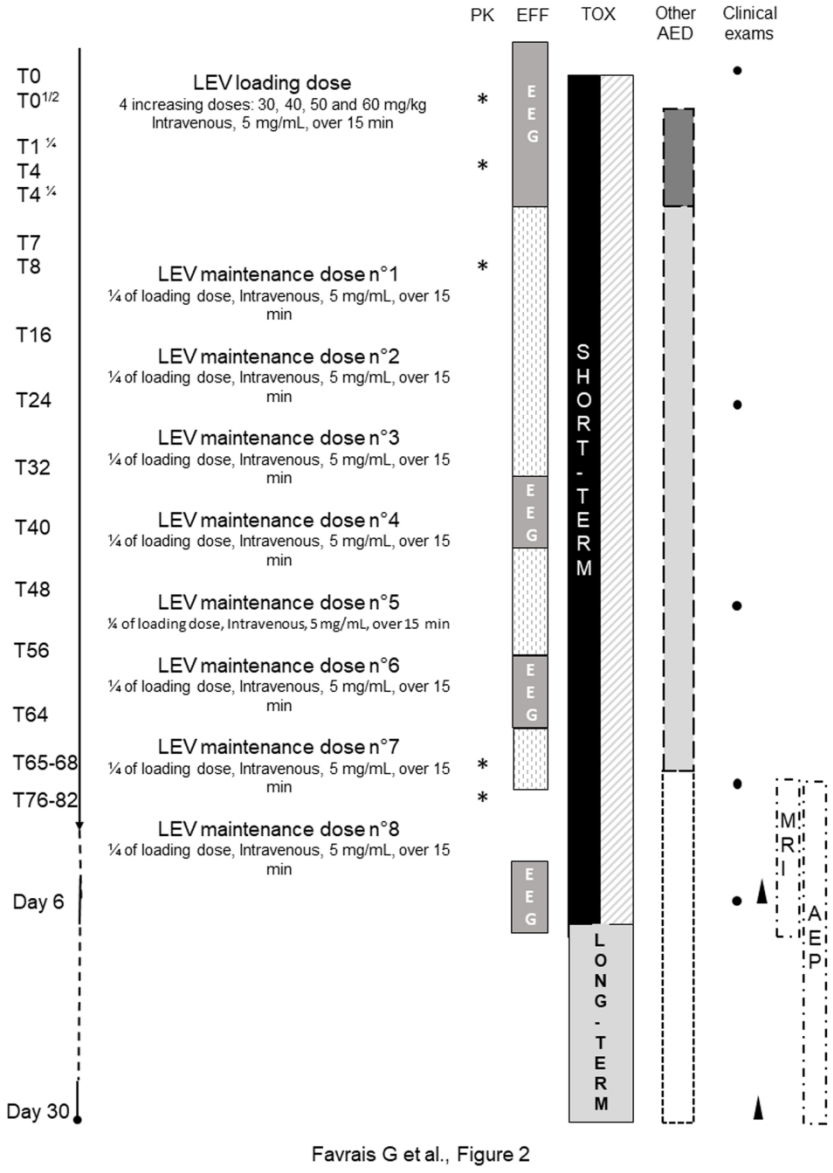

Favrais G et al., Figure 2

Figure 2: LEVNEONAT-1 experimental schedule and time-line. LEV: Levetiracetam; PK: Pharmacokinetic; EFF: Efficacy; TOX: Toxicities; AED: Anti-Epileptic Drug; EEG: Electroencephalogram; MRI: Magnetic Resonance Imaging; AEP: Auditory Evoked Potentials.

210x297mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

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Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-207

		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	**1**
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	**4, 16**
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	**16 and 24, Table 1**
Protocol version	#3	Date and version identifier	**22 and 23**
Funding	#4	Sources and types of financial, material, and other support	**22**
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	**1, 22**

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	**2**
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	**22**
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	**15-17**
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	**6-8**
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	**NA** **Single arm**
Objectives	#7	Specific objectives or hypotheses	**10-13**
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	**14 and 15**
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	**8**
Eligibility criteria	#10	Inclusion and exclusion criteria for participants.	**8 and 9**

		If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	**9 and 10**
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	**14**
Interventions: adherence	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	**NA**
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	**14**
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	**10-14**
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	**13 and 14, Fig. 1 and 2**
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	**5, 14 and 15**

Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	**5,8,18 and 19**
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	**NA** **Open study, single arm**
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	**NA** **Open study, single arm**
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	**NA** **Open study, single arm**
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	**NA** **Open study**
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	**NA** **Open study**
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	**16 and 17**

Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	**NA** **Short follow-up**
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	**17**
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	**14 and 15**
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	**13** **Retrospective subgroup analysis: Seizure burden intensity effect on LEV efficacy**
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	**NA** **Single arm**
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	**15**
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have	**15 and 16**

		access to these interim results and make the final decision to terminate the trial	
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	**11 and 12**
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	**17** **Not scheduled but possible through the French Drug Safety Agency**
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	**16**
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	**17, 22 and 23**
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	**5, 16, 18 and 19**
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	**NA**
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	**16 and 17**
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	**22**
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for	**16 and 17**

		investigators	
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	**NA**
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	**17 and 18**
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	**NA** **Pre-result step**
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	**NA**
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	**Not joined** **Available on demand (in French)**
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	**12**

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Levetiracetam optimal dose-finding as first-line treatment for neonatal seizures occurring in the context of hypoxic-ischaemic encephalopathy (LEVNEONAT-1): study protocol of a phase II trial

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Levetiracetam optimal dose-finding as first-line treatment for neonatal seizures occurring in the context of hypoxic-ischaemic encephalopathy (LEVNEONAT-1): study protocol of a phase II trial

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ABSTRACT

INTRODUCTION: Therapeutic schedules for treating neonatal seizures remain elusive. First-line treatment with phenobarbital is widely supported but without strong scientific evidence. Levetiracetam is an emerging and promising anti-epileptic drug (AED). The aim of this phase II trial is to determine the benefits of levetiracetam by applying a strict methodology and to estimate the optimal dose of levetiracetam as a first-line AED to treat seizures in newborns suffering from hypoxic-ischaemic encephalopathy.

METHODS AND ANALYSIS: LEVNEONAT-1 is an open and sequential levetiracetam dose-finding study. The optimal dose is that which is estimated to be associated with a toxicity not exceeding 10% and an efficacy higher than 60%. Efficacy is defined by a seizure burden reduction of 80% after the loading dose. Four increasing dose regimens will be assessed including one loading dose of 30, 40, 50 or 60 mg/kg followed by eight maintenance doses (i.e., a quarter of the loading dose) injected every eight hours. A two-patient cohort will be necessary at each dose level to consider an upper dose level assignment. The maximal sample size expected is 50 participants with a minimum of 24 patients or fewer in the case of a high rate of toxicity. Patients will be recruited in five neonatal intensive care units beginning in October 2017 and continuing for two years. In parallel, the levetiracetam pharmacokinetics will be measured five times (i.e., 30 minutes and four and seven hours after the loading dose and one to three hours and 12 hours to 18 hours after the last maintenance dose).

ETHICS AND DISSEMINATION: Ethics approval has been obtained from the regional ethical committee (2016-R25) and the French Drug Safety Agency (160652A-31). The results will be published in a peer-reviewed journal. The results will also be presented at medical meetings.

TRIAL REGISTRATION NUMBER: Eudra CT, 2014-000791-26.

ClinicalTrial.gov, NCT02229123, Pre-results.

Strengths and limitations of this study

- For the first time, levetiracetam will be used as first-line treatment for neonatal seizures rather than as an add-on therapy.
- The targeted population (i.e., newborns less than 3 days old) is particularly vulnerable, and the ethical authority requires the written consent of both parents before levetiracetam administration.
- The restricted availability of EEG devices in each investigating centre led after the cessation of seizure burden to a minimal requirement of a one-hour EEG recording on days one, two, three and six.
- The statistical model is designed for a rare clinical situation with a sequential adaptive method, which updates in real time the dose allocation for the next patient based on all available data from previous participants.
- On average, the proposed design prompts recommendations of the correct dose at approximately 60% of efficacy for a sample size of 30, increasing to over 80% in many scenarios for a sample size of 50.

INTRODUCTION

Neonatal seizures occur in approximately one to five per 1000 live births [1]. Hypoxic-ischaemic encephalopathy (HIE) represents the first aetiology of neonatal seizures [1,2]. In 80% of cases, seizures associated with HIE occur in the first three days of life [2]. Seizure treatment during the neonatal period creates issues concerning the developing brain facing deleterious effects of seizure burden to the potential toxicity of conventional anti-epileptic drugs (AED). Van Rooig et al. demonstrated that the seizure duration was correlated with brain lesions through MRI [3]. Therefore, the World Health Organisation recommended in 2011 that anticonvulsant treatment should be initiated immediately when clinically apparent seizures have lasted for longer than three minutes or in the case of brief repeated seizures. This recommendation was supported strongly but no gradation was attributable due to the lack of scientific evidence [4]. Furthermore, conventional AEDs such as phenobarbital (PHB) are employed in an off-label manner in neonates. Epidemiologic studies have illustrated that PHB is widely used as the first-line treatment across the world with a lack of consensus concerning subsequent add-on lines of treatment, including phenytoin (PHT), lidocaine, midazolam and other benzodiazepines [5]. A meta-analysis from the Cochrane Database concluded in 2004 that 'there is little evidence from randomised controlled trials to support the use of any of the anticonvulsants currently used in the neonatal period'. This statement was recently reinforced by a systematic review published in 2015, which presumed that 'there is an urgent need for more evidence-based studies to guide neonatal seizure management' [5,6]. This vague position resulted from the lack of well-conducted trials and from the limited available data regarding the efficacy and safety of AED use during the neonatal period. Only one randomised controlled trial was performed focusing on AED efficacy in neonates. In this trial, PHB as PHT precipitated seizure cessation in only 44% of cases in monotherapy. The seizure burden intensity appeared to be inversely related to therapeutic success [7]. This restricted efficacy

might be explained by the signalling pathway of PHB on GABA receptors, which are paradoxically excitatory in the immature brain before transitioning to their inhibitory function [8,9]. Furthermore, recurrent seizures induced an intracellular chloride accumulation, reinforcing the excitatory function of GABA receptors and then creating PHB inefficiency in treating intense seizure burden during the neonatal period [10]. Moreover, some concerns about the safety of PHB exist. Experimental data have demonstrated that PHB increased in a dose-dependent manner neuronal apoptosis in the immature brain [11]. Therefore, a necessity for new efficient and safe AEDs for newborns has emerged. Levetiracetam (LEV) might be the appropriate candidate to fulfil these criteria. First, LEV exhibits an original means of action by reducing, through the SV2a protein, glutamate release by presynaptic neurons and then regulating the intracellular calcium of postsynaptic neurons through NMDA and AMPA receptors [12]. Second, LEV appears to be free of toxicity in relation to the neonatal brain. Experimental data have demonstrated that LEV did not induce neuronal apoptosis in the neonatal brain [13,14]. Furthermore, an observational study illustrated that LEV cumulative doses received during the neonatal period were not associated with the probability of subsequently developing a cerebral palsy [15]. Third, an intravenous galenic form of LEV is available, allowing for the treatment of non-fed newborns. Fourth, an off-label use of LEV as second-line treatment after PHB has now been widely observed [16,17]. Retrospective studies reported levetiracetam use in neonates with various dose regimens and administration schedules such as increasing doses until seizure cessation, similar doses twice a day, or a loading dose with subsequent maintenance doses. Loading doses infused to neonates varied from 10 mg/kg to 60 mg/kg [17–25]. The maximal amount of LEV infused in a newborn was 150 mg/kg within a 24-hour period [17]. Fifth, to date, the treatment of approximatively 445 newborns through LEV has been reported with limited side effects including one case of anaphylactic shock and a rare sleepy state fostered by a simultaneous PHB treatment [19,22,23,26,27]. Similarly, LEV

efficacy for significantly reducing or eliminating neonatal seizures has been recently estimated at 77% of cases in first-line treatment and 66% of cases in add-on therapy in a cohort of 102 patients from five retrospective studies [28]. In this context, it is highly important to determine the benefits of levetiracetam for treating neonatal seizures and to determine the most effective and safest dose of LEV in neonates following a rigorous and prospective methodology. In this study, a phase II trial has been designed to identify the ideal LEV loading and maintenance doses in newborns suffering from HIE. An original approach has been adopted by using LEV as the first-line treatment.

METHODS AND ANALYSIS

Study Settings

Patient recruitment will be performed in five French Neonatal Intensive Care Units (NICU) (Angers, Lille, Rouen, Rennes, and Tours) beginning in October 2017 and continuing for two years. These centres have been selected for their expertise in managing neonatal seizures. The coordinating site for this study is the University Hospital Center of Tours (France). The protocol has been written according to the SPIRIT reporting guidelines [29].

Participants

Eligible patients are term newborns with hypoxic ischaemic encephalopathy (HIE) who are less than 72 hours old (Figure 1). Briefly, three inclusion criteria have been selected: i) birth term above 36 gestational weeks and birth weight above 1800 grams; ii) perinatal asphyxia, defined as Apgar score equal to or below five at five minutes, resuscitation required at birth, or metabolic acidosis on umbilical arterial blood gas or until one hour after birth (pH < 7.1, Excess Base ≥ 16 mmol/L or lactate ≥ 11); and iii) neurologic impairment in the first six hours of life, including consciousness, tone, sucking, archaic reflexes, or pupillary alterations.

Brain cooling implementation is delegated entirely to the discretion of investigators according to the French guidelines [30]. Therapeutic hypothermia implementation and the rewarming time

will be reported in the e-CRF form. Since seizures generally occur during moderate and severe HIE requiring therapeutic hypothermia, it is expected that most participants are on brain cooling. Therapeutic hypothermia is regarded as a variable in the pharmacokinetic analysis.

Inclusion should be considered when clinical signs or an EEG pattern compatible with seizures are recognised by the investigator and when monitoring with a continuous 8-electrode EEG recording is possible. A seizure lasting more than three minutes or more than two seizures lasting more than 20 seconds within a one-hour period on a standard EEG recording fulfils the inclusion criteria. Critical-activity recognition on EEG recording is based on the investigator's experience. A specific training for recognising critical activity on standard EEG has been provided to the investigators in each centre. An inclusion could be diagnosed incorrectly by a retrospective interpretation of an EEG recording by a local electrophysiologist planned as soon as possible (cf. *Efficacy Criteria*). A second interpretation by an independent and blind reader will be organised in a brief delay to assess the lack of critical activity on the EEG recording. If the lack of critical activity on the EEG is confirmed, the patient will not be included in the statistical model, and the experimental treatment will be immediately disrupted, but pharmacokinetic and safety analysis will be performed. Finally, written consent of both parents or authorised guardians and a subscription to social security health insurance are required to complete inclusion.

Exclusion criteria concern patients already treated with an AED aside from a midazolam bolus required for intubation, patients suffering from seizures due to a treatable metabolic aetiology such as hypoglycemia and hypocalcemia, patients with severe renal failure associated with serum creatinine above 150 µmol/L, patients with evident signs of genetic or congenital malformations or infectious embryofoetopathy or patients who have already been recruited in another interventional research trial.

Intervention

A therapeutic schedule consists of a loading dose (T0) followed by eight maintenance doses every eight hours resulting in a three-day treatment period (Figure 2). Eight-hour intervals between doses were implemented based on the LEV pharmacokinetics obtained from 18 newborns, demonstrating a shorter half-life of 8.9 hours relative to older patients [26]. Four increasing loading doses were selected as follows: i) 30 mg/kg; ii) 40 mg/kg; iii) 50 mg/kg; and iv) 60 mg/kg. Each maintenance dose corresponds with a quarter of the loading dose (7.5, 10, 12.5 and 15 mg/kg, respectively). Levetiracetam (levetiracetam Mylan®, 100 mg/ml) will be diluted in a 5% glucose solution to a final concentration of 5 mg/mL. LEV will be administered intravenously over a fixed time of 15 minutes through a central or peripheral line.

Principal Aim

The principal aim is to determine the most efficient dose regimen under toxicity restrictions of LEV for neonates while accounting for efficacy, toxicity, and pharmacokinetics.

Efficacy criteria

Efficacy has been defined as an 80% reduction of seizure burden in an EEG recording between the period immediately before the LEV loading dose (from 20 minutes to three hours) and the three-hour time interval from one hour and 15 minutes ($T1^{1/4}$) to four hours and 15 minutes ($T4^{1/4}$) after the beginning of loading dose infusion (T0) (Figure 2). Seizure burden corresponds with the cumulative time of ictal electric activity on the EEG- analysed time lap. A first analysis of the EEG recording will be performed locally at each investigator centre and will be reported in e-CRF format on the sixth day following T0. A second blinded and centralised analysis is scheduled to occur subsequently, every six months. If there is more than a 10% difference between EEG interpreters or an opposite conclusion, a third EEG analysis will be performed. A subsequent correction of efficacy criteria in the statistical model can be performed (whenever it is identified). Efficacy criteria will not be accounted for in the dose allocation process in case

of a second AED requirement before T4[1/4] or an unexpected event in LEV preparation or infusion precipitating an unknown dose injection.

Toxicity criteria and safety monitoring

Toxicity will be assessed according to both of the following modalities: i) short-term toxicity; and ii) long-term toxicity (Figure 2). Short-term toxicity has been designed to rapidly trigger a decreasing dose allocation for the next potential participant through an e-CRF alert. Short-term toxicity focuses on four adverse events potentially attributable to LEV: i) severe apnoea that leads to mechanical ventilation during the four-hour period following the LEV infusion [19,22,23,26]; ii) anaphylactic shock occurring during the 30 minutes following the LEV infusion [27]; iii) toxic epidermic necrosis; and iv) Stevens-Johnson syndrome. Investigators must declare the occurrence of one of these adverse events immediately to the pharmacovigilance unit and in the e-CRF. On day six, if none of these adverse events have been observed, the investigator ticks the 'no' box that corresponds to each effect in the e-CRF and short-term toxicity will therefore be regarded as negative. Long-term toxicity encompasses all the adverse events observed and declared to the pharmacovigilance unit up to hospital discharge, or the 30th day of life at the latest. A short-term toxicity alert or any serious unexpected suspected adverse reaction (SUSAR) will immediately trigger a meeting of the scientific committee, which consists of a pharmacist (CM) and a neonatologist (GF), and the LEV treatment will be discontinued. If no severe or unexpected adverse reactions are declared, a systematic meeting of the scientific committee will be planned during the 10 days following the participant discharge or the participant's 30th day of life at the latest. The scientific committee will then determine the imputability (unrelated/possible/probable) and acceptability of each declared adverse event based on the severity at the acute phase, the quality of recovery (partial or complete) with potential subsequent disability, and the frequency of occurrence. Ultimately, a single adverse event regarded as imputable to LEV and inacceptable precipitates

a declaration of toxicity as positive in the statistical model. The requirement of another AED will also be included in the statistical model as well as the delay between T0 and treatment beginning to the extent that it could alter proper LEV efficacy and toxicity.

Secondary objectives

Pharmacokinetics of levetiracetam: blood samples

LEV pharmacokinetics (PK) in participant's blood will be measured five times at 30 minutes, four hours, and seven hours after the end of LEV loading dose infusion and at one to three hours and 12 hours to 18 hours after the last LEV maintenance dose (Figure 2). Each PK sample requires 500 µL of total blood (i.e., 2.5 mL in total). After centrifugation, the plasma will be harvested, and samples will be frozen at -20 or -80 degrees before sending for measurement (VJ). The pharmacokinetics of LEV in the population of the study will be investigated through a population approach [31]. The mean values of the PK parameters (elimination clearance, central and peripheral distribution volumes, distribution clearance) and their respective interindividual variability will be estimated. Possible relationships between the covariates (birth bodyweight, gestational age, therapeutic hypothermia) and the interindividual variability of the PK parameters will be investigated. Individual PK parameters will be estimated and used to calculated the maximum concentration and the area under curve (AUC) corresponding with the loading dose, after the first maintenance dose, and the cumulative AUC of the entire treatment. Potential relationships between these PK parameters and the efficacy and safety criteria will be investigated, and these pharmacokinetic/pharmacodynamic relationships will be used to determine the optimal dosing regimen.

Seizure recurrence from $T4^{1/4}$ to day 6

Clinical or electric seizures occurrence and frequency during LEV treatment (i.e., from $T4^{1/4}$ to T72) and until complete LEV elimination (i.e., day six) will be reported in the e-CRF in addition to concomitant AED treatment. The complete and definitive cessation of seizures will be

recorded in the e-CRF. Continuous EEG monitoring is scheduled during the three-day treatment by LEV as well as a one-hour recording on day six. Unfortunately, the restricted availability of EEG devices in each investigating centre after the cessation of seizure burden prompted a minimal requirement of a one-hour EEG recording on day one, two, three and six after the LEV treatment initiation. Then, the detection of seizure recurrence and the duration of EEG monitoring are then under the responsibility of each investigator.

Pre-treatment seizure burden and LEV efficacy

PB and PHT efficacy in relation to complete seizure control have been directly associated with the pretreatment burden seizure intensity [7]. Therefore, to explore this association with LEV, a new analysis will be performed retrospectively by adjusting the efficacy criteria to the seizure burden on the pretreatment EEG. Two subgroups will be considered based on the seizure burden (SB) intensity on the pretreatment EEG equal to or above 50% of the EEG recording duration (high SB group) and strictly under 50% of the duration (low SB group). LEV efficacy will be deemed positive when a SB reduction of 50% is observed on the post-treatment EEG recording in the high SB group, whereas a reduction of 80% will still be valid for the low SB group.

Patient follow-up

The participant follow-up will continue until hospital discharge or until the 30th day of life. An assessment has been planned that consists of repeated clinical examinations, hemodynamic monitoring, brain-imaging, and auditory and electroencephalographic recordings (Figure 2). Clinical examinations will be performed at days one, two, three and six through the Thompson score, [32] which measures the neurological distress depth. In addition, Amiel-Tison scoring, [33] focusing on neurological status of the newborn, is planned to occur upon hospital discharge or on the 30th day of life. Arterial pressure and heart rate will be measured immediately before each LEV injection and every five minutes for 15 minutes, and then every 15 minutes for 45 minutes after the LEV injection. Apnoea, bradycardia under 80 beats per minute, and oxygen

saturation drops below 85% will be reported. Brain MRI will be performed between the fourth and eighth day of life. An auditory evoked potential measurement will also be required before hospital discharge.

Other AED requirements

If the persistence or recurrence of seizures is observed after the LEV loading dose, the investigators are completely free to initiate another anti-epileptic treatment. The drug name, administered dose, therapeutic schedule, and treatment duration will be reported in the e-CRF. If another AED is required during the four hours following the LEV loading dose ends, the efficacy data will not be included in the statistical model.

LEV treatment cessation rules

LEV treatment will have to be discontinued in any of the following cases : i) a short-term toxicity or a SUSAR occurs ; ii) serum creatinine raises above 150 µmol/L in the seven- to 36-hour interval following the LEV loading dose ; iii) a complete unknown LEV loading dose was infused due to a hazardous event; iv) a mistaken maintenance dose above 60 mg/kg was infused; v) a limitation of intensive cares begins before the third day of LEV treatment ; or vi) at least one of the two parents or authorised guardians withdraws his consent.

Statistical model and dose allocation

LEVNEONAT-1 is an open-label, single group, sequential dose-finding study with four increasing dosage levels. The short-term toxicity, long-term toxicity, and the efficacy endpoints were modelled under Bayesian inference. The optimal dose of LEV was defined as the highest efficient dose under the toxicity restrictions. Before the beginning of the trial, efficacy and toxicity thresholds associated with the desirable optimal dose have been selected. The optimal dose should not be associated with less than 60% of efficacy probability and no more than 10% of short-term and long-term toxicity probabilities. After the inclusion of successive cohorts of two patients, the endpoint observations are binarised as follows: efficacy (yes or no), short-term

toxicity (yes or no and, if yes, when), long-term toxicity (yes or no), other AED use (yes or no and if yes, when) and the number of infused maintenance doses with timing (Figure 1). A statistical model was designed specifically for this trial, because no other dose-allocation method was available for this indication. The model is a sequential adaptive method since it incorporates all the available information before the trial onset and all the data from the trial that have been accumulated for each new cohort inclusion. Based on updated data, probabilities of efficacy, short-term and long-term toxicities are re-estimated after each cohort. The dose allocated to each further cohort is the estimated as the optimal dose known thus far. The first cohort of patients will receive the lowest dose level, and doses will be increased one-by-one based on the model estimates (no dose-skipping will be allowed if the dose was not yet evaluated). Moreover, since long-term toxicity will take an extended time to be observed, a time to event approach will be considered to avoid ceasing inclusions between two successive cohorts.

When a short-term toxicity alert occurs, a reduction of the current loading dose allocated to the lower level is planned until the scientific committee's conclusion concerning LEV imputability or lack thereof. The maximal sample size is expected to be 50 participants with a minimum of 24 patients. However, the trial will be terminated prematurely if all doses do not reach the efficacy threshold or the lowest dose exceeds the toxicity threshold. When a patient is eligible, the current dose regimen is available on the trial sponsor's web site. This dose will be renewed in real time according to the previous participants' data.

Independent data-monitoring committee

An independent data-monitoring committee (DMC) has been established, which includes a neonatologist, a neuropaediatrician and a pharmacologist. A DMC opinion concerning the trial continuation will be solicited every six patients or in the case of an emergency upon the request of the scientific committee.

Trial interruption criteria

Three criteria have been identified for trial interruption: i) a high probability of incorrect dose range (either for efficacy or for toxicity) will cause a temporary interruption of the trial. After the IMC consultation, a new range of doses could be proposed; ii) new valid information is published during LEVNEONAT-1, which addresses the principal aim and render this trial outdated; or iii) the scientific committee can decide to terminate the trial at any time if an unacceptable toxicity is assigned to LEV.

Patient and public involvement

Patients and the public were not involved in the design of the study.

ETHICS

LEV cannot be infused prior to obtaining the written parental or authorised guardian's consent. One of two parents or authorized guardians can withdraw their consent at any time, prompting the interruption of the newborn participation in LEVNEONAT-1. Safety monitoring would still be performed to ensure adequate treatment of potential LEV side effects but it will not be recorded in the database. An authorisation from parents or authorised guardians will be necessary to use the data obtained before the agreement withdrawal.

Ethical approval for this study (version 4, 06-06-2017) has been obtained from the regional ethical committee (CPP Ouest 1) under the reference 2016-R25 on the 9th of November 2016. The French drug safety agency (Agence Nationale de la Sécurité du Médicament) approved LEVNEONAT-1 (version 4, 06-06-2017) under the reference 160652A-31 on the 5th of October 2016.

This trial has been registered on EudraCT (20 February 2014) and on Clinical Trial.gov (1 September 2014). The Eudra CT reference is 2014-000791-26 and the Clinical Trail.gov reference is NCT02229123. Trial registration data are reported in Table 1.

DATA QUALITY

Standardised LEV prescriptions have been designed in an Excel format. These documents have been joined to the allocation-dose web site. Therefore, the investigator will only fill in the allocated dose and the participant's birth weight to obtain personalised LEV prescriptions (dilution, quantity, infusion speed, monitoring). For each included participant, all data will be anonymised under a specific code (centre city and number of inclusion), and a personalised file (e-CRF) will be created on the trial sponsor's web site. Data will be reported in the participant's e-CRF accessible with a personal code (lead investigator). Lead investigators will only have access to the e-CRFs of their own centre. Only the coordinating investigator (GF), scientific committee members (GF, CM) and data manager (EB) have access to all participants' data. Data will be checked by faxing original paper documents (drug prescriptions, vital-sign monitorings, biological measurements, EEG interpretations) to the trial sponsor. An agent (EB) will be assigned by the sponsor (i.e., Universitary Hospital Center of Tours) for meeting the lead investigators and local research teams regularly, based on the inclusion dynamic. These on-site visits aim to monitor the regular filing of consent forms, the compliance with the protocol and the accuracy of the recorded data from source documents. An audit trigger by the French Drug Safety Agency could be possible at any time during the trial course. Data management have been validated through the MR-001 reference methodology.

DISSEMINATION

Any modifications to the protocol that might impact the conduct of the study or affect patient safety will precipitate a substantive protocol amendment and will be reviewed by the regional Ethics Committee and the French Drug Safety Agency. These substantive changes will be communicated to relevant stakeholders (trial registries, regulatory agencies, investigators). The results of the LEVNEONAT-1 study will be published in a peer-reviewed journal following the Uniform Requirements for Manuscripts Submitted to Bio-medical Journals (htttp://www.icmje.org/). Publications will be distributed to investigational centres and to all

relevant persons or organisations. The LEVNEONAT-1 study will also be presented at relevant national and international medical and scientific meetings related to both of the following elements: i) methodology and biostatistics and ii) brain development and seizure treatment during the neonatal period. At the end of the study, a summary of the results will be produced for the non-medical public and will be provided to the participants' parents on demand.

TIME-LINE

Investigational centres were open from the 21st of September 2017 to the 20th of October 2017. Patient recruitment was effective from the 20th of October 2017 for two years. The first enrolment occurred in February 2018.

DISCUSSION

Seizure management during the neonatal period remains elusive, and PHB is not completely efficient in terminating critical activity and is not truly safe for the immature brain. LEV might be promising and more suitable in this condition. However, although LEV is widely used in neonatal care units worldwide, no dose regimen has been clearly established.

LEVNEONAT-1 is a particularly original study using LEV as the first-line treatment and not as add-on treatment after PHB, resulting in purer efficacy and safety data and allowing the possibility of a new therapeutic schedule in neonates.

The other original characteristic of LEVNEONAT-1 is the design of a statistical model allowing for a restricted sample size to determine the optimal LEV dose in neonates by integrating data in real time of each participant. The design performances were assessed through extensive simulation studies. On average, the proposed design prompts recommendations of the correct dose at approximately 60% of efficacy for a sample size of 30, increasing to over 80% in many scenarios for a sample size of 50. Moreover, this method maintains an acceptable number of neonates with toxicities.

The first LEVNEONAT-1 weakness is the targeted population, which consists of newborns with less than three days of life who suffer from HIE complicated with seizures. The seizure incidence in the HIE context varies according to studies from 29% to 65% of cases [34–36]. This discrepancy might be partially due to the caregiver's ability to recognise clinical signs of seizure and ictal activity on an EEG recording [37–39]. In parallel, the relative urgency to obtain parental consent in this stressful context remains a sensitive issue for investigators. This fact could be a critical point for inclusion because seizures can occur early after birth and therefore require anti-epileptic drug (AED) treatment in emergency. Both parents should then be informed soon after birth even prior to seizure occurrence, and the reflection period of participants' guardians could be brief. However, the median time of the first seizures reported in the literature was around nine to 13 hours of life [34,40], allowing for time for reflection to the parents. The second critical point is the opportunity to monitor the newborns through a standard EEG as soon as seizures are identified with various logistical problems according to each investigational centre, including a variable delay or the inability to implement standard EEG monitoring outside of working hours. Further, continuous EEG monitoring is scheduled during the three day-treatment with levetiracetam and a one-hour recording on day six. The restricted availability of EEG devices in each investigating centre led after the cessation of seizure burden to a minimal requirement of a one-hour EEG recording on days one, two, three and six.

In the case of promising efficacy results, a randomised study should be performed further to confirm the findings.

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AUTHORS CONTRIBUTION

GF and ES were responsible for the study design including aims, assessment criteria and intervention schedule; SZ and MU provided statistical expertise in trial design, developed the dose-finding model, and conducted the statistical analysis; CM and GF assumed the pharmacovigilance procedure ; EB handled the legal aspects and authorisations and helped with implementation; and VJ designed the pharmacokinetic analysis and performed the LEV

pharmacokinetic measurements. All authors contributed to the refinement of the study protocol and approved the final manuscript.

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COMPETING INTEREST STATEMENT

The authors declare that they have no competing interests.

PROTOCOL VERSION

Issue date: 6 Jun 2017

Protocol Amendment number: 04

Authors: GF, ES, SZ, CM and EB

Revision chronology

2016-Jul-1 Original

2016-Nov-3 Amendment N°1

At the request of French Drug Safety Agency, details about the procedure triggered by the occurrence of a side effect and the allocation-dose process have been added to the protocol.

2017-May-26 Amendment N°2

At the request of French Drug Safety Agency, the upper dose regimen including a loading dose of 60 mg/kg was withdrawn considering the lack of significant data justifying this dosage in newborns.

2017-Jun-6 Amendment N°3

The upper dose level including a loading dose of 60 mg/kg was validated by the French Drug Safety Agency in light of Venkatesan et al. [17].

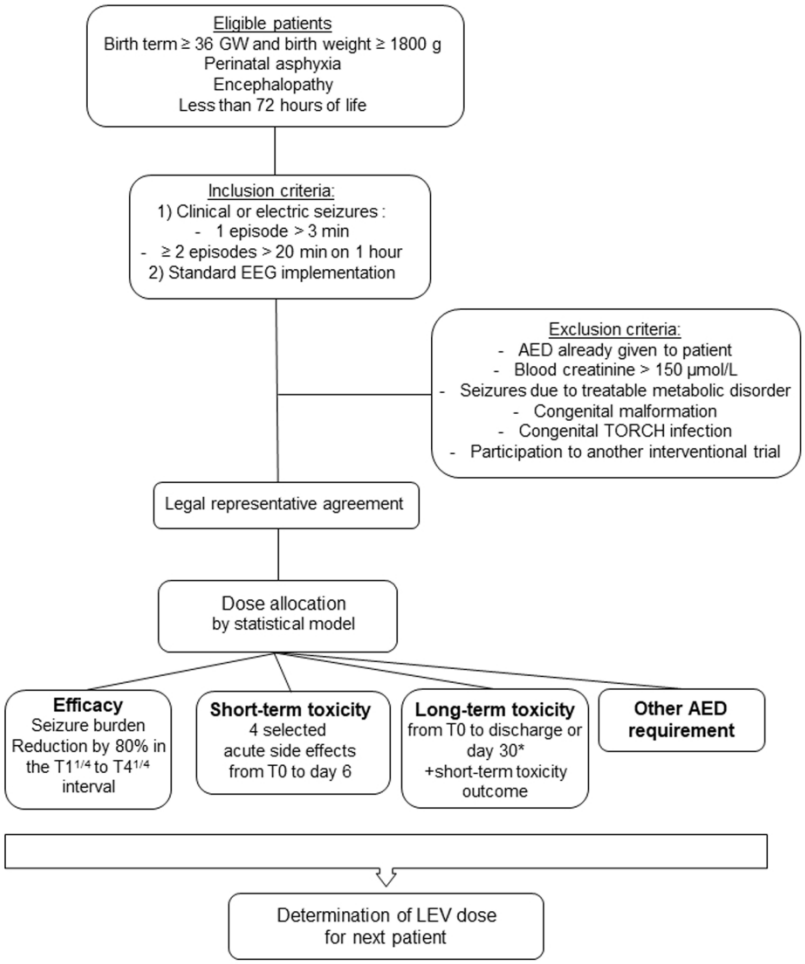
Table 1: LEVNEONAT-1 trial registration data

Data category	Information
Primary registry and trial identifying number	ClinicalTrial.gov NCT02229123
Date of registration in primary registry	1 September, 2014
Secondary identifying numbers	EudraCT 2014-000791-26
Source of monetary or material support	French Ministry of Health
Primary sponsor	French Ministry of Health
Secondary sponsor	European Union's Seventh Framework Programme for research
Contact for public queries	GF [email address]
Contact for scientific queries	GF
Public title	Levetiracetam efficacy and safety as first-line treatment of neonatal seizures occurring in hypoxic-ischaemic encephalopathy context
Scientific title	Levetiracetam optimal dose-finding as first-line treatment for neonatal seizures occurring in the context of hypoxic-ischaemic encephalopathy (LEVNEONAT-1): study protocol of a phase II trial
Country of recruitment	France
Health condition(s) or problem(s) studied	Anti-epileptic drug, neonatal seizures
Intervention	Experimental drug: levetiracetam
Key inclusion and exclusion criteria	Age eligible for study: newborns born after 36 gestational weeks and weighting more than 1800 g at birth
	Inclusion criteria: perinatal asphyxia signs, abnormal neurological examination on the first six hours of life; clinical or electrical seizures occurring before 72 hours of life; 8-electrode standard EEG available
	Exclusion criteria: newborns already treated with an anti-epileptic drug, seizures secondary to treatable metabolic abnormalities (i.e., hypoglycemia, hypocalcemia), serum creatine concentration above 150 µmol/l ; congenital malformation or genetic syndrome, proven infectious embryofoetopathy, participation to another interventional trial
Study type	Interventional
	Allocation: single arm, open study, four increasing dose regimens, two-patient cohort per dose level
	Primary purpose: optimal-dose finding, efficacy and safety
	Phase II
Date of first enrolment	February 2018
Target sample size	50
Recruitment status	Recruiting
Primary outcomes	Efficacy: seizure-burden reduction of 80% after loading dose on EEG recording
	Safety: short-term and long-term toxicities
Key secondary outcomes	Pharmacokinetic analysis through 5 times (i.e., 30 minutes, four hours and seven hours from the loading dose and one to three hours and 12 hours to 18 hours from the last maintenance dose)
	Seizure recurrence
	Pre-treatment seizure burden and levetiracetam efficacy

FIGURE LEGENDS

Figure 1: LEVNEONAT-1 Study Flowchart. GW: Gestational Weeks; EEG: Electroencephalogram; AED: Anti-Epileptic Drug; T0: levetiracetam loading dose infusion start; $T1^{1/4}$: one hour and 15 minutes after the levetiracetam loading dose infusion start; $T4^{1/4}$: four hours and 15 minutes after the levetiracetam loading dose infusion start; LEV: Levetiracetam

Figure 2: LEVNEONAT-1 experimental schedule and time-line. LEV: Levetiracetam; PK: Pharmacokinetic; EFF: Efficacy; TOX: Toxicities; AED: Anti-Epileptic Drug; EEG: Electroencephalogram; MRI: Magnetic Resonance Imaging; AEP: Auditory Evoked Potentials.



Favrais G et al., Figure 1

Figure 1: LEVNEONAT-1 Study Flowchart. GW: Gestational Weeks; EEG: Electroencephalogram; AED: Anti-Epileptic Drug; T0: levetiracetam loading dose infusion start; T11/4: one hour and 15 minutes after the levetiracetam loading dose infusion start; T41/4: four hours and 15 minutes after the levetiracetam loading dose infusion start; LEV: Levetiracetam

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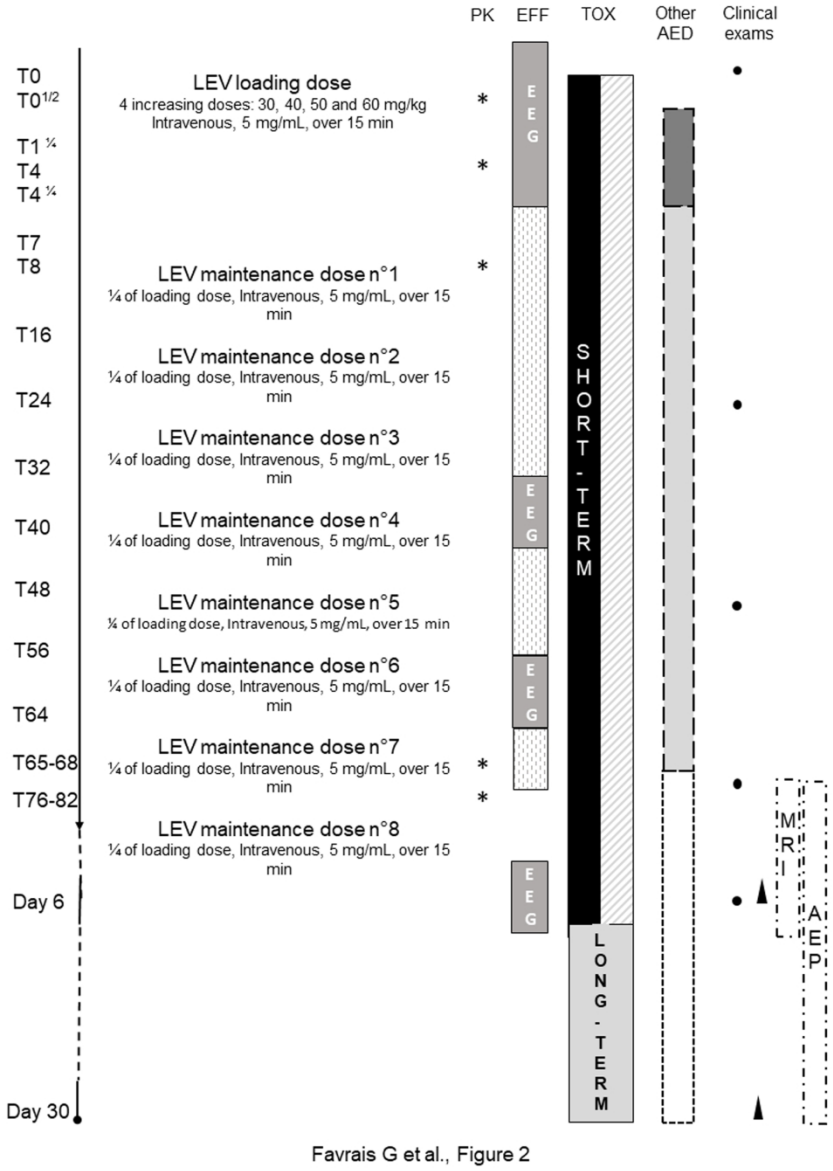

Figure 2: LEVNEONAT-1 experimental schedule and time-line. LEV: Levetiracetam; PK: Pharmacokinetic; EFF: Efficacy; TOX: Toxicities; AED: Anti-Epileptic Drug; EEG: Electroencephalogram; MRI: Magnetic Resonance Imaging; AEP: Auditory Evoked Potentials.

210x297mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	**1**
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	**4, 16**
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	**16 and 24, Table 1**
Protocol version	#3	Date and version identifier	**22 and 23**
Funding	#4	Sources and types of financial, material, and other support	**22**
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	**1, 22**

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	**2**
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	**22**
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	**15-17**
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	**6-8**
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	**NA** **Single arm**
Objectives	#7	Specific objectives or hypotheses	**10-13**
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	**14 and 15**
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	**8**
Eligibility criteria	#10	Inclusion and exclusion criteria for participants.	**8 and 9**

		If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	**9 and 10**
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	**14**
Interventions: adherence	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	**NA**
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	**14**
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	**10-14**
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	**13 and 14, Fig. 1 and 2**
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	**5, 14 and 15**

Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	**5,8,18 and 19**
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	**NA** **Open study, single arm**
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	**NA** **Open study, single arm**
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	**NA** **Open study, single arm**
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	**NA** **Open study**
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	**NA** **Open study**
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	**16 and 17**

Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	**NA** **Short follow-up**
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	**17**
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	**14 and 15**
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	**13** **Retrospective subgroup analysis: Seizure burden intensity effect on LEV efficacy**
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	**NA** **Single arm**
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	**15**
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have	**15 and 16**

		access to these interim results and make the final decision to terminate the trial	
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	**11 and 12**
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	**17** **Not scheduled but possible through the French Drug Safety Agency**
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	**16**
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	**17, 22 and 23**
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	**5, 16, 18 and 19**
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	**NA**
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	**16 and 17**
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	**22**
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for	**16 and 17**

		investigators	
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	**NA**
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	**17 and 18**
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	**NA** **Pre-result step**
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	**NA**
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	**Not joined** **Available on demand (in French)**
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	**12**