Published in final edited form as:
Epilepsia. 2013 August ; 54(0 4): 13–23. doi:10.1111/epi.12295.

Epilepsy Therapy Development: Technical and Methodological Issues in Studies with Animal Models

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SUMMARY

The search for new treatments for seizures, epilepsies and their comorbidities faces considerable challenges. Partly, this is due to gaps in our understanding of the etiology and pathophysiology of most forms of epilepsy. An additional challenge is the difficulty to predict the efficacy, tolerability and impact of potential new treatments on epilepsies and comorbidities in humans, using the available resources. Here we provide a summary of the discussions and proposals of the Working Group 2 as presented in the Joint American Epilepsy Society and International League Against Epilepsy Translational Workshop in London (September 2012). We propose methodological and reporting practices that will enhance the uniformity, reliability and reporting of early stage preclinical studies with animal seizure and epilepsy models that aim to develop and evaluate new therapies for seizures or epilepsies, using multi-disciplinary approaches. The topics considered include: (a) implementation of better study design and reporting practices, (b) incorporation in the study design and analysis of covariants that may impact outcomes (including species, age, sex), (c) utilization of approaches to document target relevance, exposure and engagement by the tested treatment, (d) utilization of clinically relevant treatment protocols, (e) optimization of the use of video-EEG recordings to best meet the study goals, and (f) inclusion of outcome measures that address the tolerability of the treatment or study endpoints apart from seizures. We further discuss the different expectations for studies aiming to meet regulatory requirements to obtain approval for clinical testing in humans. Implementation of the rigorous practices discussed in this report will require considerable investment in time, funds and other research resources, which may create challenges for academic researchers seeking to contribute to epilepsy therapy discovery and development. We propose several infrastructure initiatives to overcome these barriers.

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Keywords

Pharmacokinetics; video-electroencephalography; tolerability; regulatory requirements; non-pharmacological treatment; infrastructure

INTRODUCTION

Anti-epilepsy therapy (AET) development occurs in various stages. *Target identification studies* identify mechanisms of disease that may be amenable to modification by treatments. *Assay development* involves the creation of in vitro and animal-based test systems with which to identify therapeutic agents. *Lead generation* is then conducted by testing agents hypothesized to have therapeutic activity in the animal models or against the targets, which generally involves the in vitro assays. "Lead compounds" may also be discovered empirically by s*creening* for activity in the animal models or in in vitro assays. Toxicity screens are used to assess tolerability. A panel of efficacy models may be used to provide more detailed characterization of efficacy. Use of biomarkers may be appropriate at this stage. Some leads may require *optimization* through medicinal chemistry. Potential clinical candidates then undergo evaluation for toxicity and safety. Clinically relevant formulations are developed. *Pharmacokinetic (PK)* and *target engagement* studies are conducted with the formulation. *Toxicity and safety testing* according to *Good Laboratory Practices* (GLP) is then conducted in anticipation of filing of an exemption with regulatory agencies (Investigational New Drug (IND) application) to conduct human clinical studies.

From 30,000 compounds screened by the Anticonvulsant Screening Program (ASP) of the National Institute of Neurological Disorders and Stroke, only 9 have acquired an indication for seizures (2012). The cost of bringing a new drug to the market by a pharmaceutical company is significantly multiplied by the failure rates in drug development (Herper 2012). This reality, along with the increasing concerns about the reproducibility and reliability of preclinical studies, across multiple research fields (Fang, et al. 2012, Galanopoulou, et al. 2012, Kahle and Bix 2012, Landis, et al. 2012, Steward, et al. 2012) has prompted efforts to improve the current practices and de-risk the process of preclinical AET discovery.

Here, we address issues related to general study design, methodology, and reporting of research with animal models. Recommendations are made regarding best practices that will facilitate the interpretation and evaluation of studies, allow comparisons between studies conducted in different laboratories, and increase the likelihood that results can be replicated. Special emphasis is given to issues related to (1) experimentation with animals, (2) measurement of outcomes, including behavioral and electrographic characterization of seizures, (3) drug formulation, and (4) PK characterization.

The primary focus of this report is the preclinical studies that aim to determine the efficacy and tolerability of new therapies. These studies are often conducted in academic research laboratories. Such studies are not mandated by regulators and are not conducted according to GLP. We present here the views on best practices as provided by Working Group 2 of the joint American Epilepsy Society (AES) and International League Against Epilepsy (ILAE) Workshop to Optimize Preclinical Epilepsy Therapy Discovery that met in London in September 2012. These views are designated as "proposed", as we intend them to be a starting point for discussion among the larger community of researchers, which we hope will refine them. Due to their observational or hypothesis-driven nature, these expectations may not be always be realizable in exploratory, early-stage preclinical studies. We also discuss ways of optimizing the available infrastructure, to accelerate and de-risk the discovery and optimization of epilepsy therapies.

STUDY DESIGN AND REPORTING OF GENERAL METHODS

Methodological rigor

Prior publications have underscored the importance of maintaining high standards of rigor and detail in the design and reporting of preclinical studies. The key points are summarized in Table 1.

Animal issues

One-third of the toxic side effects seen in humans cannot be predicted in any of the utilized non-human species (Baillie and Rettie 2011). Poor predictability can arise because of species differences in absorption, distribution, metabolism or excretion (ADME), PK, drug effects, or factors that affect the biological activity of a drug, such as immunogenic reactions (Baillie and Rettie 2011, Loscher, et al. 1991, Vugmeyster, et al. 2012). As a result, regulatory agencies generally require that toxicological testing be conducted in two species (one rodent, one non-rodent).

It is unclear, however, to what degree higher non-rodent species better predict the specific target mechanisms involved in human epilepsies and seizures. Animal acquisition, care and associated costs, as well as the more stringent regulatory requirements have generally precluded the use of such species in experimental studies. In the absence of target validation, it is unclear whether discarding across-species discordant findings would filter out treatments likely to fail in humans or miss out on potential beneficial treatments.

AETs may have different effects in animals of different species, strain, genetic background, age, sex or hormonal state, housing conditions, seizure/epilepsy models, or in the presence of other co-existing health issues (Table 1). Randomizing animals across treatment groups with respect to these variables and reporting the incidence of the variables in each treatment group is an alternative.

Proposal

- There is insufficient information available at present to recommend efficacy testing in more than one species. Tolerability testing in disease models may identify relevant toxicities that do not occur in normal animals.
- One sex may be sufficient for a particular study, but both male and female animals should be tested prior to IND-enabling studies.
- AETs intended for use in neonates or children should be tested in age-specific models.
- Covariants that may influence the biologic effect of the drug and its PK properties should be randomized across treatment groups and included in the analyses. Covariant characteristics of each study group should be specified in publications and their balance across treatment groups should be statistically verified.

Drug formulation and delivery

Drug formulations usually include ingredients, referred to as *vehicle* in laboratory studies or *excipients* in pharmaceutical preparations that act as a carrier for the active drug. The excipients may exert their own effects, either directly or by altering the properties of the active drug (e.g., through changes in pH). A drug formulation may affect blinding (e.g., color change due to drug-vehicle interaction) or may create a placebo effect (e.g., pleasant taste). As a result, selection of drug dosing, the timing of drug administration, and the endpoint of testing should be adapted for the specific formulation used.

In preclinical studies, a variety of drug delivery routes are used, which may be acceptable for the specific goals of the proof-of-concept preclinical studies but may not always be clinically applicable (e.g., intraperitoneal or intracerebral injections). However, for treatments that are intended for clinical evaluation, it is important to demonstrate, at some stage of preclinical development, that a clinically relevant delivery method and treatment protocol is effective and well tolerated. These issues are further discussed in relation to specific models or treatment indications in other publications (Brooks-Kayal, et al. 2013, Galanopoulou, Buckmaster, Staley, Moshe, Perucca, Engel, Loscher, Noebels, Pitkanen, Stables, White, O'Brien and Simonato 2012, Pitkänen, et al. 2013, Wilcox, et al. 2013).

Dose-response studies allow the identification of the population average dose-response curves for both efficacy and adverse side effects. Dose-response experiments are relatively easy to perform in studies of acute or short-term efficacy (i.e. antiseizure effects). However, in studies requiring prolonged treatment or monitoring (e.g., disease-modifying, antiepileptogenesis studies), dose-response experiments may be prohibitive in terms of the time, cost, animal numbers and resources required.

Proposal

- Before proceeding to clinical testing, studies designed to evaluate clinically relevant treatment protocols should be conducted. To guide the planning of clinical trials, these studies should report (a) the appropriate dosing schedule, (b) route of administration, and (c) the time windows to obtain the intended therapeutic effect.
- Safety, toxicity and PK studies of the active drug should be conducted with the proposed clinical formulation at some stage of the preclinical drug development.
- Reporting of the composition of the vehicle and drug formulation as well as the purity and sources of their ingredients is strongly encouraged. Physical properties of the vehicle and drug formulation (such as color, viscosity and pH) should be similar. Potential differences between the vehicle and study drug (such as taste, odor, change in urine color) that may affect blinding should be considered. The potential that the vehicle may affect efficacy measures should be assessed. The safety and toxicity of the vehicle should be assessed.

PK, target relevance and evidence for target engagement

Single and/or repeat-dose PK help define the kinetics of a drug and its main metabolites in plasma or other biological fluids. Establishing the drug exposure-response relationship may (a) define the therapeutic dose window and its separation from toxic doses, (b) help predict the probability of adverse effects as a function of exposure and covariants, (c) identify the mechanism of action, and (d) predict optimal route and protocol of delivery. When used, PK should preferably be performed using animals of similar characteristics as those included in the study (i.e., strain, sex, age, concurrent drugs). When the underlying pathology or disease may affect PK or the response to a drug, these studies would be more informative if performed in the animal model of the studied seizure or epilepsy.

Sufficient detail should be provided on the method of drug delivery, anesthesia and concomitant drugs, ambient temperature, strain and source, age, sex, weight, and type of animals (healthy or experimental). The sampling methods should describe (a) the site and method of sample collection, (b) timing, number of samples per time-point, and volume of samples, (c) whether sampling was serial from the same animal or whether one sample was obtained per animal, (d) the transport and processing of the samples, and (e) the analysis methods. The software, mathematical methods and parameters used (model, initial values, settings, minimization algorithms, weighting, model discrimination and diagnostics

methods) should also be provided. The range of therapeutic and toxic levels, if found, would also be important to report. Overall, despite the significant species-related PK differences, the therapeutic plasma levels of anti-seizure drugs generally show significant similarities between rodents and humans (Loscher 2007).

Drug levels can be determined in plasma, cerebrospinal fluid (CSF) and/or brain-derived tissue extracts or extracellular fluids. The method of brain tissue collection may be amenable to contamination by drug in the blood. For drugs that act on an extracellular or plasma membrane target, *extracellular drug levels* more accurately reflect the concentration of drug in the target tissue. However, if the drug target is intracellular (as with certain hormones), brain tissue levels may be more appropriate. Neither brain nor extracellular drug levels may necessarily correspond to CSF or blood levels, which are more clinically relevant.

Demonstration of brain levels is obviously only valuable if the drug target is in the brain. Evidence of drug-induced toxicity or other anticipated physiologic effects (e.g., sedation or weight loss) also suggest exposure to the drug. However, these parallel drug exposure effects may not be related to the targeted disease mechanism and are useful only in delimiting the tolerability threshold of the drug. If evidence for target engagement is provided (i.e., with a functional assay), drug levels may not be necessary in proof-of-concept studies, but would be useful in future drug development studies to optimize the dosing and frequency of the treatment protocol.

Proposal

- In PK studies, reporting of the characteristics and demographics of the target population (preferably to match the study population), methods of sample collection and processing, mathematical analysis and modeling is encouraged. PK and evidence of target engagement should preferably be done in the animal model of the disease.
- Interpretation of results will depend on the presence or absence of evidence for treatment exposure, target relevance and engagement (if known).
- Prior to clinical testing, it is highly recommended to determine whether brain levels correlate with levels at clinically accessible compartments (i.e., blood or CSF) or with other in vivo biomarkers.

ISSUES TO CONSIDER FOR VIDEO-EEG MONITORING OF SEIZURES IN PRECLINICAL RESEARCH ON ANIMAL MODELS OF ADULT EPILEPSY

This section addresses experimental approaches for monitoring the process of epileptogenesis in adult animal models of epilepsy using spontaneous recurrent seizures as an outcome measure. While no single set of methodologies is appropriate in all circumstances, potential pitfalls in the quantification of epilepsy are discussed with a particular focus on trials that involve disease modification. Clear definition of outcome criteria is encouraged. In addition, best practices for the reporting of research results are provided.

Two issues that every investigator must consider during the design of these studies are: first, what constitutes an epileptic seizure in an animal? This has been controversial, but the definition accepted by the ILAE Commission on Classification and Terminology defines epileptic seizure for humans as “a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain” (Berg, et al. 2010, Fisher, et al. 2005). Even this definition could be questioned, and a critical concern for our purpose here is to consider a seizure in terms of the type of epilepsy being studied (i.e., the epilepsy

syndrome). Second, the strength of the conclusions to be drawn from even the most robust quantification of seizure activity still depends on the degree to which the animal model reflects the properties of the targeted epilepsy syndrome. In turn, this depends on how well the model has been characterized and validated in terms of similarities with the human condition.

Surface versus depth electrodes, location and number of channels

Intracranial or “depth” electrodes that penetrate the brain parenchyma have several advantages: they generally have better signal-to-noise ratio and can be located close to the hypothetical seizure-onset zone. However, intracranial electrodes are more likely to cause injury, which may confound the study. Depth electrodes are more difficult to replace if they malfunction. Confirmation of the intra-cerebral recording site is probably best done with an electrolytic lesion, although this approach can be problematic. Electrodes on the “surface” of the brain are less likely to cause brain damage and they are faster and easier to apply. The “surface” recordings may be accomplished with or without a burr hole in the skull. A craniotomy enables a lower-noise and more reliable epidural electrode placement, but may also cause damage to the brain, so many laboratories avoid them. Compared to intracranial recordings, epicranial surface recordings generally have lower signal-to-noise ratio and allow for less localization with risk of missing focal seizures arising in deeper structures. Scalp recordings are also possible, but they are more prone to noise and overall less stable.

In theory, one only needs a single channel (i.e., electrode pair) to monitor for the *occurrence* of seizures, assuming that the area invaded by the seizure is known to extend to the recording field of the electrodes and common mode rejection does not cancel out the activity. If some or all of the seizures cause focal field potential activity, it is important to have at least one channel recording from the seizure field. This may be an important issue in cases where the localization and field of the seizure cannot be predicted or may change in time or decrease as a result of the investigational treatment. Consequently, the likelihood of false-positive therapeutic effects increases, which would overestimate the treatment efficacy. With a single channel, one obviously cannot localize the seizure focus or study seizure propagation to different brain regions. One may also risk losing the utility of the animal if the electrode is lost, unlike studies using multiple electrodes. With too few electrodes, proper distinction of seizures from other forms of rhythmic behavior or artifacts or evaluation of the EEG background (i.e., focal versus diffuse slowing or background suppression; hypsarrhythmia) may be compromised. Utilization of more channels, however, will also increase the amount of data to be stored and analyzed, and increase the drain on batteries in telemetric systems.

The selection of the electrode recording site(s) is often challenging, particularly in animal models with multiple types and locations of seizures and in models with underlying lesions that may alter the propagation of seizure activity to the surface. On the other hand, for many well-characterized rodent models, several studies where multiple electrodes were used suggest that a single pair of appropriately positioned electrodes (i.e., one channel) would have captured all of the seizures (Kadam, et al. 2010, Williams, et al. 2009). However, extrapolation to other animal models should be justified by similar EEG studies in those models. Ultimately, decisions on the location and number of recording electrodes, and how the data are analyzed, will depend on the animal model, the extent of its prior characterization in regards to EEG findings, and the specific question being asked.

Proposal

- For long-term seizure monitoring in preclinical studies, the use of epicranial electrodes generally provides a good balance of seizure detection and quantification

with minimal damage to the brain. However, other approaches may have advantages for specific issues.

- Use of four surface electrodes/channels with bilateral coverage, if possible, is generally better than fewer electrodes, but a single channel may be adequate in many cases. Initial studies to identify and quantify seizures should be based on visual pattern recognition. Selection of a single channel that consistently demonstrates the EEG activity of interest (i.e., seizures) for computer-based analysis may then be sufficient. Justification of the recording site(s) should be included in the publications.

Seizure definition and detection

There has been considerable debate on what is a seizure. Authors should therefore (1) define quantitatively what was considered a seizure and what was not, and (2) show a sufficient number of examples of the seizures to demonstrate the full range of variability. Seizures should be illustrated at several time scales, to appreciate the overall structure of the entire seizure and also the waveforms of the individual components. Ideally, oscillatory activities that could be mistaken for a seizure should also be illustrated and the incidence of these events in the control group should be specified.

Studies using computer-based seizure detection should provide sufficient description of the types of seizures or interictal epileptic activities detected, the parameters and algorithms used to define them and differentiate them from non-epileptic patterns.

Inter-rater variability may lead to different interpretations of certain seizure-like events by visual pattern recognition but also by seizure detection software that use different algorithms or settings. In general, visual pattern recognition is preferred when the identification of seizures is complicated and *requires* subjective assessment of characteristics that cannot be captured by computer algorithms. This should not be a problem if the reviewer is appropriately blinded. The speed of computer-based analyses is several orders-of-magnitude faster and more objective than human review *when seizures can be unambiguously identified with computational techniques*. It is important that both the false-positive and false-negative error rate be determined for the algorithm based on “gold standard” (human) review of limited EEG samples from appropriate stages of epileptogenesis (White, et al. 2006). Both visual- and computer-based algorithms have decreased effectiveness as signal-to-noise ratio decreases and electrical artifacts become larger and more frequent. Although computer-based analyses can be extremely useful, they must be checked closely and regularly; this is particularly true when the seizures, electrical artifacts and signal-to-noise ratio may change.

The methods for detecting seizures and interictal activity should provide the following details: (1) the types of seizures or interictal epileptic activity detected, (2) the method (visual versus computer-based) and criteria for seizure recognition, (3) the number of hours of EEG recording that were analyzed, (4) the length of wake versus sleep periods analyzed (if done), (5) the number of channels and the speed of review, and (6) the sampling method (continuous versus intermittent; if intermittent, the intervals at which the subjects were sampled).

Seizure reporting should also include description of the observed seizure frequencies and duration, their behavioral correlates, as well as a more detailed description of the number of seizures and the overall period of recording and analysis. Where possible, plots of number of seizures per day versus time should be included in the report so that readers can assess the degree of clustering of seizures and the appropriateness of the chosen monitoring durations

and intervals. When seizure clustering occurs, sufficient description of the criteria used to identify the individual seizure events (i.e., return to baseline) should be provided, as well as measures of time-in-seizure, if background does not return completely to baseline between seizures.

Proposal

- The behavioral and electrographic criteria used to define seizures should be precisely specified in descriptive and quantitative terms. In particular, the characteristic electrographic features used to define seizures, including waveform patterns and their durations should be stated. The full range of variability of electrographic seizures should be illustrated; views should be provided on time scales that allow the overall structure of the seizure as well as the individual components to be discerned.
- Detailed description of computer-based algorithms and the parameters used to define an electrographic seizure should be included.
- Reporting of the inter-rater reliability in both human- and computer-based analyses is encouraged (i.e., comparing human versus computer-based analyses, comparing different human reviewers, or comparing different algorithms). Experimental evidence that these are not seen in the normal (non-epileptic) age- and sex-matched control population should be provided.

Duration of monitoring

The issues of *how long to record* and *when to record* are intertwined. Continuous “24/7” recording is the *ideal*, but continuous recording means that each animal requires a recording unit for the *entire duration* of the study, which can be several months, or even longer. One has to not only consider the variance across animals, but also variance *within* animals. In the studies reported by the Dudek lab (Kadam, White, Staley and Dudek 2010, Williams, White, Clark, Ferraro, Swiercz, Staley and Dudek 2009), virtually every animal with epilepsy (at least with severe epilepsy) showed progressive worsening if one monitored for sufficiently long enough periods, but the progressive change in seizure frequency and severity could be highly variable. It is critical to report when staggered recordings were conducted, particularly in reference to the time of occurrence of the insult. Seizures are often non-convulsive when the seizure rate is quite low at early times after an insult (i.e., false negatives as missed seizures are quite possible with inadequate monitoring); seizures can occur in large clusters later after an insult, leading to high variability at longer times after an insult. Clear criteria distinguishing a seizure cluster [i.e., recurrent seizures with interictal return to baseline within a defined period of time] from status epilepticus [i.e., seizures without interictal recovery for at least 30 min (or less)] need to be preset as they are likely to affect the seizure frequency measurements and interpretation. Several practical and economic issues concern how much recording can be obtained per animal, and how long does one need to board an animal. Issues such as: Are experimental animals recorded in an animal facility in isolation? Or are they recorded intermittently in a laboratory setting, where noise and interruptions can be an issue? These issues depend directly on various national and institutional guidelines and regulatory requirements (e.g., Institutional Animal Care and Use Committee (IACUC) policies in the USA). Furthermore, the size of video files can also be a limiting problem in continuous long-term monitoring, particularly when one uses a single camera per animal.

Proposal

- In anti-epileptogenesis studies, continuous EEG recording, from the time of insult and for as long as possible, is preferred. Staggered recordings at later times may be

sufficient, depending on the study protocol. Provide detailed information regarding monitoring duration and intervals in the methods, and report changes in terms of the minimum seizure frequency that can be resolved at the monitoring duration. For example, if the monitoring is for 48 hours, the lowest frequency that can be resolved is 1 seizure / 48 hours, i.e., 0.02 seizures per hour or 0.48 seizures per day.

Frequency response and digitization

The recognition of the importance of high-frequency oscillations (HFOs) and very slow changes in local field potentials emphasizes the importance of considering and reporting frequency response (band-pass) and digitization rate in any EEG study aimed at monitoring seizures. However, to simply assess the frequency, duration, and severity of seizures, one should not have to record either very high frequencies or very low frequencies; this issue can become a “red herring” for studies that simply aim to quantify the presence/absence of seizures. Recall the Nyquist theorem, which states that a signal must be sampled at least twice as fast as the highest frequency component of interest in order to reconstruct the waveform accurately; if not, the high-frequency content will be aliased to a frequency within the bandpass.

Proposal

- Frequency response (bandpass) and digitization rate should be published.

Video analysis of behavior

Video can define the behavioral seizure type (e.g., distinguish between convulsive and non-convulsive seizures) and help to determine if apparent seizures are due to movement artifact. It is important for the reader to be able to understand the degree to which video was used in the analyses. For example, the number and location of the cameras should be specified.

Proposal

- If video is used, publications should specify how the video was recorded, including the number and location of cameras, and how it was implemented in the data analyses.

MONITORING AET TOLERABILITY IN ANIMAL MODELS

In addition to being effective in humans, the therapeutic cannot produce unwanted side effects that outweigh the benefits of the treatment. *Minimal essential outcomes* (Table 2) require minimal effort to acquire, but provide critical information on the safety and side effects of both the animal model and the treatment. The most obvious adverse outcome seen in animal studies is failure to thrive that, for small animals such as rodents, is best measured by weight loss. Monitoring of eating or sleeping can also be done with simple systemic physiological measures. Tissue studies to determine whether the treatment acted on the desired target during treatment administration, when that target is known.

Early incorporation of a brief battery of tolerability and/or functional tests in early preclinical studies may help (a) define the benefit-safety therapeutic window within the targeted population, (b) provide evidence for biological activity of the treatment which can be useful to support treatment exposure, particularly in negative studies, or (c) facilitate early randomization and longitudinal clinical follow up in lesional models of epilepsy according to the observed clinical deficits, similar to the clinical practice. A battery of such tests is given in Supplementary Table 2. Depending on the study goals, more specialized tests can be incorporated (Supplementary Table 2 and (Brooks-Kayal, Bath, Berg,

Galanopoulou, Holmes, Jensen, Kanner, O'Brien, Whittemore, Winawer, Patel and Scharfman 2013, Engel, et al. 2013)).

NON-PHARMACOLOGICAL TREATMENT STRATEGIES

Non-pharmacological treatment strategies comprise those that are not traditional antiseizure drugs. Some examples include but are not limited to: (1) gene therapy with viral vectors, (2) cell / stem cell therapies, (3) deep brain stimulation (DBS) or surgery, (4) dietary, and (5) traditional medicine or herbal compounds. In general, these approaches and associated preclinical research with such novel strategies should share the overall design of the experiments and translational roadmaps of pharmacological treatments, but there could be some specific aspects and challenges that need to be outlined and addressed. In this section, we will discuss some of these specific features of non-pharmacological treatment strategies and provide our general views on what would be important to implement while designing such studies with translation in mind.

Gene therapy

Based on animal studies, gene therapy for epilepsy has proven to be a promising alternative for developing novel treatment strategies (Noe, et al. 2012, Weinberg and McCown 2013). Gene delivery vehicles may vary but viral vectors, such as adeno-associated virus (AAV), lentivirus, and herpes simplex virus, seem to have advantageous translational potential. Preclinical studies using viral vectors as a gene delivery system need to address several important points to document translational value of the approach. Apart from the efficacy of the transgene in various animal models of epilepsy, the studies need to address the following issues.

- **a.** The expression and distribution of the transgene in the brain. In most cases, viral vector-based gene therapy approach strives to deliver the transgene into the focus of seizure origin. Spreading of the transgene to other areas may lead to unpredictable and unwanted side effects.
- **b.** The inflammatory response in the brain caused by viral vector injection (activation of microglia, astrocytes, cytokine expression). In the brain, viral vector injection may cause an inflammatory reaction (Lowenstein, et al. 2007), which could promote epileptogenesis (Vezzani, et al. 2012).
- **c.** The effect of transgene overexpression on related endogenous proteins, such as receptors of transgenes, or the proteins of the similar function or the same family of proteins, or the proteins that they interact with. Often alteration of protein or peptide expression levels are accompanied by compensatory changes in levels of proteins related to the transgene, which may interfere with or alter the transgene effect.
- **d.** Possible tumorigenesis caused by the transgene transduction over reasonably long period of time. Insertional mutagenesis is an inherent risk factor for some viral vector-based gene therapy approaches, and may become a concern for using them in humans. This needs to be explored either by examining the tissue for brain tumor formation, and/or by investigating the expression of various proliferative factors in the transduced cells.
- **e.** EEG monitoring is necessary to estimate both short-term, and particularly the long-term effects of the transgenes (4 weeks - 2 months).
- **f.** Finally, the side effects of the transgenes (e.g., effect on behavior, memory, weight loss, and anxiety) need to be also evaluated [see, e.g., (Sorensen, et al. 2008)].

Not all such gene transduction studies may be feasible to apply for early postnatal rodent models of the epilepsies, since, e.g., AAV vectors require 3–4 weeks in full expression of the transgene. Thus, the resulting treatment, even if applied at an early postnatal age, would also overlap and affect the adult period. Lentiviral vectors however express transgenes in a shorter period of 4–7days, and could be used instead.

Cell/Stem Cell Therapies

Similar considerations apply for cell / stem cell therapies. Although cell therapy may lag behind gene therapy approaches in terms of advances towards translational applications, there are certainly stem cell lines that hold the potential to be developed into a treatment alternative [see, e.g., (Shetty 2012)]. Such cells include, but are not limited to, embryonic stem (ES) cells, cord blood cells, inducible pluripotent stem (iPS) and induced neuronal (iN) cells. Specific aspects that need to be explored and demonstrated for cell therapy are the same as for gene therapy. In addition, one experimental evidence should contain: (a) degree of survival and distribution / migration of the grafted cells in the brain; (b) phenotype of the cells, their neuronal properties, and synaptic integration into the host circuitry; and finally (c) the long-term stability of neuronal phenotype (4–12 weeks).

Deep Brain Stimulation (DBS)

Translationally, DBS is the most advanced non-pharmacological approach that has already been used in clinical applications. DBS has been applied with variable outcomes and success, but still considerable research is dedicated to further advancement of this field for clinical applications. The important aspects that need to be addressed experimentally when designing such studies include but are not limited to: (a) electrode localization(s), (b) possible inflammatory response (activation of microglia, astrocytes, cytokine expression), (c) variety of stimulation paradigms (frequency, strength, duration and brain region), (d) EEG monitoring, (e) efficacy in relevant models, (f) long-term effect during and after DBS, and (g) side effects during and after DBS (e.g., effect on behavior, memory, weight loss, anxiety, motor function).

Dietary Therapies

Dietary approaches have gained increasing attention recently. Metabolic changes may regulate susceptibility to seizures in both children (Freeman and Kossoff 2010) and adults (Lambrechts, et al. 2012). An additional specific point that needs to be addressed in animal studies with dietary therapies is the documentation of changes in metabolism induced by the treatment. Even in these studies, continuous EEG monitoring is necessary to estimate efficacy in relevant animal models. The long-term effects during the diet, their persistence after the termination of the diet, as well as side effects during and after the treatment need to be explored.

Traditional Medicine and Herbal Compounds

Traditional medicines, including various herbal compounds and products, are currently increasing in use to complement or replace conventional medical therapies (McElroy-Cox 2009). Preclinical studies based on these approaches should not differ in format from those with other conventional pharmacological antiseizure drugs, and requirements for such studies should be similar.

MEETING REGULATORY REQUIREMENTS

The goals and expectations of studies aiming at obtaining regulatory approval for clinical testing in humans are focused upon ensuring the safety and quality assurance of the IND formulation and treatment protocol that will eventually be tested in humans. IND-enabling

studies are beyond the focus of this manuscript, as they are governed by the regulatory agencies and are done in specialized GLP laboratories. The main global regulatory agencies are the Food and Drug Administration (FDA) in the United States, the European Medicines Agency (EMA) in Europe, and the Ministry of Health, Labor and Welfare (working in conjunction with the Pharmaceuticals and Medical Devices Agency) in Japan. IND-enabling studies are expected to provide adequate information on pharmacology and toxicology obtained from healthy experimental animals or in vitro. IND applications include: (1) a clinical study "first-in-humans" protocol; (2) in vivo or in vitro pharmacology to provide a rationale for the expected human benefit, extrapolate human doses and drug levels, identify unintended actions of the IND, and estimate its therapeutic index; (3) toxicology studies; (4) assurance of quality of the IND in regards to chemistry, manufacturing and controls; and (5) information on prior human experience. IND enabling studies are not performed on animal models of a disease. Therefore, the utilization of clinically relevant methods and treatment protocols during the early preclinical studies assessing the efficacy and tolerability of a treatment in animal models would help guide the design of the proposed "first-in-humans" study protocol, as well as capture disease-related safety issues.

INITIATIVES TO OPTIMIZE INFRASTRUCTURE

Improvements in the following domains are deemed important to achieve.

Expertise

1) Creation of a uniform classification and interpretation system for rodent EEGs, throughout their life span.

2) Training courses for preclinical AET research.

3) Creation of a translational consulting center to provide guidance and facilitate access to centers with specialized expertise or resources (e.g., PK-PDs, toxicology, compound resources).

Technology

4) *Centralized repositories for annotated EEG and video-EEG recordings* to standardize video-EEG analysis and promote the validation of motion and EEG-based software that detect behavioral and electrographic seizures.

5) *Improving EEG technology and detection for neonatal rodents,* including the creation of lighter and smaller EEG electrodes for both tethered and telemetric monitoring systems.

6) *The development of clinically relevant in vivo biomarkers of target relevance and engagement* to guide treatment validation and implementation in humans.

Publications and dissemination of information

7) *Public access registry of preclinical AET studies and eventually their results.* However, conflicts of interests, intellectual property issues, the significant cost of maintenance (Kimmelman and Anderson 2012), and issues related to the compliance of the investigators to timely report their results (Ross, et al. 2012) will need to be addressed.

8) *Journal of negative or fragmentary studies* to provide equal opportunity for the publication of both negative and positive results and report fragmentary studies that cannot be completed. Caution however is warranted to avoid a negative bias, by over-representing negative, preliminary and underpowered studies that

are faster to report at the expense of well-designed and therefore time-consuming positive studies.

9) *Open access to all preclinical AET development data,* at least for those pertaining to publicly-funded research or AET candidates for further development, as recently requested for clinical trials that have been publicly-funded (The Cochrane Collaboration 2012).

10) *Open access archive of the quality control experiments for preclinical research resources and tools* (e.g., antibodies, siRNAs, chemicals, specialized assays).

Funding and advocacy for epilepsy research

11) *Advocating an increase in funding sources for epilepsy research and optimizing the strategic allocation of available funds from all sources to research areas in need.* Adherence to rigorous research study designs will also require significant increase in associated costs. In fiscal year 2013, the National Institutes of Health (USA) are expected to allocate 3–5 times less research funds per individual with epilepsy than per patient with Alzheimer's or Parkinson's disease, or autism (information kindly provided by H. Steve White and Julie Milder, on behalf of CURE). The increasing competition for the limited available research budget and the importance of a strong advocacy group to secure research funds have been recently highlighted with the National Alzheimer's Project Act (NAPA) initiative (Kaiser 2012). Shared databases to facilitate information sharing among funders and strategic re-allocation of funds to research areas in need would also be worth considering.

CONCLUSIONS

We have highlighted some of the methodological and technological issues that may improve the validity and translatability of preclinical AET discovery research. It is important to maintain clinically relevant practices and study goals, anticipate the impact of confounders (e.g., species, age, sex), utilize PK data and ensure exposure to the treatment and target engagement, and optimize the monitoring for seizures or other safety and efficacy outcomes. We have attempted to provide recommendations for some of these practices. Undeniably, there are additional issues that merit future attention, such as those that relate to the development of treatments for age-specific epilepsies and seizure syndromes. It is equally critical, however, to improve the infrastructure to train researchers, provide the necessary tools, disseminate, support, and accelerate these efforts. The collaboration among academic investigators, pharmaceutical industry, regulatory agencies, funders, and publishers will be the key to the success.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to acknowledge the invaluable advice during our proceedings by H. Steve White (University of Utah, Salt lake City, Utah), James Cloyd (Center for Orphan Drug Research, University of Minnesota, Minneapolis, Minnesota, USA), and Elisabetta Vaudano (Innovative Medicines Initiative, Brussels, Belgium). We would also like to thank the AES, ILAE, CURE, Epilepsy Therapy Project, and Autism Speaks for their financial support of the AES/ILAE Joint workshop in London (September 2012).

ASG has received research grant support from NINDS (NS078333 and NS020253), Autism Speaks, CURE, and the Heffer and Siegel Families Foundations. ASG has received speaker honorarium from Novartis and royalties from

Morgan and Claypool Publishers and John Libbey Eurotext Ltd. MK has no conflicts to disclose. JAL has received the research grants from NIH/NINDS (R01NS045207 and R01NS058802) and has no conflicts of interest. AN has no conflicts of interest to disclose. AP has received research grants from the Academy of Finland and the Sigrid Juselius Foundation. AP has no conflicts of interest to disclose. MAR has received support from NINDS grants NS072094, NS079202, NS077582, and from the Epilepsy Therapy Project, Gilead Sciences, and Eisai Inc. KJS has no conflicts to disclose. VW has no conflicts of interests to disclose. FED receives support from grants NS072258, NS079274, NS079135, NS064661, and N01-NS42359 from the NIH and from W81X W81XWH-12-2-0122 and WH-11-1-0752 from the Department of Defense. FED has received financial support in the form of grants, gifts, and/or consulting fees from Epitel, Inc., Johnson Pharmaceutical Research Institute, Johnson-Ethicon, and Neurotherapeutics Pharma, and he also has equity interest in Epitel, Inc.

We confirm that we have read the Journal's position on issues relating to ethical publication. This review is consistent with these guidelines.

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Table 1

Standards for study design and reporting of early preclinical studies

Aspect of study	Preferred study design and reporting practices
Experimental design	Describe the species, strains, and genetic background, if known, for both experimental groups and controls
	Justification of selected animal models or experimental setting
	Statement of adherence to ethical and animal care guidelines
	Justification for route of treatment delivery/dosing
	Report the timing of treatment delivery in regards to time of the day and endpoint assessment
	Justification of the timing of treatment based on expected PK-PDs or anticipated time of action of the treatment in the studied strain
	Use consistent housing, breeding and handling conditions across groups
	Randomized, placebo/vehicle-controlled, blinded design
	State criteria for dose selection; dose-response studies may inform on therapeutic window
	Age, sex, stage of the disease, length of treatment and observation for outcomes, and other covariants that may affect outcomes (handling, litter effect etc) to be randomly distributed across groups
	Apply uniform handling procedures across groups
	Inclusion of methods to assess target relevance and target engagement
	Select outcomes that are appropriate for the symptom/disease target or target population
	Select measurable and quantifiable endpoints that allow rigorous objective comparisons
	The purity, stability, manufacturing reproducibility of selected biologicals (viruses, cell lines, etc) should be described
Data collection and analysis	Describe the power analyses that set the sample size requirements
	Pre-define and report the inclusion and exclusion criteria and criteria for outliers
	Pre-define and report rules for sampling data or stopping data collection
	Describe causes and extent of lost data
	Report interim analyses
	Report number of replicates per group and statistics for each experiment; clarify if replicates are biological or technical and from how many experiments or animals they are derived from
	Report the reproducibility of results across the experiments in the study and the distribution of values (scattergrams), if possible
	Description and justification of the selected statistical methods
Minimizing bias	Data collection and analyses to be done blinded to group allocation
	Description of blinding methods and randomization of groups; report issues that hinder blinding
	Strategies for randomization and/or stratification to be described
	Inclusions and exclusions to be applied blindly to group allocation
	Report missing data
	Report both positive and negative data
	Report conflicts of interests
Reporting of results	Experimental methods need to be described in sufficient detail to permit replication
	Describe housing, breeding and handling conditions: light/dark cycle, number of animals per cage, time of day of the experiment, type and sequence of experimental procedures per rat or group
	Information on materials, tests, and assays used should be included to support their validation for their specificity and appropriateness for the studied population and study aims
	Describe vehicle composition and any properties that might influence the demonstrated efficacy and tolerability of the AET

Aspect of study	Preferred study design and reporting practices
	Report number of replicates per group and statistics for each experiment
Interpretation	Discuss clinical relevance of findings: target population, clinical applicability of treatment protocol (therapeutic window, tolerability of recommended doses, prior positive or adverse clinical experience with similar treatments)
	Discuss evidence for target relevance and target engagement
	Discuss evidence for reproducibility, robustness or limitations of current study
	Discuss effect size in regards to potential clinical impact
	Discuss evidence in favor or against replication/validation of results
	Discuss alternative interpretations of the findings
	Discuss available concurring or discordant studies from the literature

These recommendations are discussed extensively in several recent publications (Galanopoulou, Buckmaster, Staley, Moshe, Perucca, Engel, Loscher, Noebels, Pitkanen, Stables, White, O'Brien and Simonato 2012, Hooijmans, et al. 2010, Kahle and Bix 2012, Kilkenny, et al. 2010, Landis, Amara, Asadullah, Austin, Blumenstein, Bradley, Crystal, Darnell, Ferrante, Fillit, Finkelstein, Fisher, Gendelman, Golub, Goudreau, Gross, Gubitz, Hesterlee, Howells, Huguenard, Kelner, Koroshetz, Krainc, Lazic, Levine, Macleod, McCall, Moxley, Narasimhan, Noble, Perrin, Porter, Steward, Unger, Utz and Silberberg 2012, Ludolph, et al. 2010, Nature Neuroscience 2013, Rigor in Science Working Group 2011, Shineman, et al. 2011)

Table 2

Minimal Essential Outcomes to Measure in Animal Models of Epileptogenesis

Other Outcome	Measurements
1. Failure to Thrive	Weight, eating, sleeping
2. Drug tissue distribution and targeting (when possible for treatments with known targets)	Brain concentration and target validation
3. CNS Dysfunction	Simple behavioral testing