## Title

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# *Preface* - Practical and theoretical considerations for performing a multicenter preclinical biomarker discovery study of post-traumatic epileptogenesis: lessons learned from the EpiBioS4Rx consortium⋆

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## Abstract

The Epilepsy Bioinformatics Study for Antiepileptogenic Therapy (EpiBioS4Rx) is a NINDS funded Center-Without-Walls international study aimed at preventing epileptogenesis after traumatic brain injury (TBI). One objective of EpiBioS4Rx relates to preclinical biomarker discovery for post-traumatic epilepsy. In order to perform a statistically appropriately powered biomarker discovery study, EpiBioS4Rx has made a rigorous attempt to harmonize the preclinical procedures performed at the three EpiBioS4Rx centers, located in Finland, Australia, and the USA. Moreover, we have also performed a rigorous *interim analysis* of the success of procedural harmonization, which is reported in this virtual special issue. The analysis included harmonization of the production of animal model, blood sampling, electroencephalogram analyses (seizures, high-frequency oscillations) and magnetic resonance imaging analysis. Based on lessons learned, we propose a 3-stage protocol to facilitate the success of preclinical multicenter studies: preparation ⇨ testing ⇨ multicenter study. The need of funding for preparation and testing phases, which precede the actual multicenter study and are necessary for its success, should be taken into account in the design of funding schemes

### Keywords

Biomarker; Case report form; Common data element; Electroencephalogram; Harmonization; Lateral fluid-percussion injury; Magnetic resonance imaging; Plasma; Post-traumatic epilepsy; Preclinical; Traumatic brain injury

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## 1. Towards preclinical randomized controlled multicenter trials for biomarker discovery – EpiBioS4Rx is carrying the torch

Several research areas, particularly stroke, spinal cord injury and TBI, have over the recent years raised concerns regarding the “reproducibility crisis” in preclinical therapy discovery studies (DeWitt et al., 2018; Dirnagl et al., 2013; Steward et al., 2012). These concerns have kindled discussion regarding the need for preclinical randomized controlled multicenter trials (pRCTs) to increase animal numbers and methodological rigor, and consequently achieve statistical power and experimental reproducibility, in order to enhance the chance of a successful translation of preclinical findings to the clinic (Balduini et al., 2016). The discussion regarding such a multi-center approach has been accompanied by the development of methodologies, including the generation of preclinical common data elements (CDEs) and case report forms (CRFs), intended to facilitate harmonization of preclinical procedures, data analysis, and reporting in participating centers (Landis et al., 2012; McNutt, 2014; Nielson et al., 2014; Smith et al., 2015).

The challenges in translating preclinical discoveries to the clinic have also been recognized in the epilepsy research community (Galanopoulou et al., 2012; Simonato et al., 2014). The first attempt to develop and apply CDEs and CRFs in a preclinical multi-center epilepsy study was performed in the European FP7-funded study EPITARGET (Lapinlampi et al., 2016). More recently, the joint Working Group of the International League Against Epilepsy (ILAE), National Institutes of Neurological Diseases and Stoke (NINDS), and American Epilepsy Society (AES) initiated an ambitious activity, aimed at developing procedures for harmonization of data collection and analysis, including multi-center study designs in preclinical epilepsy research. One aspect of this activity focuses on developing a library of CDEs and CRFs for different epilepsy models and procedures, which can be adapted as needed for a given experiment (Harte-Hargrove et al., 2017; Scharfman et al., 2018).

The Epilepsy Bioinformatics Study for Antiepileptogenic Therapy (EpiBioS4Rx) is a NINDS funded Center-Without-Walls international study aimed at preventing epileptogenesis after traumatic brain injury (TBI) (https://epibios.loni.usc.edu/). The preclinical biomarker discovery in EpiBioS4Rx is performed by three centers located in Finland, Australia, and the USA, applying a multicenter study-design. All centers use the same rat model of PTE induced with lateral fluid-percussion injury (FPI). Rats are extensively investigated by using multidisciplinary methodologies with the aim to identify biomarkers, including plasma analysis, electroencephalography (EEG), and magnetic resonance imaging (MRI) which are performed in all participating centers.

EpiBioS4Rx is the first preclinical multicenter biomarker discovery study that has made a rigorous attempt to harmonize preclinical procedures and data analyses accross all study sites by using the CDEs and CRFs, which have recently become available for TBI and epilepsy studies (Harte-Hargrove et al., 2017; Lapinlampi et al., 2016; Scharfman et al., 2018; Smith et al., 2015). However, application of the same experimental protocols at different study sites does not necessarily result in true procedural harmonization, as noted by the pioneering study of Llovera et al. (Llovera et al., 2015), reporting data from a preclinical randomized controlled 6-center European therapy-development study for stroke.

To move the field forward, we not only harmonized preclinical procedures for biomarker discovery in the three EpiBioS4Rx centers, but also performed a rigorous *interim analysis* of the success of procedural harmonization, which is reported in this virtual special issue. The analysis included success of harmonization of the production of animal model (Ekolle Ndode-Ekane et al., 2019), blood sampling (Kamnaksh et al., 2018), EEG analyses (seizures, high-frequency oscillations) (Casillas-Espinosa et al., 2019; Santana-Gomez et al., 2019), and MRI analysis (Immonen et al., 2019). We also present an informatics approach that developed parameters and applied visualization tools to assess the overall success of harmonization (Ciszek et al., 2018). Data were collected from animals included in the study between February 1, 2017 and April 30, 2018. In the spirit of transparency, we present both the hurdles and successes encountered in the data collection and analyses to provide a realistic view to those individuals planning a preclinical multicenter trial in the future. With the experience gained, we propose a pipeline with three sequential study phases, summarizing the issues, which might help to optimize the quality of the data collected at different study sites. We believe that the pipeline will be applicable not only in preclinical biomarker discovery, but also in therapy discovery, both of which require large animal numbers to achieve statistical power.

## 2. Setting-up a multicenter preclinical biomarker discovery study - lessons learned

The planning, conduction, and analysis of harmonization of a preclinical multicenter study can be divided into three phases summarized in Fig. 1.

### 2.1. Phase I – preparation

The objective of the Preparatory Phase is to design and set-up the basic instruments for preclinical harmonization and to confirm that all sites have the capacity and infrastructure needed to perform the study. Preparations should be done before initiation of the actual collaborative project and may require several months to complete.

Generation of project-specific CDEs and CRFs requires understanding of the critical elements of the procedures, logistics of study work-flow, and anticipation of their variability and its effect on biomarker analysis. For example, what is the effect of the number and duration of anesthetic exposures or time of the day at blood sampling on plasma biomarkers? Therefore, experienced research team members play an important role in the design of study-specific CDEs. The team needs a person with prior expertise in the generation of a data dictionary and design of the electronic database (*e.g.,* RedCap). In international consortia, the location of the database is important to consider. So is the accessibility of the database from different countries as well as issues related to data download and access even beyond the project lifetime (Duncan et al., 2018).

During the Preparatory Phase the investigators should anticipate the time needed for obtaining the animal research licenses necessary to perform the preliminary experiment to test the study platforms (Phase II, see below). Investigators need to confirm that their center has hardware and personnel capacity to flexibly perform tightly scheduled investigations, for

example, time-consuming MRIs. Also, investigators need to prepare contingency plans for unexpected complications such as diseases in local or vendor animal colonies or a breakdown of expensive equipment (*e.g.,* MRI coil), which could significantly delay the project completion.

It is important to agree in advance on the quality control criteria to be applied at each site for each experimental procedure. Also, it is important to consider the effect(s) of conflicting standards on case inclusions and exclusions. It is critical to identify an expert in the consortium for each procedure who can perform hands-on training of other consortium members (*e.g.,* blood sampling according to 3R principles, lateral FPI injury surgery). This may require traveling over long distances and needs to be appropriately budgeted. Equally important is to agree on the project manager who continuously oversees the performance at each study site and notifies the project leadership team regarding missing data and protocol deviations. For this aspect of study planning and performance the electronic database is a key instrument as it can be programmed to send reminders and reports on missing values.

Sometimes it may not be possible to become completely harmonized. For example, the regulatory requirements for the timing of administration of analgesics relative to TBI may vary among countries, which can influence the harmonization of TBI procedures and acute post-impact follow-up (*e.g.,* analysis of righting reflex). Hardware needed for the project such as EEG amplifiers for high-density EEG or animal MRIs can vary. However, when the investigators are aware of differences such as these, their effect on harmonization procedures, data collection, and data analysis can be anticipated.

### 2.2. Phase II - testing

The objective testing phase is to learn the procedures needed in the study and to obtain preliminary data to assess the feasibility of the project. Phase II should also be completed before initiation of the actual multicenter study.

Phase II should include a small animal cohort at all study sites that is used to practice all experimental protocols, utilizing the planned study flow and data collection procedures. During Phase II the investigators can also refine the CDEs and the design of the electronic database, practice data entry, and download data between the study site and the database. Phase II is critical for hands-on learning, harmonization of procedures, and obtaining rapid feedback between the study sites, and therefore, should be closely monitored by the project manager. Exchange of videos can facilitate the procedural learning but should not replace face-to-face meetings. Media and procedures for communication between the study sites over different time zones needs to be established. It is important to perform a systematic analysis of the success of harmonization and data quality to identify the procedures that need more training, changes in on-site practices, and to estimate the effect of study site-related variability for the final power calculations.

### 2.3. Phase III – multicenter study

By the time of initiation of the actual multicenter study, the issues related to database construction and management, animal research licenses, contracts to use specific infrastructure, training in and practice of different procedures, and study monitoring should

have been solved. Each site should be able to perform the study within the planned time window. It is important to continue to monitor the procedures and database completeness. The consortium should be prepared to train newly hired staff. Development of study-tailored metrics for analysis of the success of harmonization should be developed and applied.

## 3. Conclusions

So far, there are two reports available from preclinical multicenter trials. In TBI, the Operation Brain Trauma Therapy study investigated various therapies to improve post-TBI recovery (Kochanek et al., 2011). In stoke, a large pRCT investigated the efficacy of anti-CD49d treatment for acute brain ischemia (Llovera et al., 2015). These studies, however, did not report the use of CDEs/CRFs, and no detailed analyses of the harmonization procedures were presented. The six articles in this virtual special issue give a detailed description of harmonization procedures in the EpiBioS4Rx preclinical multicenter trial focusing on biomarker discovery – both successes and failures to this point. We show that harmonization is possible, but training and monitoring are necessary to optimize the harmonization procedures within an acceptable time window. Further analyses will show whether the level of harmonization will influence the outcome measures such as rate of epileptogenesis in different centers. The data also emphasize that without rigorous analysis of the success of harmonization, it is difficult to judge the true level of harmonization between the study sites.

We propose a 3-phase process when planning a preclinical multicenter biomarker discovery study. The preparation (Phase I) and testing (Phase II) phases should precede the initiation of preclinical multicenter study (Phase III) and they should be clearly defined and benchmarked in funding applications. Phases I-II should be appropriately budgeted in terms of time and money, and it should be made clear this is separate from the research, yet essential to its success, even though it may require a special funding instrument to be developed to cover the costs.

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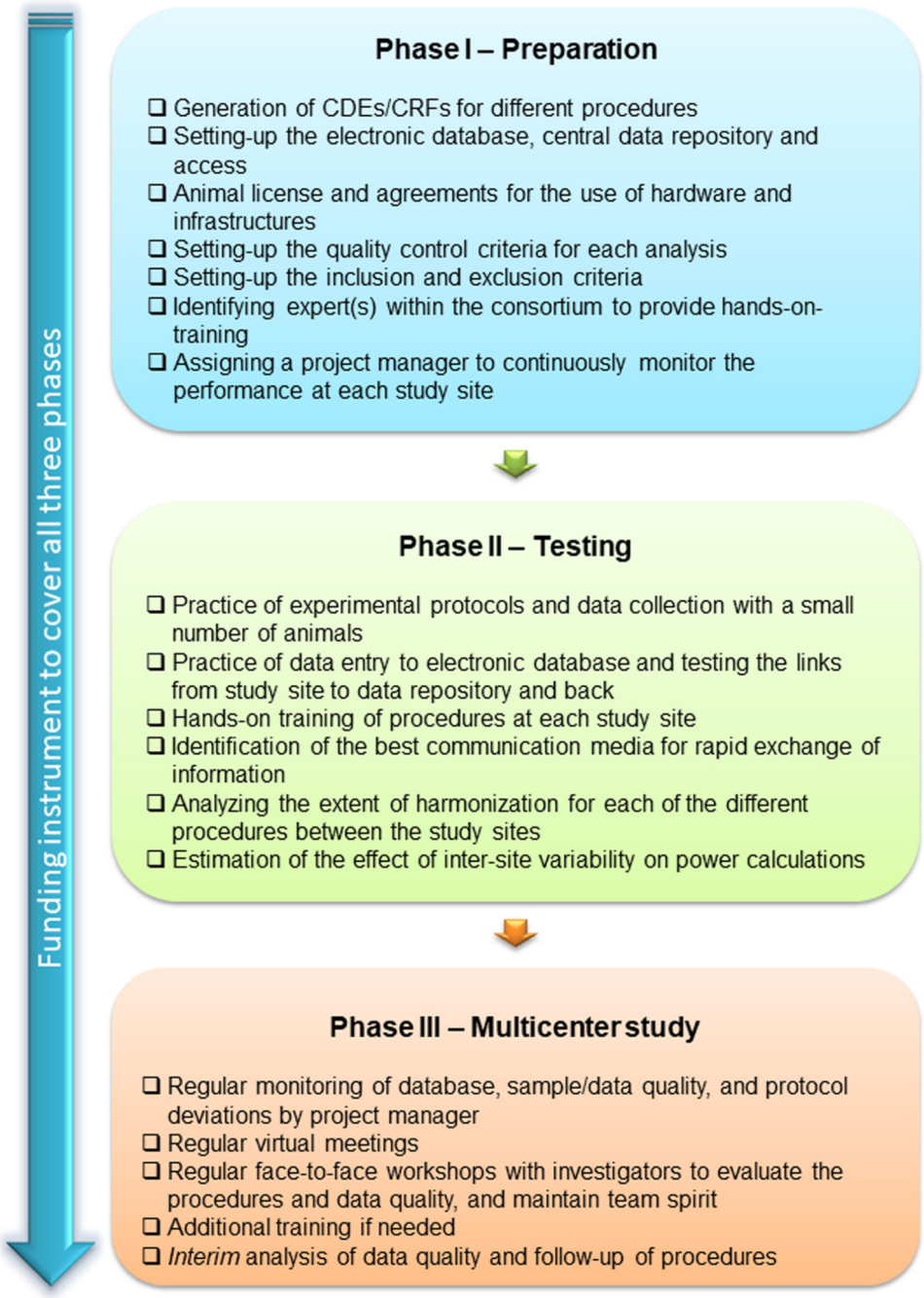


**Fig. 1.**

A proposed 3-phase process for harmonization of procedures in biomarker discovery. **Phase I**: During the preparation phase, each study site will prepare the paper work and contracts and will become prepared for personnel training and procedural monitoring. One important aspect is to design project-specific common data elements and set-up the electronic database. **Phase II**: During the testing phase each site will perform the planned experiments with a small number of animals, enter the data into the database, analyze the success of harmonization and its effect on power analysis, train personnel, and practice the monitoring

of procedures. **Phase III**: During the multicenter study the training and monitoring and analysis of procedural harmonization continue. Funding is needed also for preparation and testing phases, which precede the actual multicenter study and are necessary for its success. This should be taken into account in the design of funding instruments.