UNIVERSIDADE D
COIMBRA

Luís Carlos Pais Alves

The Role of Pharmacodynamic Endpoints in the Early Clinical Development of CNS-Active Medicines

Dissertação no âmbito do Mestrado em Biotecnologia Farmacêutica, orientada pelo Professor Doutor Sérgio Paulo de Magalhães Simões e pelo Professor Doutor José Luís de Almeida e apresentada à Faculdade de Farmácia da Universidade de Coimbra.

Outubro de 2021

The Role of Pharmacodynamic Endpoints in the Early Clinical Development of CNS-Active Medicines

Luís Carlos Pais Alves

Dissertação no âmbito do Mestrado em Biotecnologia Farmacêutica, orientada pelo Professor Doutor Sérgio Paulo de Magalhães Simões e pelo Professor Doutor José Luís de Almeida e apresentada à Faculdade de Farmácia da Universidade de Coimbra.

Outubro de 2021

AGRADECIMENTOS

Ao Professor Doutor Luís Almeida e à Susana Brandão pela oportunidade de estágio na BlueClinical.

Ao Professor Doutor Sérgio Simões pelos conhecimentos transmitidos no Mestrado em Biotecnologia Farmacêutica.

Ao Dr. Filipe Pinto por todos os conhecimentos transmitidos e pela constante disponibilidade e apoio. Por acreditar em mim e por me incentivar a exigir sempre mais de mim próprio, um enorme obrigado.

À Luiza por me ter acompanhado neste percurso, por estar sempre disponível para todas as dúvidas e pelo apoio incondicional.

Aos meus colegas, por personificarem a alegria no dia-a-dia.

Aos meus pais, por serem o meu porto de abrigo. Por todos os ensinamentos e conselhos que me dão, e me por me terem permitido ser tudo aquilo sou hoje. Aos meus avós, por quem mais quero ser um motivo de orgulho. Por todos os afetos e brincadeiras. Que em todos os momentos da minha vida vos tenha como exemplo, pois sei que, em qualquer lugar, estarão lá a olhar por mim.

Aos Campinácios, por me centrarem no essencial. Pelos valores transmitidos, pelas vivências partilhadas e pelo coração cheio com que regresso sempre. À Laura, por ter sido um enorme apoio ao longo desta aventura.

À minha namorada, por todo o amor e carinho, pelo seu apoio e conselhos, e por todos os dias me incentivar a ser uma versão melhor de mim próprio. Longe ou perto, agradeço por estar sempre lá e por me continuar a acompanhar em cada etapa da minha vida.

À minha família e amigos, por me terem acompanhado incessantemente ao longo da minha vida. Por me motivarem e incentivarem sempre a não desistir dos meus objetivos e por me ajudarem a superar todos os desafios.

A todos um enorme obrigado!

“The best way to predict the future is to create it.”

Peter Drucker

ABSTRACT

The pharmaceutical industry has been suffering from low success rates on the approval of new drugs. One of the main attrition is found in the early clinical development. This problem especially affects new CNS-active medicines. CNS biomarker techniques and inclusion of PD assessments in early clinical trials have a significant potential to reduce the drug development failure rate by providing more robust PK/PD data to support early "go, no-go" decisions to be made.

It is extremely important to have well defined PD endpoints to support a certain drug development. Resourceful CNS test batteries are presented, including assessments such as eye tracking measurements, body sway and subjective tests. Pharmaco-EEG concerns the quantitative analysis of the effects of substances on the CNS by means of neurophysiological and electrophysiological methods. The importance of pharmaco-EEG requirements is detailed, namely data acquisition (equipment and procedure) and data processing. The study of this clinical biomarker is complex, as it is affected by a high variability and the translatability is not universal across the spectrum of CNS-active drugs. A comprehensive review of this method, with particular emphasis on psychotropic drugs, is needed for an accurate validation and standardization of this approach in early clinical research.

The main area of interest will be the application of this tool in FIH clinical trials. EEG is still one of the cheapest methods that can be used to assess drug effects on brain activity and can easily be included in early-stage studies in healthy subjects. The application of this predictive biomarker is of high interest for early drug development, as it can be used to classify psychotropic drugs, evaluate drug-to-drug interactions, and monitor side effects. Moreover, the use of quantitative methods for data analysis provides a description of the PD effects of active compounds on brain functions, that may be used to study PK/PD relationships.

The main objective of this thesis is to provide a state-of-the-art on CNS pharmacodynamic assessments and, specifically, pharmaco-EEG and its application in early-stage studies with CNS-active drugs.

Keywords: Biomarkers; Central Nervous System; Phase I clinical trials; Pharmaco-EEG; Pharmacodynamic

RESUMO

A indústria farmacêutica tem vindo a ser afetada pelas baixas taxas de sucesso na aprovação de novos medicamentos. Um dos principais atritos é encontrado na fase inicial de desenvolvimento clínico. Este problema afeta especialmente fármacos ativos no SNC. A inclusão de biomarcadores do SNC e de avaliações farmacodinâmicas na fase inicial de ensaios clínicos têm um potencial significativo para reduzir a taxa de insucesso, fornecendo dados de farmacocinética/farmacodinâmica robustos para suportar decisões acerca da continuação do estudo.

É extremamente importante utilizar parâmetros farmacodinâmicos bem definidos para validar o desenvolvimento farmacêutico. Uma bateria de testes indicada para o SNC é apresentada, incluindo avaliações tais como métodos de rastreamento do movimento ocular, oscilação corporal e testes subjetivos. A fármaco-eletroencefalografia inclui a análise quantitativa dos efeitos de fármacos no SNC, através de métodos neurofisiológicos e eletrofisiológicos. A importância de cumprir requisitos específicos aquando da realização do fármaco-EEG é também detalhada, nomeadamente na aquisição de dados (equipamento e procedimento) e processamento de dados. O estudo deste biomarcador clínico é complexo, pois é afetado por uma elevada variabilidade individual e a compatibilidade não é universal em todo o espectro de fármacos direcionados para o SNC. Como tal, é necessária uma revisão abrangente deste método, com particular ênfase nos fármacos psicotrópicos, para uma validação e uniformização desta abordagem na investigação clínica.

A principal área de interesse será a aplicação desta ferramenta em ensaios clínicos de entrada no Homem com voluntários saudáveis. O EEG é um método barato para avaliar o efeito de fármacos na atividade cerebral e pode ser integrado de forma viável nestes estudos. A aplicação deste biomarcador preditivo é de grande interesse para o desenvolvimento clínico, uma vez que pode ser utilizado para classificar fármacos psicotrópicos, avaliar interações entre estes, assim como monitorizar os efeitos secundários. Além disso, a utilização de métodos quantitativos para análise de dados fornece uma descrição dos efeitos farmacodinâmicos de fármacos nas funções cerebrais, que podem ser utilizados para estudar a sua farmacologia.

O principal objetivo deste trabalho é fornecer um estado da arte sobre avaliações farmacodinâmicas no SNC e, especificamente, a fármaco-EEG e a sua aplicação em ensaios clínicos com fármacos ativos neste sistema.

Palavras-chave: Biomarcadores; Sistema Nervoso Central; Ensaios clínicos de Fase 1; Fármaco-EEG; Farmacodinâmica

ABBREVIATIONS

AE	Adverse Event
BBB	Blood Brain Barrier
CNS	Central Nervous System
ECG	Electroencephalography
EEG	Electroencephalography
EMG	Electromyography
EOG	Electrooculography
FDA	Food and Drug Administration
FIH	First in Human
fMRI	Functional Magnetic Resonance Imaging
HV	Healthy volunteer
IPEG	International Pharmaco-EEG Society
LORETA	Low Resolution Electromagnetic Tomography
MAD	Multiple Ascending Dose
MD	Multiple Dose
MTD	Maximum Tolerated Dose
NME	New Molecular Entity
PD	Pharmacodynamic
PET	Positron Emission Tomography
PK	Pharmacokinetic
POC	Proof-of-Concept
PSG	Polysomnography
QEEG	Quantitative Electroencephalography
SAD	Single Ascending Dose
SD	Single Dose

TABLE OF CONTENTS

INDEX OF FIGURES AND TABLES

FIGURES

TABLES

1 - INTRODUCTION TO CNS DRUG DEVELOPMENT

1.1 CNS Drug Development Landscape

The pharmaceutical industry has struggled with low clinical success rates for new drugs, with particularly high drug-failure rate in early clinical development. This especially affects new central nervous system (CNS)-active medicines.

Translating new therapeutic approaches for CNS diseases from animals to humans remains difficult, with a high attrition rate in CNS drug development. When compared to non-CNS drugs, this therapeutic area has a lower clinical approval rate and overall clinical success. CNS drugs entering Phase I studies have decreased significantly over the last years, accounting for 8% of all novel drugs entering clinical trials. Also, in Phase III development, CNS drugs were significantly more likely to fail than non-CNS drugs, with 45% less likeliness to obtain regulatory approval.

The higher unsuccess rate in CNS drug development raises significant strategical and financial risks for companies developing new drugs. This is reflected by a reduction in the number of CNS drugs under development over the last decades, and translates to fewer clinical trials for such drugs, both in early-stage and late-stage [1].

The low success rate in CNS clinical development, especially when it occurs late in the pipeline, leads to an exacerbation of the already high costs and time to market (12 years for CNS drugs, about double that of other drugs) and poses high risks for companies, which sometimes opt for withdrawing drug development programs in the neurosciences, accounting for the high risks involved [2].

On this matter, several causes have been highlighted as the reason for such high risks and failures in this area, mainly related with inadequate understanding of diverse critical factors. Some of these factors are related to (1) chemical and biological knowledge of the CNS, namely the challenging complexity of brain anatomy and function, (2) incomplete information and understanding of the complex nature of CNS diseases and (3) intra-brain distribution as well as the measures to study these processes (the inaccessibility of the human brain for sampling)[3]. On the other hand, the vast majority of high attrition is still linked to pharmacokinetics (PK) and pharmacodynamics (PD) that, unlike the previously discussed pool of attrition factors, have not seen significant advancements in past two decades. Inadequate or insufficient

knowledge on neuro-PK, blood-brain barrier (BBB) transport mechanisms and biomarkers of drug effects (neuro-PD), difficulty selecting initial drug dosage, the occurrence of hard to predict untoward toxicities and lack of efficacy are cited as major factors behind the high attrition rate of new CNS therapies [2].

Several researchers have highlighted lack of efficacy as the most common cause for drug development program discontinuation[1,2,4]. Most failures to demonstrate efficacy have occurred more often in Phase III than in Phase II, supporting the notion that many drugs may have been improperly assessed by sponsors in early-stage development [1]. These findings expose the need for more robust and accurate clinical testing in the early stages of CNS clinical development to mitigate the higher safety and financial risks inherent to later stages of development.

One possible way to reduce these risks is to identify earlier in the development pipeline which drugs are unlikely to succeed and terminate these projects before entering larger and costly clinical trials. New CSN-active drugs should undergo testing through valid preclinical and early clinical PD models as a precondition before higher investments are made to develop new therapies. This would allow resources to be better allocated on drugs with a greater likelihood of success. Such an effort can potentially accelerate development and market entry, significantly reducing the overall development cost and bringing obvious benefits for patients, the pharmaceutical industry and the community as a whole[4].

1.2 First-in-Human Studies

The reasons for attrition and possible causes explored in the early stages of CNS clinical development have a significant impact on the pipeline's subsequent stages. The clinical pipeline for drug development is typically divided into four phases: phase I (which is the focus of this thesis), phase II, phase III, and phase IV. Phase I trials are the first human trials of an investigational drug to assess its safety and tolerability profile. Phase II studies are intended to evaluate the safety and efficacy of a drug in a small number of patients at a specific therapeutic dosage. Phase III studies are designed to validate a novel drug's efficacy and safety in a larger patient population and to support regulatory approval. Finally, after approval, Phase IV studies are conducted in a real-world setting to provide further information on a new drug's risk-benefit profile [5–7].

Phase I studies are usually referred to as "first-in-human" (FIH) studies and traditionally represent the transition from non-clinical to clinical research. The FIH studies are those that study the first interaction between the new drug and humans. FIH research will provide the first human data on new therapies and, as a result, aid in the design of future studies [8]. The primary goals of FIH studies are to collect data on safety, tolerability, PK, and PD. These studies are non-therapeutic since they do not provide a therapeutic value assessment [9]. These trials are used to characterize the safety and tolerability profile in humans across a range of doses, and to detect possible adverse reactions at each dose level. Also, the mechanisms by which the drug is absorbed, metabolized, and excreted must be determined, translating to a bioavailability and PK profile. In what concerns PD, Phase I studies may provide preliminary data on how the drug affect the human body, provide an initial measure of efficacy, and serve as a guide for dose selection in subsequent trials [10].

Biomarkers, defined as any characteristic that is measured as an indicator of health, disease, or a response to an intervention, have been discovered to play a critical role in improving drug development efficiency and speed. Clinical endpoints directly measure how a subject feels and functions, being a highly reliable tool to show that benefits, as measured by clinical outcomes, outweigh adverse events (AEs). Later, the importance of clinical endpoints in CNS drug development will be discussed, since it is increasingly recognized, with the advance of science and technology, that the use of biomarkers can promote a more efficient development of safe and effective medicines. Thus, it is critical to implement biomarker programs in this phase [11].

FIH trials can have different designs. With the integration of non-clinical data available and the increasing robustness of data emerging during a trial in humans, FIH and early phase clinical trials are frequently conducted under integrated protocols that combine different study parts (e.g., Single Ascending Dose, Multiple Ascending Dose, and food effect). It is important to understand the complexity of these studies and how well planned they must be since, at this stage, little is known about the new product and only nonclinical evidence is available to support their conduct. These early trials are pivotal to achieve success in subsequent studies[8].

1.3 Study Population

To conduct a proper FIH study, the risk management strategy should consider various aspects of the trial. The choice of the study population is one of the most important issues that must be addressed, whereas these trials are often undertaken in healthy subjects but can

also include patients. Healthy volunteers (HVs), defined by the National Institutes of Health as "someone with no known significant health problems who participates in research to test a new drug, device, or intervention [12]", must satisfy an array of key inclusion and exclusion criteria, that include an adequate set of vital signs (with ECG), laboratory values and clinical assessments that must be within normal ranges [8].

HVs represent the ideal model for conducting this type of early clinical research, and prove a good model for tolerability and pharmacological activity (PK and PD) assessment of new compounds [7]. Several advantages are recognized in the enrolment of HVs in early-stage clinical trials [7,9]:

- Eliminates the interference of concomitant comorbidities or medication;
- Provide a pool of subjects with greater tolerability to potential intensive interventions and adverse effects;
- Allows the possibility of simultaneous treatment with multiple investigational products;
- Generates data that may be useful for several indications;
- Promotes study feasibility, allowing easier and quicker recruitment.

Despite the obvious practical advantages, these studies also raise a variety of ethical questions, as HVs are exposed to risks without any expectation of a health benefit [6]. Several authors have assessed the ethical concerns around FIH trials, considering AEs evaluations in various clinical trials. These assessments highlighted that, in HVs whose non-oncology test drug was administrated, most of AEs were mild or moderate, with very few severe or serious AEs [13–15]. Therefore, these conclusions emphasize the overall favorable safety profile of investigational non-oncology drugs observed in HVs studies. When the potential therapeutic effects of the experimental drug are expected to outweigh its well-known toxicity or when the expected risks are not acceptable in healthy subjects, trials testing high-risk drugs (e.g., oncology, anti-HIV drugs) are usually conducted in patients [7, 9].

HV studies benefit both promoters and patients when they are tailored to specific study objectives and can be applicable to most CNS drug development. Enrolling HVs also alleviates the ethical concern of enrolling patients with advanced disease in a short-term study at subtherapeutic doses when other studies (like Phase 2 or Phase 3 studies) might be more suitable for the patient.

1.4 Early-Stage Pharmacodynamics

The unraveling of clinical biomarkers as a useful tool in early-stage development has shifted Phase I conduct, allowing the emergence of clinical endpoints, and establishing itself as a powerful approach to lower CNS attrition rates.

Healthy subjects also play an important role in early-stage PD assessments, owning several advantages (and some limitations) when compared to patients.

Firstly, HVs represent a far more homogeneous population, accounting for a low PD profile variability and enabling a robust dose-effect and PK/PD estimation. Also, a clear safety evaluation (including biomarkers) and a full PK profile (including drug-drug interactions and food effect early assessment) are usually obtainable, allowing early risk-benefit and safety margin assessment. HVs enrolment promote operational flexibility, making it easier to setup complex biomarkers at a full time-course and consequently speed up the results. Specialized Clinical Pharmacology Units can effectively conduct these studies in relatively large sample sizes, in the order of dozens to few hundreds of subjects.

Even so, there could be some limitations of using HVs, which can be addressed in the form of three questions:

- The relative presence of the study target in the healthy population - Does the target exist? – and, if so, what is its distribution.
- The target expression – Is it sufficiently expressed in healthy subjects? – and, if so, to what extent?
- The biomarker dynamics – Does the dynamics of that biomarker allow to detect an effect? – with target occupancy and modulation being crucial measures to answer this question.

Differently, performing early-stage PD assessments in patients allows access to targets that may only exist in this population (normally overexpressed), altered downstream pathways, large biomarkers dynamics, and pathophysiological biomarkers. Still, patient enrolment results in a large interindividual variability, thus only large/qualitative effects are detectable, and rarely can robust/effect nor PK/PD be estimated. Additionally, there is a significantly higher operational burden due to operational complexity, ultimately leading to limited sample sizes. Also, safety interference, arising from disease and concomitant medication, is a major downside in early-phase PD assessments [16,17]. Several PROs and CONs regarding the selection

of either healthy subjects or patients for biomarkers assessments in early clinical development are depicted in Table I [16].

Table I. Biomarkers in Early Clinical Development (Healthy Subjects vs Patients)

	PROs	CONs

Healthy Subjects	• Homogeneous → Low variability
	• Recruitment, setup and study conduct time
	• Operational flexibility
	• Early risk-benefit and safety margin
		• Target relative presence
		• Target expression
		• Biomarker dynamics
		  ○ Target occupancy
		  ○ Target modulation

Patients	• Target only in patient • Overexpressed target • Altered downstream pathway(s) • Large biomarkers dynamics • Pathophysiological biomarkers	• Large variability • Safety interference (disease, concomitant medication) • Operational burden • Recruitment, setup and study conduct time

2. PHARMACODYNAMIC BIOMARKERS IN PHASE I

2.1 Pharmacodynamic Biomarkers Landscape

Pharmaceutical companies should avoid entering resource and cost-intensive late phase clinical trials with drugs that are unlikely to be therapeutically effective or that have low chance of being superior to existing treatments. To decrease attrition rates in late stages of development and prevent wasting time, energy, and money, a rigorous pre-selection process shall be implemented. Such process shall allow the positive selection of good candidate compounds from research and a rapid killing process for candidate drugs that do not show good results in earlier stages of development. In other words, the pharmaceutical industry must be far more selective earlier in the research and development process (as soon as in Phase I to Phase II studies) to ensure that only optimal candidates receive full development funding [17].

The lack of etiopathological knowledge, unclear markers of target engagement and costly failures when drugs do not achieve clinically relevant outcomes in large-scale studies have all hampered drug development for CNS conditions [18]. In a significant proportion of cases, the assessment of CNS effects of a candidate drug could help overcome those challenges.

CNS drug development needs to evolve in a way that minimizes the impact of lack of scientific knowledge in the related fields, namely psychiatry and neurology. Phase I clinical trials should be adapted by incorporating new ideas and techniques that more efficiently identify medicines that are not viable before they reach the final phases of testing (i.e., Phase III). Hence, the conventional Phase I clinical trial methodology is not "wrong," but instead is a starting point that must be adjusted and enhanced to establish a "customized" and efficient framework for the development of new CNS therapeutics [19]. The use of PD biomarkers in Phase I studies may have a powerful effect in the enhancement, as they cover a wide range of potential pharmacological effects and can contribute to guide the decision-making process.

Demonstrating target engagement can lead to earlier proof-of-concept (POC) studies, whether directly or by a downstream functional marker. Phase II attrition can be reduced by up to 50% with a combination of more extensive target validation and early POC trials, which, in turn, can reduce the cost of a novel molecular entity by up to 30% [20]. In early clinical drug

development, biomarkers can be useful in translating information from Phase I to Phase II studies.

In order to obtain a proper CNS effect, the drug must be able to enter the CNS at the right place, at the right time, and at the right concentration. Understanding the biological mechanisms behind the PK/PD relationships of CNS drugs is one of the key difficulties in developing treatments with higher safety and efficacy. The importance of PK/PD interaction assessments is still greatly overlooked in CNS early drug development and traditional early-phase trials consist of single- or multiple-dose designs, often merely focused on determining the safety and tolerability of an investigational compound in healthy volunteers [21]. PK/PD information obtained in Phase I studies includes:

- Identification and separation of PK and PD variability sources.
- Rational selection of potential biomarkers that correlates with clinical outcomes.
- Selection of appropriate clinical dosing regimens.

To know which CNS PD assessments to choose and how to assess the results is not an easy task. The great majority of CNS PD research is conducted to determine the clinical effects of well-known medications on brain functions, rather than to answer questions about brain penetration or target engagement, or to forecast a novel drug's effective dose range [22]. There are very few sets of PD data generated from human studies over a wide range of doses or concentrations [2,23], because results are frequently not published or publications are delayed to later development stages [22]. The clinical biomarkers and their use in drug development are summarized in section 2.3.

Although animals are also used to study concentration-effect relationships, animal models do not always precisely predict human disease, especially in the case of CNS disorders [24]. Disparities in blood-brain barrier (BBB) permeability, drug-metabolizing enzymes, and transporters can result in differences in drug exposure in the human brain compared to animals [25]. Furthermore, animal models may only mimic some pathways and mechanisms of human CNS disease or contain targets not seen in humans, hampering the translation efficacy and/or toxicity of novel therapeutic.

To overcome these concerns, a careful PK/PD assessment in humans at the clinical stage is essential to identify discrepancies from animal models and adjust dosing from the pre-clinical stage [2].

Vital information about a compound's dose–response effects can be obtained in the non-clinical studies and considered for early phase clinical planning, through a translational approach [21]. With early PK/PD data in hands, the 'Learn and Confirm' phase of drug development is anticipated, increasing the chance of a novel drug with sound and favourable previous data of (1) penetrating the CNS, (2) binding to the target, and (3) having a functional effect that is reflective of efficacy[20].

CNS biomarker advances have provided the tools and techniques needed to better understand and treat CNS diseases. Biomarkers help in understanding the cellular and molecular processes that underpin them. Additionally, with this knowledge it is possible to have a deeper comprehension of the neurocircuitry that needs to be targeted to treat some psychiatric and neurological disorders. With a deeper understanding of how neurocircuits relate to specific symptoms, a brain-based taxonomy can be used to identify CNS disorders. This, in turn, permits new targets to be identified, as well as biomarkers that can be used to guide the development of drugs specific for those targets [20].

The methodologies used to establish neurocircuitry engagement may add significant clinical complexity and, depending on the approach, it may be feasible to include more simplistic behavioural assessments that are known to be correlated to specific circuit modulation [20].

The assessment of novel therapy's therapeutic potential in smaller, better-designed POC studies, shifts the PD assessment to earlier-stages of development[21]. The use of PD biomarkers in early-phase clinical trials (low-population-variability setting) will assist in the identification of important clinical PK/PD covariates, through the evaluation of intraindividual PK and PK/PD variability. The identification of variability in these parameters can also lead to a better notion of the inclusion and exclusion criteria in Phase IIa and dose individualization in Phase IIb. These studies' results may also contribute to the rationale behind the selection of potential biomarkers to monitor during dose escalation (Phase IIa) and help correlate target engagement to functional outcomes (Phase IIb). Finally, establishing exposure-response relationship for biomarkers may support the rational for Phase II dose regimens, starting dose, dose increments and dosing intervals[26].

Going to late Phase II and Phase III clinical trials, one shall consider that changes in biomarkers usually follow a different time course than changes in clinical endpoints and are often more closely related to the time course of plasma drug concentrations. As a result,

biomarker-based exposure–response relationships can aid in better establish dose ranges for clinical testing, and, in some cases indicate when to assess drug titration [17]. This process is an integral part of dose-ranging studies, and consists in adjusting the dose of a medication for the maximum benefit without adverse effects, where the experimental drug is given in increasing dosages until side effects become intolerable [27]. For this reason, insight into potential AEs is also provided at this point.

Clinical biomarkers also have the possibility of altering the trajectory of CNS drug development by helping to stratify future patient populations, allowing for smaller patient studies [20], and to clarify which populations may benefit from specific targets engaged by novel therapeutics [18], ultimately contributing to an earlier decision-making.

The potential to 'de-risk' a clinical development program through a PK/PD early approach frequently more than offsets the added complexity and cost required to include biomarker assessments in early stage studies [21], and even if a biomarker fails in the validation process, it may still be beneficial to have employed it because more knowledge about the disease's pathophysiology and the drug has been acquired [17]. Ultimately, PD data will help in the decision-making process.

The decision-making process is intrinsically related to effective "go-no go" trial decisions, since for many CNS disorders, clinical trials are difficult and sometimes apparently adequately powered clinical trials may fail to show efficacy and provoke unnecessary cost burden. Many biomarkers will never undergo the rigorous statistical evaluation that would establish their value as a surrogate endpoint, even so they are substantially decisive to support the decision to commit (or not) to a major clinical trial program [17].

2.2 Three Pillars of Drug Development

In early clinical development, fundamental data and information is required to assess if an NME has the ability to elicit a pharmacological effect and so test the mechanism of action in humans. These key aspects of data and knowledge were defined as the "three pillars of survival".

PK/PD knowledge must be improved to support the three pillars of drug development that can inform key decisions related to a compound's subsequent clinical development plan [28]:

- Pilar 1 - Exposure at the target site of action;
- Pilar 2 - Binding to the pharmacological target;
- Pilar 3 - Expression of pharmacological activity.

Pillar 1, related to biophase exposure, encompasses the topics about the drug's pharmacokinetics, eliciting the importance of drug exposure at the target site of action over a desired period of time. This pillar focus on the PK parameters, such as PK profile and the rationale for drug dose regimen, which are crucial for drug´s binding to the pharmacologic target as expected from the mechanism of action and to elicit pharmacological effect over an appropriate span of time.

Pillar 2 concerns to the effective drug binding to the intended target, according to its theoretical mechanism of action. Target engagement is a prerequisite for expression of pharmacology and target modulation, and therefore it is necessary to assess not only dose/effect and PK/PD relationships but also the specificity of target occupancy (on-target vs. off-target).

Pillar 3, the pillar more related to the drugs' pharmacodynamics, follows the fundamental principle that the functional modulation of the target is a prerequisite for potential therapeutic activities and to test the mechanism of action of a new molecular entity (NME). PK/PD studies of biomarkers that indicate expression of pharmacology at the site of action are most likely to provide the highest level of confidence and direct proof that sufficient levels of target and downstream pathway modulation are being accomplished. Thus, if the compound's functional pharmacological properties and mode of action are well characterized, commensurate with the demonstrated target exposure and target binding (Pillar 1 and 2 respectively), some assurance can be drawn indirectly [28].

On practical perspective, the assessment of the three pillars for a certain new drug can help determine the confidence that can be attributed to its exposure and pharmacological effect. Four levels of confidence and four different outcomes emerge from this system and guide the clinical development of a drug (Table 2) [28].

Table 2. Use of Three Pillars of survival to manage risk in early clinical development. Adapted from Morgan *et al* [28].

		Pharmacology confidence	
		LOW	**HIGH**
Exposure confidence	**HIGH**	• **Pillar 1 and 2** Risk in relying only on exposure and binding; study design and decision-making from clinical endpoint needs to be clear	• **Pillar 1,2,3** Maximum confidence in translation of drug exposure and pharmacology & of testing the mechanism
	LOW	• **None or partial Pillars** Serious concerns that mechanism will not be tested & clinical studies unlikely to be definitive	• **Pillar 2 and 3** Reasonable risk being carried forward if confident that drug reaches target in humans & clinical endpoint relevant to site of action

Four possible scenarios reflect exposure and pharmacology confidence and therefore guide the decision for further studies. The matrix was developed so the alignment with the three pillars of survival could be used to assess the likelihood that the new drug will attain a positive PK/PD result.

Several drug development programs have been used to determine whether the alignment with the three pillars of survival provides a positive correlation with program progression or termination. According to the findings, the three pillars of survival are the core building elements of a compound's profile, and collectively, they are highly correlated with the likelihood of a candidate drug's success in development and the ability to test the mechanism.

If none of the pillars discussed above are assured, exposure will probably only be observed in plasma, not at the target site (e.g., CNS), suggesting that the PK/PD relationship is not well established and that there is no data to show relevant downstream pharmacology effect. In this case, clinical studies are unlikely to be definitive. Given that only pillars 1 and 2 or pillars 2 and 3 are established a reasonable risk is being carried forward and the drug success depends on further well-established study designs. In an optimal scenario, pillars 1, 2, and 3 are secured and such demonstration would result in maximum confidence in the translation of drug exposure and pharmacology, and also of a properly tested mechanism of action [28].

The aforementioned pillars serve as early guidance on how to reduce the uncertainty in Phase 1. In this matter, clinical biomarkers are of great interest to understand if the pillars are being adequately assessed. Relying on a mechanism-based level of classification, proposed by

Danhof *et al.* [29], it is possible to choose the best biomarker for the related pillar assessments. According to this classification, the type 2 (target occupancy), type 3 (target activation) and type 4 (physiologic measure) biomarkers can be used to predict the biophase exposure, binding to target, and target modulation, respectively. At this stage is important to have a limited but robust fit for purpose validation regarding the chosen biomarkers, with a special focus on variability (reproducibility and repeatability), dynamics, and operational feasibility [16].

This analysis indicates that paying close attention to basic principles of PK and pharmacology throughout drug discovery and clinical development can be of high value in the improvement of candidate survival.

2.3 Clinical Biomarkers

Phase I studies are expanding to examine the effects of drugs, recurring to novel strategies that explore both traditional endpoints and newer endpoints selected specifically to test the potential utility of the drug on the target illness. This "expansion" of Phase I trials is driven by the need to collect data in a time- and cost-effective manner that will allow sponsors to be more efficient in making "go/no-go" decisions regarding a new investigational molecule at the different checkpoints of its clinical development [19].

On this subject and referring to the already discussed importance of the PK/PD data, one can note a discrepancy between the inclusion of PK and PD assessments in early development programs. While increased consideration of the PK profile's suitability for drug survival assessment has resulted in a decrease in early program terminations [30], it has also highlighted the other relevant causes for compounds being deemed unsuitable for drug development, such as inadequate clinical safety and efficacy. The selection of suitable safety and efficacy biomarkers can help to improve the rationale development of new drug molecules. In combination, or as an alternative to such approaches, consideration of the required drug exposure providing the desired and/or undesired pharmacological effect can contribute to a quantitative assessment of the potential safety/risk relation and efficacy [31].

Recent advances in biomarkers and translational medicine have increased the understanding of the cellular and molecular processes involved in neuropsychiatric and neurological disorders, as well as the underlying neurocircuitry. Enhanced imaging techniques, patient selection tools, digital technology, liquid biomarkers, and electrophysiology have all contributed to advancements in this particular area [20]. Novel diagnostic and enrichment biomarkers will allow more integrated application of discovery and development information

[17], leading to better patient selection and improved clinical trial design [20]. PD endpoints that go beyond traditional animal behavioural endpoints are required, particularly if these measures can be translated into meaningful signals in early-phase healthy volunteer trials [21].

According to the National Institutes of Health (NIH) Biomarkers Definitions Working Group, biomarkers can have a wide classification [32,33]:

- Biological marker (biomarker): a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention;
- Clinical endpoint: a characteristic or variable that reflects how a patient feels, functions, or survives;
- Surrogate endpoint: a biomarker that is intended to substitute for a clinical endpoint. A surrogate endpoint is expected to predict clinical benefit (or harm or lack of benefit) based on epidemiological, therapeutic, pathophysiological, or other scientific evidence.

A clinical biomarker of drug effect should reflect a process on the critical path between the pharmacological action of the drug and its effect on a disease [17], acting as a quantitative indicator of a biological process. In drug development and pharmacology, influencing such a process is done exclusively to attain a clinical endpoint, which is an improvement of the feelings, functioning, or survival of a patient [33].

Also, it can be divided into three distinctive groups: pharmacological, which can be observed in healthy volunteers; toxicological, which can also be observed in patients; and pathological, which can only be observed in patients having the disease. Clinical biomarkers may not only be divided into distinct groups but also be classified based on the "location" in the chain of events, from underlying subject genotype or phenotype through to clinical scales. This mechanism-based approach consists of a differentiation of seven types of biomarkers [29], on which there will be a particular emphasis on type 2, type 3, and type 4, as already explained in section 2.2. Since the main aim here is to discuss Phase 1 clinical trials in healthy subjects, the focus will be the group of pharmacological clinical biomarkers.

The capability of neurophysiological measures to serve as biomarkers is critical to their utility in guiding CNS drug development. According to *Joshi et al.* [18], neurophysiological biomarkers must have three desirable qualities to succeed in clinical studies – translatability, validity, and scalability. Firstly, valuable biomarkers must be translatable, so that they must be

accessible and measurable in preclinical models, as well as sufficiently well studied so that they can be linked to relevant underlying neural circuits and known mechanisms of cognitive dysfunction in CNS disease. To be useful in human trials, ideal biomarkers must also be (1) reliably assessed in both healthy subjects and patients, (2) suitable for use as a repeated measure (i.e., insensitivity to order or practice effects) and (3) responsive to pharmacological agents or cognitive training interventions. If these candidate biomarkers ultimately prove useful for predicting or monitoring clinical effectiveness, biomarker acquisition should also be (1) low-cost, (2) scalable, and (3) suitable for use in multicenter studies with no need for a specialized testing environment (i.e., real-world clinical settings).

A biomarker is likely to be of greatest use if the therapeutic effect is difficult to measure, if there is a considerable delay between drug exposure and effect, and/or if the novel drug affects a pathway for whose role in disease is not well understood or unknown. In the early stages of clinical development, when measuring clinical endpoints is too time-consuming or cumbersome to deliver timely proof of concept or dose-ranging information, clinical biomarkers are the most effective solution [17].

Thus, the development of NMEs may be accelerated by incorporating measurements of target engagement into Phase I studies. A target engagement marker (also known as a pharmacodynamic biomarker) indicates if the NME is successfully targeting the intended disease pathway, but it is not itself automatically predictive of a clinical outcome. Therefore, validated biomarkers that have already been compared to well-established clinical endpoints should be used to obtain the desired results [19]. In this early stage, establishing POC via changes in clinical biomarkers is only as good as the conceptual approach for the clinical biomarker. The scientific program for assessing biomarkers should be developed as early as possible in the drug discovery and preclinical development phases, to bring the biomarker into clinical trials and establishing a link between the biomarker and the clinical outcome [17].

The development of a prototypical compound is always based on some balance between target and off-target effects. Especially in early development, the same biomarkers can reflect, in some cases, an off-target effect and, in other cases, the desired pharmacology of a drug. Thus, it is very important to be careful in the selection process for biomarkers, so as to select those that are fit-to-purpose for the stage of drug development in which they are used. This question must be assessed with a structured and practical approach, something that is widely applicable and particularly useful for the early stages of innovative drug development [33].

Considering these matters, the applicability of clinical biomarkers must go "hand in hand" with the three pillars of drug development [33] so that the testing of mechanistic aspects of new compounds gets improved and the usually high chance of failure [28] gets mitigated.

2.4 Functional Biomarkers

As referred in the previous topic, for CNS drug candidates, biomarker-guided drug development assures target engagement, offers early proof of efficacy, and helps guide future dose. Concerning clinical biomarkers, one may distinguish from mechanistic biomarkers and functional biomarkers, according to their own characteristics and purpose for which they are being assessed.

Mechanistic biomarkers can demonstrate target engagement via pharmacodynamic data such as receptor occupancy, enzyme binding, and enzyme activity. Mechanistic markers used to demonstrate target engagement are usually linked to the mechanism of action of the drug, and the tools needed to directly demonstrate target engagement may not always be available in clinical trials. In non-clinical studies confidence may come from tissue level measurements and in vivo receptor occupancy assays using a vast range of highly validated techniques. Liquid chromatography-mass spectrometry technology, PK/PD modelling and simulation for dosing are some of the most used, but fewer tools are available when it comes to the clinic context. PET imaging emerged as a potential solution, and if a PET ligand is available, it becomes a useful tool that can be applied to both non-clinical and clinical trials and provide a proper correlation between both phases. In the absence of a ligand, the approach becomes more limited, but it can still be utilized to demonstrate CNS penetration. Functional magnetic resonance imaging (fMRI) and cerebral spinal fluid measurements are also appropriate methods for demonstrating CNS penetration [20].

Functional biomarkers, on the other hand, can assess a functional change in the brain as a result of target engagement. Functional markers, such as quantitative EEG (QEEG), and event-related potential, can be utilized to show a physiological response that corresponds to target binding and functionalization. The demonstration of downstream functional effects is of great value at this early development stages, since it can be used as an indirect measure of target engagement. Moreover, the information retrieved from studies including functional biomarkers back translates to non-clinical research, making the process more robust and reliable. This provides reasonable evidence of CNS penetration and target engagement downstream, but it falls short of taking into account the complex brain activity, since

neurotransmitter changes are also crucial to the neurocircuit. To that end, and in light of recent advances in the field, neuroimaging may serve as an alternative or complement to QEEG analysis. Neuroimaging provides a comprehensive spatiotemporal resolution, allowing for real-time neurotransmitter measurements and a better understanding of neurotransmitter dynamics. However, this type of method requires the use of specialized imaging centers, which may not be feasible and may result in lengthy response times and increased costs. Overall, each of these markers provide additional insight into the neurocircuitry that is being modulated by the NME [20].

Ideally, receptor occupancy and target engagement in non-clinical models can be correlated with a pharmacodynamic effect, and an analogous measure can be used in healthy volunteers or early patient studies for proof-of-concept trials. In patients, functional CNS measurements are used to characterize psychiatric or neurological disorders for research and diagnosis purposes, as well as to assess treatment response in clinical practice. Functional measurements, on the other hand, are employed in the early stages of drug development to learn enough about the new medicine's effects to improve the success of subsequent clinical trials. In drug development, these two goals are frequently combined, however the focus shifts as the program progresses. However, it is vital to keep in mind that the conditions for measuring clinical endpoints in neurobehavioral diseases differ from those for PD assessments of neuropsychiatric drug effects in early-phase development. In the latter, being that the focus of the research is on the properties of the drug rather than the disease, measurements are more frequently performed in healthy volunteers rather than in patients [22].

Even if the biomarkers are exploratory and not surrogate markers of the disease, observed biomarker alterations may correlate to relevant clinical outcomes. As a result, functional markers can be used to predict clinical response in the target patient population, allowing for more timely decision-making. A biomarker-guided drug development methodology can improve research and development productivity, which is necessary to keep innovation going and avoid revenue loss [34].

2.5 Integration of PD biomarkers in Phase I

Traditional Phase I studies typically involve single (SAD) and multiple ascending dose (MAD) trials in healthy subjects to establish safety, characterize PK and to identify the maximally tolerated dose. Contrastingly, studies assessing the pharmacodynamics of CNS novel drugs in

a Phase I context are scarce, and those that are performed not only do not use validated functional pharmacological biomarkers but also are not usually an integral part of the early development program [22]. That said, it is also possible to particularize these major shortcomings for psychotropics development, where the tendency is to oversimplify relevant factors underlying disease mechanisms and pharmacological effects, since neuro-PK is mainly studied without evaluating and correlating to appropriate neuro-PD. This limits the evaluation of the practical significance and the predictive value of specific tests for this particular phase and class of drugs.

Since the currently applied simplistic approach to produce data on multiple processes in isolation is not informative as processes are context dependent, it would be of great added value if neuro- PK and associated PD would be obtained in a single experimental context, allowing for more robust data collection [3]. Study population is also a critical aspect when deciding the study design. It is crucial to have a well-planned study population adjusted to the development scheme, since the three pillars of drug survival may not be achieved by the simplest traditional Phase I study. These key aspects can be studied in HVs, but they may also need to be studied in patients with the target illness, either to assess for the desired effect or because the dose response curve may shift in the population with the target illness compared with normal volunteers. In certain cases, it may be worth planning the study so that it incorporates a seamless transition to participants with the target illness (symptomatic volunteers) to characterize the dose-response curve in that specific population [19].

As discussed in Section 1.1 the probability of success for novel treatments of CNS disorders leaving Phase I trials is markedly lower than in other therapeutic areas. To avoid costly failures associated with larger Phase II/III trials, many pharmaceutical companies have increasingly incorporated translational medicine models, PD modelling strategies, as well as adaptive dose and study population into Phase I trials. These shifts in their development plans have been implemented to achieve detailed mechanistic and functional data to inform "go-no go" decisions on a compound's success while mitigating risk associated with developing a new compound [21].

From a regulatory perspective, there is not any published guidance that clearly defines the desirable selection criteria for neurophysiological biomarkers. Thus, there was (and still is) a unanimous lack of consensus on a well-accepted method for measuring neurocognition in clinical trials, defining the optimal trial design for such trials, and how regulatory agencies

should approve new entities in neuropsychiatry. In 2008, an initiative reuniting a variety of cognitive neuroscience experts - academics, the pharmaceutical industry and the US Food and Drug Administration (FDA) - identified the following criteria as desirable in an FDA-approved battery for use in clinical outcome measures [1835]:

- High test-retest reliability
- Utility as a repeated measure
- Relationship to functional outcome
- Tolerability and practicality
- Responsivity to therapeutic agents
- Construct validity
- Clear link to neural circuits and cognitive mechanisms
- Available animal models

A well-controlled study or studies using a clinical biomarker presenting all the above criteria may serve as confirmatory evidence to support one adequate FIH study [17], as stated in the Section II of the FDA Guidance for Industry [36], *"When the pharmacologic effect is not considered an acceptable effectiveness endpoint, but the linkage between it and the clinical outcome is strong, not merely on theoretical grounds but based on prior therapeutic experience or well-understood pathophysiology, a single adequate and well-controlled study showing clinical efficacy can sometimes be substantiated by persuasive data from a well-controlled study or studies showing the related pharmacologic effect."*.

As per this regulatory basis, there are several key points during the early part of the drug development process at which clinical biomarkers can be employed, each of them to address different questions. A general approach may translate in a design as illustrated in Figure 1. In preclinical studies PD measures can be initially implemented to provide predictions of efficacy or to develop hypothesis that can be tested later in clinical studies. Many PD-based methodologies employed in the clinical testing are typically derived from preclinical animal behavioral studies, thus, later in the pipeline, the clinical plan can include these measures either integrated in the Phase I studies or in a separate PD-based study. A parallel biomarker study can be conducted either when there are practical constraints or the required statistical power cannot be attained within a typical FIH design. Ideally, such an approach will enable the management of the numerous applicability of PD biomarkers, as depicted in Section 2.3. This can guide "go-no go" decisions and therefore the continuation to Phase II trials [4].

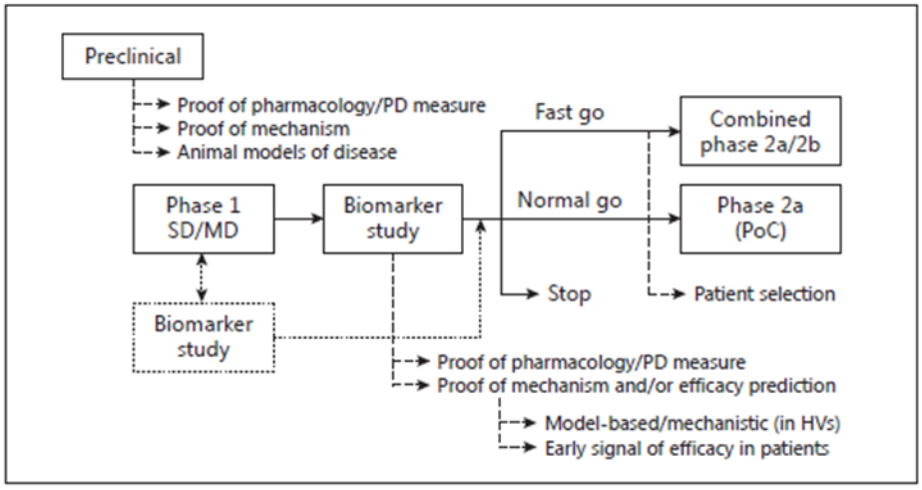

Figure 1. Points of application of PD biomarkers in early drug development. Retrieved from *Wilson et al* [4].

As depicted in Figure 1, in addition to the traditional FIH design, multiple PD methodologies are incorporated across both the SD/SAD and MD/MAD studies. According to the state of the art, the ideal design for FIH studies with PD measurements in the CNS is a crossover design with ascending doses. In this design, usually small panels of subjects are used (4–5 subject-per panel) [37]. For these types of studies, many designs are possible, and while a standard approach is frequently adopted, it is neither mandatory nor part of regulatory guidance [19]. The choice of the design largely depends on the questions that one wants to answer in the trial. Adopting an adaptative design is beneficial, with the possibility of increasing the number of subjects at several stages during the dose progression. Furthermore, dosing panels may be repeated (when additional data is required) or the overlap of doses may be increased (when a more careful progression of escalation is warranted) [37]. This provides significant adaptability and safety, particularly in the MAD study, since dose level escalation is flexibly defined (limited by toxicology limits) and the switch from HVs to patients is clearly defined in the protocol, resulting in a more rapid and less expensive investigation of the NME[21].

English et al. presented an illustrative example of a development "go-no go" plan of a psychoactive drug Phase I program incorporating various PD methodologies (Figure 2). In the SAD study, PD studies can be included as part of the SAD dosing cohorts, or separate PD focused cohorts (1c) can be conducted at 2–3 dose levels once safety and tolerability data have been obtained from the MTD cohorts (1a). In addition, a comprehensive PD battery (1d) can be included as part of the SAD and a food effect study can also be considered in this design

(1b). As with the SAD, PD methods can be included as part of the MAD cohort panels (2a), where dosing may extend beyond concentration steady-state to include additional PD measurements (2b). Similarly, a PD battery (2c) can be included as a separate cohort of the MAD to include multiple measurements [21].

The timepoints at which the PD assessments are performed are also crucial to obtain reliable data of drug effects. Figure 3 illustrates the example of combining multiple PD methods into a PD battery. In this example, a novel investigational compound is being evaluated using a ketamine-reversal, crossover model where the test compound is administered prior to ketamine. The drug administration is preceded (baseline testing) and followed (dosing period and postdose testing) by fMRI, QEEG and cognitive/behavioral assessments [21].

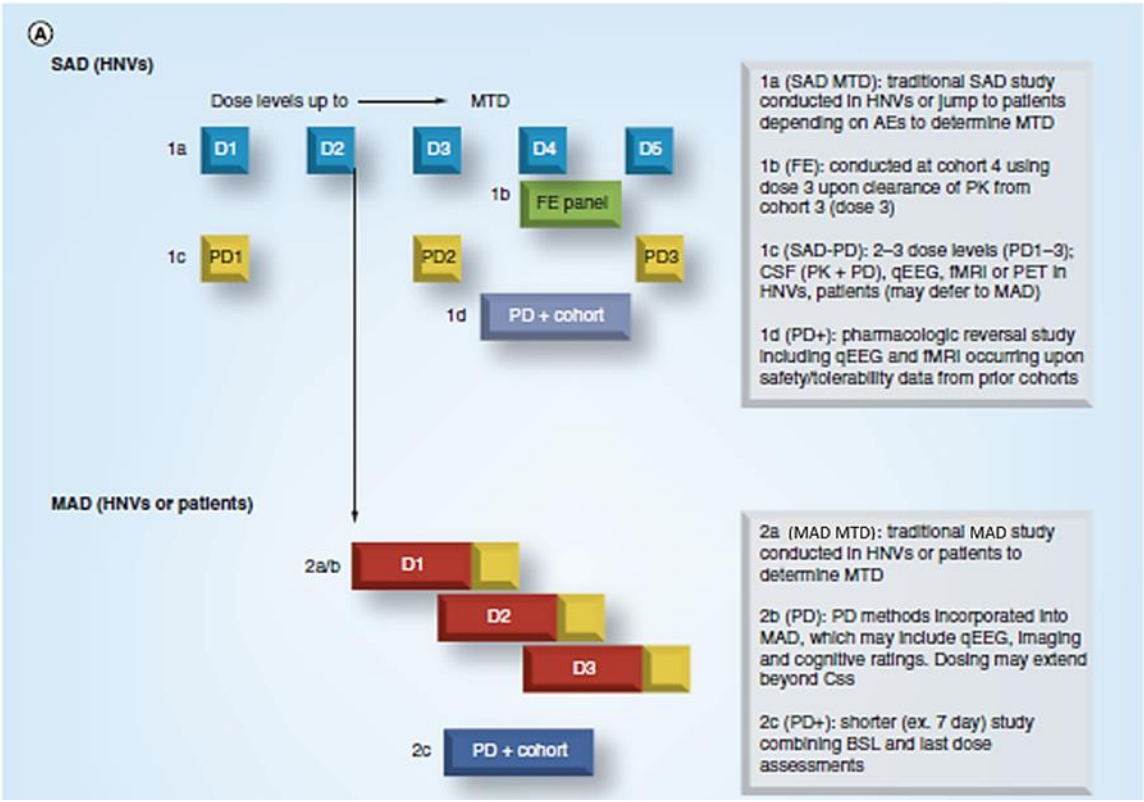

Figure 2. Illustrative development "go-no go" plan of a psychoactive Phase I program incorporating various pharmacodynamic methodologies. Adapted from *English et al* [21].

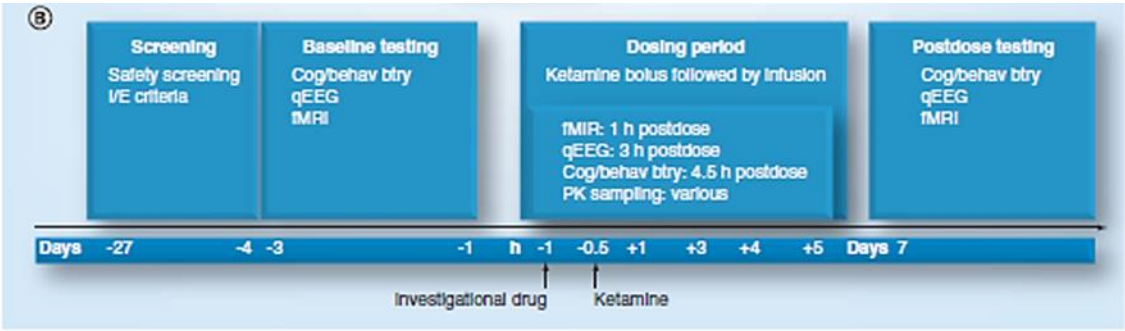

Figure 3. Illustrative example of the PD assessments timepoints in a psychoactive drug Phase I study. Adapted from *English et al* [21].

3. CNS PHARMACODYNAMIC ASSESSMENTS

3.1 CNS Test Batteries

Phase I studies are typically data-intensive, with numerous sets of assessments performed in succession to establish a novel drug's PK/PD profile. The influence of CNS drugs, either as a primary therapeutic effect or a less desirable side effect, can be evaluated by several tests specialized in the assessment of these effects [38,39]. The CNS is naturally sensitive to a variety of external and internal factors, which interact with the effects of CNS-active medicines. Before conducting research in this field, these variables must be carefully evaluated. In view of this, it is especially important to show effects which indicate penetration of the blood-brain barrier and correlation between a compound's CNS effects and both the dose and blood concentration, helping to determine whether an effect is due to specifically the compound [39]. Understanding which biological systems are activated and obtaining proof of pharmacodynamic effect is a vital factor to dispel doubts about the pharmacology of a new psychoactive agent. These test batteries allow the evaluation and comprehension of the abovementioned effects through a series of practical research questions, that closely reflect the pillars of drug development. With the application of these tests, it is possible to determine if the compound in test has the expected specific properties [40], estimate the optimal dose [41] and assess the safety and tolerability, primarily through confirmation of the therapeutic index [42].

There is evidence that various receptors are not always found in a single brain region, so that drugs acting on the CNS will usually influence more than one function and affect several different functional domains within each group[43]. This offers numerous possibilities to measure drug-induced changes in CNS activity. Thereupon, the usual core test battery comprises several domains to test both neurophysiological and neuropsychological functions (Table 3).

Neurophysiological assessments comprehend a range of objective tests sensitive to detect effects in motor coordination, visuomotor functioning and coordination, alertness/attention, sleep characteristics and also in the autonomic nervous system. The most common tests that are capable to measure these effects are body sway, smooth pursuit eye movement, adaptive tracking, saccadic eye movement, polysomnography (PSG) and pupil size. Pharmaco-EEG is also part of this group of tests, since it provides a general overview of CNS activity. Neuropsychological assessments, also denominated as subjective tests, detect changes in subjective conditions, which are important aspects of drug effects. These tests are often among

the most sensitive drug effects measures and, even though the instruments partly overlap, they are easily interpretable. Visual analogue scales and questionnaires, memory testing and cognitive tests are examples of measurements used to assess subjective drug effects, memory, and cognitive performance, respectively [44].

Table 3. CNS test and measures.

Functional domain	Test or measure
Alertness	Saccadic eye movement
Visuomotor coordination	Adaptative tracking
Motor coordination	Smooth pursuit eye movement Body sway
General CNS activity	Electroencephalography (EEG)
Sleep	Polysomnography
Autonomic nervous system	Pupil size
Subjective drug effects	Visual analogue scale Questionnaires
Memory	Memory tests
Cognitive performance	Cognitive tests

The tests can be jointly employed in an adapted design (*e.g.,* Figure 3) depending on the specific needs and objectives of the clinical trial. This allows for a complete coverage of all relevant domains of CNS activities, improving not only the information but also the safety of a trial, because it shows CNS effects even before these unravel as AEs. The feature that the test battery can be adjusted with different tests according to the protocol allows it to be suitable for use in studies with different types of drugs, but also in different populations. The tests have a short duration and can be readily repeated with negligible learning effects. This enables continuous testing throughout the day (both before and after drug administration), which is essential for comparing CNS effects to plasma drug levels or adverse effects. Following that, full dose-effect curves may be easily measured. These assessments are characterized for being highly sensitive, robustly detecting even subtle effects, in most cases associated with class-specific profiles [43,44].

In the following topics several of the aforementioned assessments will be further detailed.

3.2 Eye Tracking

The study of eye movements is increasingly being employed as a model to study the many-body interactions of a large variety of complex systems, ranging from the brain to collaboration networks. It can reveal information about neurodegenerative processes and cognitive function in addition to measuring motor control. These processes are still poorly understood, especially in the early stages of disease, making new treatment strategies difficult to implement [45]. For that reason, saccadic and smooth pursuit eye movements have been frequently used to assess CNS-drug (side) effects.

Saccadic eye movements, or simply saccades, are highly sensitive and specific measures of alertness, sedation, and tranquillity [44], so that they have become a popular means to study motor control, cognition, and memory, and are often used in conjunction with techniques such as functional imaging. A method for their measurement in a large population of healthy volunteers was originally described by Wilson *et al.* (1993) [46], being nowadays among the most well-understood movements, with easily measured dynamic properties [47]. For this test, with an average duration of 1 to 2 minutes, the subject is instructed to continuously follow a light source, which moves on a computer screen and, as the light source "jumps" from side to side, the eyes' movements - saccades - are monitored [43–45]. The majority of studies report saccadic peak velocity for measuring visually guided saccades or antisaccades (where subjects are instructed to look away from the target) [48]. Although there are different techniques to quantify eye movements [45], saccadic peak velocity is one of the most sensitive measures of alertness currently available in drug research, representing the maximum velocity of the eye when the light jumps. In addition, reaction time (s), jump size (deg) and inaccuracy (%) can also be calculated for each saccadic eye movement [43]. Smooth pursuit eye movements are very similar to saccades but presents a different outcome. This test is highly influenced by drugs that impair coordination, therefore providing an excellent assessment of the subject's motor coordination. In this test, the subject is instructed to follow a light source, that moves smoothly and horizontally on a computer screen for about 1 to 2 minutes, for measurement of smooth pursuit, indicated by the percentage of the time the subject´s eyes are in smooth pursuit of the target [43,44].

The effects of several classes of CNS-active drugs medicines on eye movements in healthy individuals are still being studied, although several studies have already contributed to understanding the full potential of these measurements to enhance CNS early-stage trials. The most consistent observations across various pharmacological classes, including

benzodiazepines (e.g., diazepam, lorazepam, and midazolam), first- and second-generation antipsychotics (e.g., haloperidol and olanzapine), anticholinergic agents, and anticonvulsant/mood-stabilizing drugs are a decrease in saccadic peak velocity and a reduction in smooth pursuit velocity (or increase in saccades during pursuit). These results mostly reflect the sedating effects of these drugs on CNS activity. Still, for other classes of drugs, such as antidepressants and stimulants (e.g., amphetamine and nicotine) there are no consistent results that indicate an adverse impact on smooth pursuit and saccadic eye movements in healthy individuals [49].

Adaptive tracking, even though not being an eye tracking measurement, primarily depends on visuomotor coordination and vigilance, having particularly sensitivity to drugs that cause ataxia. Briefly, in this test, the subject moves a dot on a computer screen with a joystick such that it stays within a constantly changing circle. The speed of the circle is adjusted in response to the subject's ability to keep the dot in the circle, ensuring that the test is adapted to the individual subject, and the percentage of time correctly tracked can be calculated to determine his performance [43,44]. According to several studies, various psychoactive drugs have shown to impair adaptive tracking in healthy subjects [43,50,51].

3.3 Body Sway

As a typical element of the battery of PD assessments in CNS, body sway measurement has been widely used to assess drug effects on motor coordination [51–54]. Body sway test measures the subject's body movements in a single plane (usually forward/backward movement), while standing with the eyes closed. To determine the body sway score and, consequently, assess postural stability, all the antero-posterior movements of the subjects are measured over 2 minutes and expressed as millimetre sway [43,44]. This assessment is employed routinely as a surrogate measure of sedation and is normally associated with drug effects in the cerebellum [55], the brain part responsible for the maintenance of body balance and postural stability, and, so being, it has already been used in studies with diverse CNS-drug classes, namely triptans [53], anticholinergic drugs [56] and anxiolytic drugs [57], such as benzodiazepines [58].

3.4 Subjective tests

Changes in subjective conditions are important aspects of drug effects. To rate subjective states, various instruments are used, and they are frequently among the most sensitive drug impact assessments. Subjective tests are easily repeatable and simple to interpret when compared to other assays, a feature that allows its application at different periods of a FIH trial. These assessments may be simultaneously applied at screening period (to familiarize subjects with the procedures and to reduce test-retest effects), at beginning of periods (to serve as baseline measurements), and at specific points during the periods, to compare their results across the study [59]. Subjective tests have the utmost importance in CNS-drugs testing, measuring relatively mild degrees of neurocognitive impairment with speed, efficiency and low cost, increasing trials productivity, efficiency and knowledge generated [60].

The use of subjective ratings in pharmaceutical development might be plagued by plenty of research issues, including instrument and control selection, timing and environment during assessment execution, and practice effects. Because Phase I studies are so operationally intensive, it is recommended that short subjective tests (15-20min) are employed, with computerized assessments being used frequently to facilitate administration. These tests (whether conducted live or through a computer) should be conducted in a quiet, distraction-free environment, with special attention paid to meals and the use of sedating or other cognitively damaging substances. Practice effects associated with a subject's repeated execution of a cognitive evaluation, as well as diurnal variation related to the schedule of the tests' execution may differ significantly from healthy volunteers to patients. As a result, maintaining consistency in the timing of cognitive test administration can assist reduce cognitive performance variability and, to minimize practice effects, it has been suggested that repeated testing trials be included at baseline. All these details can improve the interpretability of Phase I PD readouts [21].

According to the existing bibliography, even though they partly overlap, it is possible to subcategorize subjective tests in:

- Questionnaires and Visual Analogue Scales
- Memory testing
- Cognitive tests

Questionnaires and standardized interviews are particularly suited to quantify subjective conditions that have been present for a longer period (e.g., sleep questionnaires) and usually

aimed to characterize a subject at baseline or repeatedly after drug. Visual analogue scales, originally described by Norris [61], assess subjective feelings of alertness, mood, and calmness, normally following Bond and Lader scales [62]. This test, that relies on the ability of subjects to quantify a subjective state, consists of line segments and at the two ends of the line, two opposing words representing states of mind (e.g., happy – sad, tense – relaxed) are presented. Subjects put a mark on a point on the line that best represents their subjective state corresponding to the condition tested, resulting in a distance calculated from the mark on the line [43]. Visual analogue scales are commonly used to quantify subjective effects of benzodiazepines [42,63].

Memory is a function that can be affected by many CNS-active drugs, so that, testing their effects on this functional domain can be difficult. Memory is often reduced during sedation, and it may seem to be impaired by drugs that affect other systems. Therefore, in order to minimise the risk of these complications, memory tests should be performed in conjunction with other CNS PD assessments [43,44]. The most common test to measure memory impairments in CNS-active drugs studies is the Visual Verbal Learning Task. This assessment is a word learning and memory test, where subjects are presented with a series of words, one by one on the computer screen, and the words need to be pronounced and memorized [43]. Memory tests have been shown to be affected by benzodiazepines [64] and antidepressants [65].

Many CNS-acting medicines cause cognitive impairments, which are frequent in neuropsychiatric diseases. In clinical trials, demonstrating process-specific cognitive changes is becoming increasingly relevant since cognitive tests are designed to identify a drug's effects on cognition as well as to rule out potentially confounding non-cognitive factors, like sedation and low motivation. While cognitive batteries are the gold standard for antipsychotic registration trials (e.g., Phase III trials), using these measures as PD biomarkers in early-phase studies can provide valuable information about cognitive processes and safety outcomes [21]. Various cognitive tests can be incorporated in a PD assessment [44], depending on the study´s objective and design:

- Sternberg's Memory Test
- Digit Symbol Substitution Test
- Dual Task Test
- Stroop test
- Choice Reaction Time Test

The Sternberg's memory test is a working memory task designed to measure the process of information retrieval from recent memory; Digit symbol substitution test assesses visual perception but also measures other cognitive functions regarding attention, short-term memory, and psychomotor speed. This is one of the most commonly used tests in all of neuropsychology, since it offers a practical and effective method to monitor a range of cognitive operations, over time, in clinical practice [66]; Dual task test is designed to measure how much mental workload a subject can handle. In this test, the participant is required to execute two responses in close succession to two different streams of stimuli (e.g., visual and auditory stimuli), with the purpose of assessing interference between both. The ability to select appropriate responses in close succession depend on different brain structures, that may be impaired by the novel drug that is being studied [67]; Stroop test provides information regarding selective attention, perception, as well as the cognitive and neural mechanism underlying mental inhibition. This test measures the increase in response latency observed when an individual is required to identify the color of a color-word when these aspects of the stimulus are incongruent (e.g., the word RED presented in the color blue) and as proved to be sensitive to the effects of CNS-active drugs, especially those with arousing or de-arousing effects [68]; The choice reaction time test is a parametric version of the Stroop test. In this test, the patient is required to respond to one stimulus but to not respond to another, enabling the assessment of the patient's ability to maintain attention and vigilance for the target stimulus and the ability to inhibit responses to the nontarget stimuli [69].

Using subjective testing during a Phase I trial can help characterize CNS compounds by providing important PD information. When compared to PK data, these metrics have historically been used to define adverse cognitive or behavioural effects associated with novel CNS medicines and can provide important information about dose-limiting response effects. Furthermore, when carefully selected based on informed non-clinical data and applied during FIH studies, cognitive and behavioural assessments may provide significant findings suggestive of efficacy to inform dose selection.

Pharmaco-EEG will be the CNS assessment focused on the next section, due to the enormous potential for integration in Phase I clinical trials and enhancement of CNS drug development success.

4. PHARMACO-EEG

4.1 Pharmaco-EEG in FIH Studies

Pharmaco-EEG is a functional biomarker that has benefited from an increased interest in both nonclinical and clinical fields, even though it has progressed at different rates over the years, with several major events contributing to this.

The first human pharmaco-EEG recordings were depicted in 1931, with several findings in the EEG waves effects in studies with cocaine, morphine, chloroform and scopolamine. Although some discoveries started revealing the potential of this technique in the years following it was only until the 1950's that attempts were made to correlate the acute effects of psychotropic drugs on healthy volunteers' EEG with their clinical efficacy in psychiatric patients. An EEG-based classification system classified mianserin as an antidepressant, despite it being initially developed by Organon as an anti-inflammatory drug. Its antidepressant therapeutic potential was subsequently confirmed in clinical studies that supported the predictive value of the pharmaco-EEG [70]. This episode is considered a crucial moment in the pharmaco-EEG history, since the pharmaceutical industries that were active in the development of psychotropic drugs massively implemented pharmaco-EEG since.

These clinical developments also sparked interest in animal pharmaco-EEG, with the idea of developing a similar EEG-based classification to predict the psychotropic potential of novel compounds in nonclinical studies, thus becoming one of the first translational biomarkers in psychotropic drug development.

Later, and despite the emergence in the industry of several groups solely dedicated to the study of this biomarker, the science of pharmaco-EEG was partly abandoned, becoming a small focus among neurologists. The successive failures of clinical EEG to accurately predict the therapeutic value of a number of compounds (e.g., maroxepine [71]), as well as the parallel emergence of structurally and functionally revealing imaging techniques (i.e., fMRI, PET, among others) and the increasing application of molecular biology, contributed to this shift in psychiatric neuroscience's focus [70,72]. Currently, the landscape significantly evolved with the development of novel technologies that improved the processing, quantification and sophisticated analysis of clinical EEG.

Pharmaco-EEG is a PD biomarker used in clinical and experimental pharmacology, neurotoxicology and therapeutic research to assess the effects of drugs on the CNS using neurophysiological and electrophysiological approaches. Pharmaco-EEG strictly refers to human QEEG in the context of drug testing and distinguishes itself by being a non-invasive method that can be used routinely throughout a FIH trial, thus supporting the development and approval of NMEs. Nowadays, licensing for psychoactive agents is approved without evidence of brain effects, resulting in a rush of ineffective medicines on the market, a recurring struggle that could be solved by the pharmaco-EEG capability to confirm that brain alterations occurred and anticipate a novel entity's potency, dose, and clinical efficacy [72,73].

The numerous features of QEEG can make it a reference clinical biomarker in CNS-drug development, with several advantages when compared to state-of-the-art imaging techniques (Table 4). Pharmaco-EEG presents a direct measure of pharmacological action, target engagement and neuronal function, with high temporal resolution, properties that no other technique depicted in Table 4 can fulfill. Furthermore, QEEG is one of the cheapest of the various methods that can be used as a window into the brain's activity, something that, combined with its high portability and availability, makes it possible to feasibly be integrated into FIH studies. Finally, it's worth noting that the anatomical and functional architecture of the neuronal circuits in the CNS that generate EEG signals in the species used in nonclinical drug discovery and humans are quite similar. Consequently, QEEG has the distinct advantage that the same methodology can be applied in both stages of development (in contrast, rodent imaging generally requires the animal to be anaesthetized, which can difficult interpretation and translatability even when the imaging technique is similar), laying a solid foundation for biomarker translatability.

Despite their differential properties, combining pharmaco-EEG with additional PD biomarkers in a well suited study design can, in many cases, provide added benefit as illustrated by Boeijinga [74], who discusses a scope on implementation of multimodal approaches and personalized marker strategies in neuropsychiatry.

For all the aforementioned advantages, pharmaco-EEG data can be exploited not only to classify psychotropic substances and assess drug-drug interactions but also to monitor side effects and toxicity [4,75].

Table 4. Comparison of available functional CNS biomarker techniques. Adapted from *Wilson et al* [4].

	QEEG	fMRI	PET	PSG
Direct measure of molecular target engagement?	No	No	Yes	No
Measure of pharmacological action and engagement of target neural circuit?	Yes	Yes	No	Yes
Direct measure of neuronal function?	Yes (electric field)	No	n.a.	No
Temporal resolution	High	Medium	Low	Medium
Spatial resolution	Low	High	High	n.a.
Can it be integrated with SD/MD studies?	Possible in many cases	No	No	No
Portability/equipment can be standardised between sites	Yes	No	No	No
Availability	High	Medium	Medium	Medium
Cost	Very low	Medium	High to very high	Low
n.a. = not assessed;				

Historical unsuccess of pharmaco-EEG in the past years seem to justify the relative low adherence to this PD assessment. However, inconsistent results may find its foundation in the lack of standardization of the methods or guidances for recording and analysis of QEEG across different study protocols. In fact, the large inter-individual variability observed in EEG records has also been attributable to the lack of standardization, which can still be offset by increasing the sample size [75]. Overall, despite evidence of QEEG potential in the literature, there is surprisingly little material accessible to assist with the straightforward PD biomarker application since what is available is difficult to use robustly to enhance early decision-making in drug development. The most important step toward confirming the potential of pharmaco-EEG as a "go-to" method would be a significant increase in standardization across industry and academia, which may be accomplished with a concerted effort from both stakeholders [76]. Such effort has already been started with the publishing of guidelines for the recording and evaluation [75], and advanced analysis [77] of pharmaco-EEG data in humans, by the International Pharmaco-EEG Society (IPEG).

4.2 Data Acquisition and Processing

IPEG guidelines published in 2012 were developed by a global panel of EEG specialists and provide clear and concise recommendations on pharmaco-EEG recording and evaluation, with the purpose of improving the standardization of pharmaco-EEG studies in human subjects and facilitating data comparability, allowing for data pooling and meta-analyses [75]. Additionally, in 2016, IPEG published a complementary paper covering the advanced analysis of data retrieved from pharmaco-EEG in humans, highlighting the usual difficulties and the required precautions to consider when processing such recordings [77].

A brief analysis of the most crucial points depicted in these papers for a robust pharmaco-EEG procedure in FIH trials will be presented in the next topics.

4.2.1 Equipment

A major problem impairing pharmaco-EEG data has been the standardization of EEG recording equipment and data acquisition requirements, specifically electrode positioning. In order to properly obtain robust data in pharmaco-EEG studies, the EEG data acquisition process should comply with specific practical conditions (Table 5).

In what concerns EEG digital recording, the sampling frequency must be at least 500 Hz, and the digital resolution of the A/D converter must be at least 16 bits. To reduce noise and improve data quality, it should be applied a high-pass filter set below 0.5 Hz (recommended 0.01 Hz) and a low-pass filter set at 70 Hz, being that the usage of a notch filter should be avoided (otherwise 50 or 60 Hz) as it can potentially disguise an electrode problem. Also, pre-amplifier impedance should be set over 100 MΩ and electrode impedance should be balanced across all electrode sites.

Electrode configuration is a crucial factor in the success of QEEG studies, such that the appropriate number and placement of electrodes has been updated considering recent investigations. So being, while it has been previously demonstrated that the effects of specific compounds may be traced using a limited number of electrodes and derivations, a configuration of at least 21 electrodes (Ag/AgCl or equivalent) placed according to the 10-20 system (Figure 4A) is considered the minimal electrode placement. If additional EEG electrodes are needed (e.g. for topography or tomography assessments), then the extended 10–20 system (10% system [78]) should be employed (Figure 4B). It is also recommended for EEG recording to be recorded against a common reference electrode (Cz, A1 and A2).

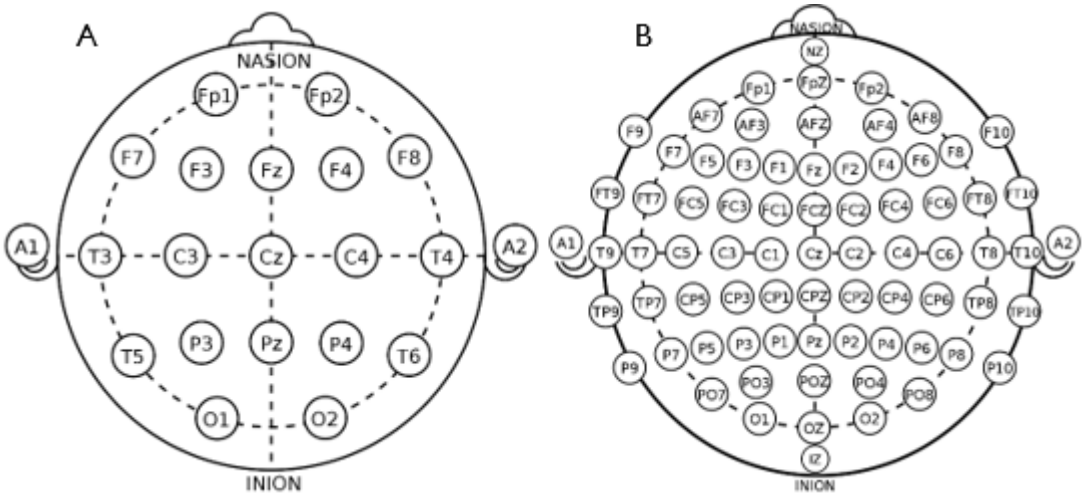

Figure 4. (A) International 10-20 system of EEG electrode placement; (B) Extended International 10-20 system (10% system) of EEG electrode placement.

Additionally, the recording of electrooculography (EOG), electrocardiography (ECG) and EMG (electromyography) is recommended, for artefacts identification, assessment of the activity of the autonomous nervous system and as additional biomarker for vigilance stages or artefacts, respectively.

Table 5. Minimum requirements for pharmaco-EEG recording equipment. Adapted from *Jobert et al* [75].

EEG recording equipment	Sampling rate	≥500 Hz
	A/D conversion	≥16 bits
	High-pass filtering	≤0.5 Hz (0.01 Hz recommended)
	Low-pass filtering	70 Hz (roll-off of at least 12 dB/octave)
	Notch filter	Usage avoided; otherwise, 50 or 60 Hz (dependent on the power supply frequency)
	Pre-amplifier impedance	≥100 MΩat 50 Hz
	Common mode rejection	≥90 dB
Electrodes	Electrode impedance	Balanced impedance across all electrode sites
	Number and placement	At least 21 electrodes placed according to the 10–20 system or the extended 10–20 system (10% system) in case >21 electrodes are used
	Type	Ag/AgCl or equivalent in terms of electrode drift and DC resistance
	Montage	Monopolar against a common reference
	Reference	Should be modifiable post hoc, (Cz, A1, A2, average mastoids)
	Ground	AFz
	EOG	Vertical and horizontal for artefact identification
	ECG	Recommended
	EMG	Recommended
Storage conditions	Local storage	The proprietary format of each EEG recording equipment
	Export/import format	European data format 'plus'
	Signals	Raw data without transformation Automatic artefact rejection is optional

4.2.2 Procedure

Many factors such as recording environment, conditions and timepoints influence the function and activity of the CNS, as well as neurophysiologic readouts of brain activity. As a result, standardizing experimental design and conditions is critical to keep these variables under control to the maximum extent possible (Table 6).

Every detail that could interfere with EEG measurement is important, thus the recording environment should be meticulously controlled. The recording should occur in a separate, sound-attenuated room with constant dimmed light (approximately 40 lx) or light level defined by the computer monitor used for task presentation, if applicable, and regulated room temperature (20–23 °C). Intermittent disturbing events must be avoided and external interactions should be limited.

Usually, clinical EEG procedures are carried out with the subject in a semi-reclined position (to reduce neck muscle tension) or in an upright position facing a computer monitor (for studies that include task presentation this position is the most amenable for its execution). In studies involving both resting and task-related recordings, it is recommended that resting recordings be performed in a situation that exactly replicates the recording conditions to be used when engaged in tasks (Figure 5).

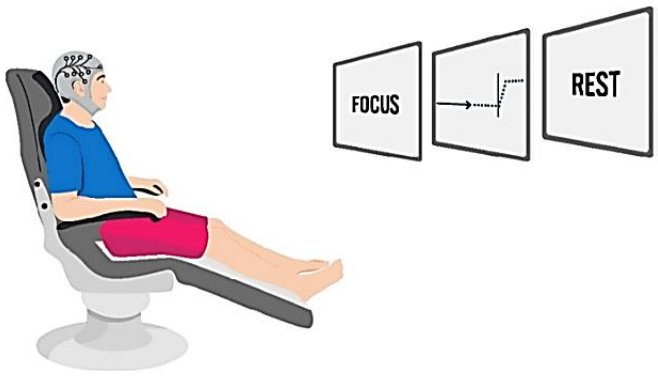

Figure 5. Illustration of the set up for clinical EEG recordings. Retrieved from *Alder et al* [79].

For the purpose of obtaining reliable and comparable data, recording conditions have also been purposed to be uniformed across clinical sites. Then, pharmaco-EEG should be recorded under one or more of the following conditions:

- 5 min vigilance controlled with eyes open. During the session the vigilance status should be controlled trough the execution of a continuous task (normally displayed at a computer monitor).
- 5 to 15 min resting condition with eyes closed. The purpose of this recording is to examine the variations in vigilance and wakefulness over time, and to quantify drug induced changes in such parameters (such as sleepiness).
- 10 min resting condition with alternate eyes open and eyes closed. Recording status must be maintained for periods of 1 min duration each, with several alternations.

In FIH clinical trials, a current primary objective is to understand and characterize the drug PK/PD relationship, and so this aspect must be taken into consideration for the design of studies with repeated measurements.

All the abovementioned requirements should be fulfilled not only at baseline recording but also at multiple timepoints following drug administration, usually one timepoint around T_{max} and at least three timepoints covering the decline in PK curve (e.g., 1, 2, 4 and 8 h). At crossover studies (recommended for this type of studies in healthy subjects), tests should be recorded at the same time of the day, preferably in the morning, and under the same conditions.

Finally, clinical EEG recording should always be conducted prior to additional testing (see Section 3), since vigilance may be reduced following strenuous tasks.

Table 6. Minimum requirements for pharmaco-EEG recording procedure. Adapted from *Jobert et al* [75].

Experimental design and conditions	Adaptation	It is recommended to make the subject familiar with the recording conditions and procedures during a separate recording session
	Recording environment	Sound-attenuated room Constant dimmed light (approximately 40 lx) or light level defined by the computer monitor used for task presentation Constant room temperature: 20–23°C Subject in a semi-reclined comfortable position or in an upright position facing a computer monitor (for studies that include a task)
	Design	Double-blind placebo-controlled cross-over design is recommended for studies in healthy subjects For multiple dose and patient studies, the design should be adapted according to the objectives
	Recording timepoints	Baseline and a number of post-drug recording time points to be driven by drug PK; at least one timepoint around Tmax plus at least 3 timepoints covering the decline in the PK curve (usually multiples – e.g., 1, 2, 4 and 8 h) If $T_{half} \geq 12$ h, then a 24-hour overnight time point should be considered
	Time of day	Preferably in the morning Cross-over repeat tests should be done at the same clock time and under the same conditions
	Recording conditions	5 min vigilance controlled; eyes open 5 to 15 min resting condition; eyes closed 10 min resting with alternate eyes open and eyes closed

4.2.3 Data Processing

QEEG signal reveals the spontaneous synchronized postsynaptic neuronal activity of the cortex with high temporal resolution, thus providing a direct measure of brain function. Figure 6 represents the spectral analysis of EEG. For each channel selected from the EEG recording, time-frequency analysis (Fast Fourier Transform) can be applied to extract the spectral distribution of the signal into frequency bands. Spectral analysis via Fast Fourier Transform is presently the most common method of choice for the parameterisation of pharmaco-EEG studies, a process that implies a tremendous data reduction. Briefly, a first data reduction comprises the transformation from time domain into the frequency domain,

followed by a second step of data reduction, consisting in the extraction of spectral parameters. In this second data reduction, the frequency range is subdivided into the standard frequency bands - delta, theta, alpha and beta (Table 7) - and the spectral power (area under the curve) is computed for each of them (expressed in μV/Hz).

There has also been an increasing interest in high-frequency gamma EEG activity in addition to the aforementioned frequency bands, paving the way for the processing and assessment of gamma EEG activity in QEEG studies. The brain activity in the gamma-frequency band (~30-40 Hz), even though it has become the focus of interest for several research groups [80], overlaps entirely with the spectral bandwidth of muscle activity (~20–300 Hz), causing contamination of the EEG with high-frequency artifacts [81]. Therefore, the high-frequency gamma band is not usually considered in pharmaco-EEG measures, although in future studies its potential may be fully exploited, without the interference of muscle artifacts.

Table 7. Frequency ranges for spectral analysis in pharmaco-EEG studies. Adapted from *Jobert et* al [75].

Frequency Band	Frequency Range (Hz)
Delta	1.5 - <6.0
Theta	6.0 - <8.5
Alpha-1	8.5 - <10.5
Alpha-2	10.5 - <12.5
Beta-1	12.5 - <18.5
Beta-2	18.5 - <21.0
Beta-3	21.0 - <30.0
Total power	1.5 - <30.0
Gamma	30.0 - <40.0

These results can also be presented in topographical maps. Each channel (corresponding to a different region in the scalp) shows a specific spectral distribution, therefore, each topographic region can be characterized by its most prominent bandwidth. Brain mapping is widely used to display activity simultaneously recorded from several electrodes, allowing to easily visualise the spatial relationships of EEG data among the scalp recording sites and, thus, constituting a well-suited method to assess the topographical changes induced by CNS-active compounds.

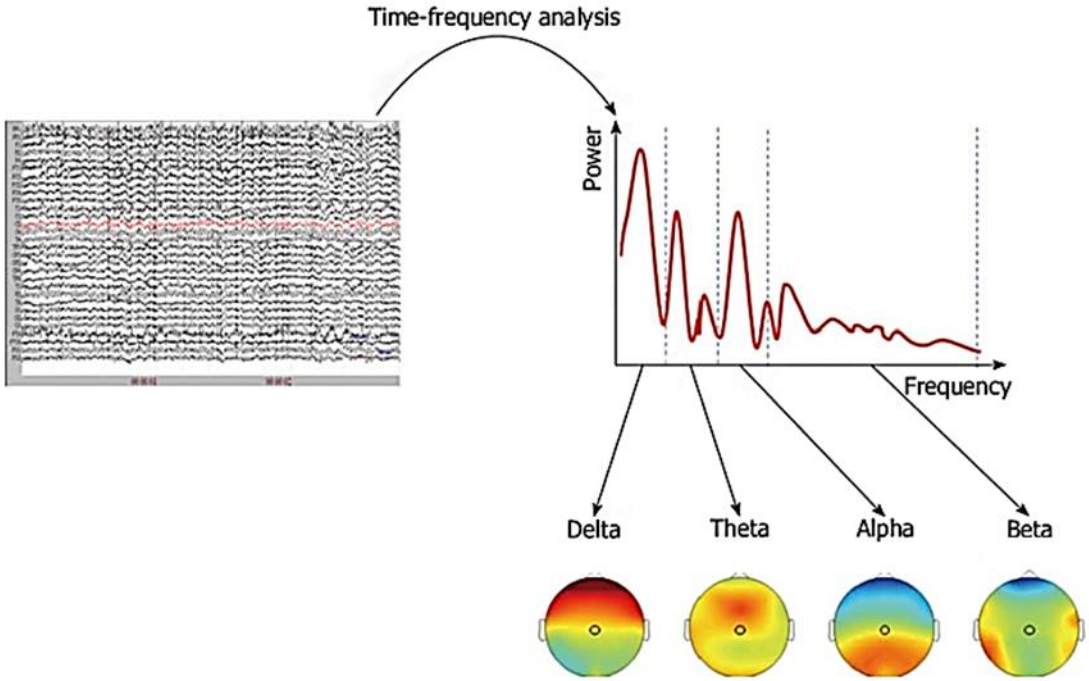

Figure 6. Spectral analysis of EEG. Adapted from *Lelic et al* [82].

Lastly, when processing QEEG data, some strategies can be applied to mitigate common pitfalls and optimally capture drug effects. These concerns usually relate to the stability, dynamic and reliability of EEG measurements. A comparison of the EEG activity at the beginning and at the end of the recording, in addition to the evaluation of the spectral parameters for the entire session, is very useful to verify the stability of the recording condition and to detect possible drug effects. As well, when assessing test-retest reliability, two different categories of factors that may have an impact on EEG reliability should be considered, such as diurnal factors (influence the EEG activity) and technical factors or variation in experimental conditions (influence the quality of EEG recordings).

4.3 Pharmaco-EEG Profiling

Representative drugs of the main psychopharmacological classes, such as antipsychotics (also known as neuroleptics), antidepressants, anxiolytic drugs (tranquilizers and hypnotics), nootropics/cognition-enhancing drugs and psychostimulants, induce (compared with placebo) significant changes in the quantitatively analysed EEG of healthy subjects, which result in different pharmaco-EEG profiles and pharmaco-EEG maps (based on multilead analysis). The most typical profiles derived from numerous placebo-controlled trials with different psychotropic substances are described and their topographical results presented in Figure 7.

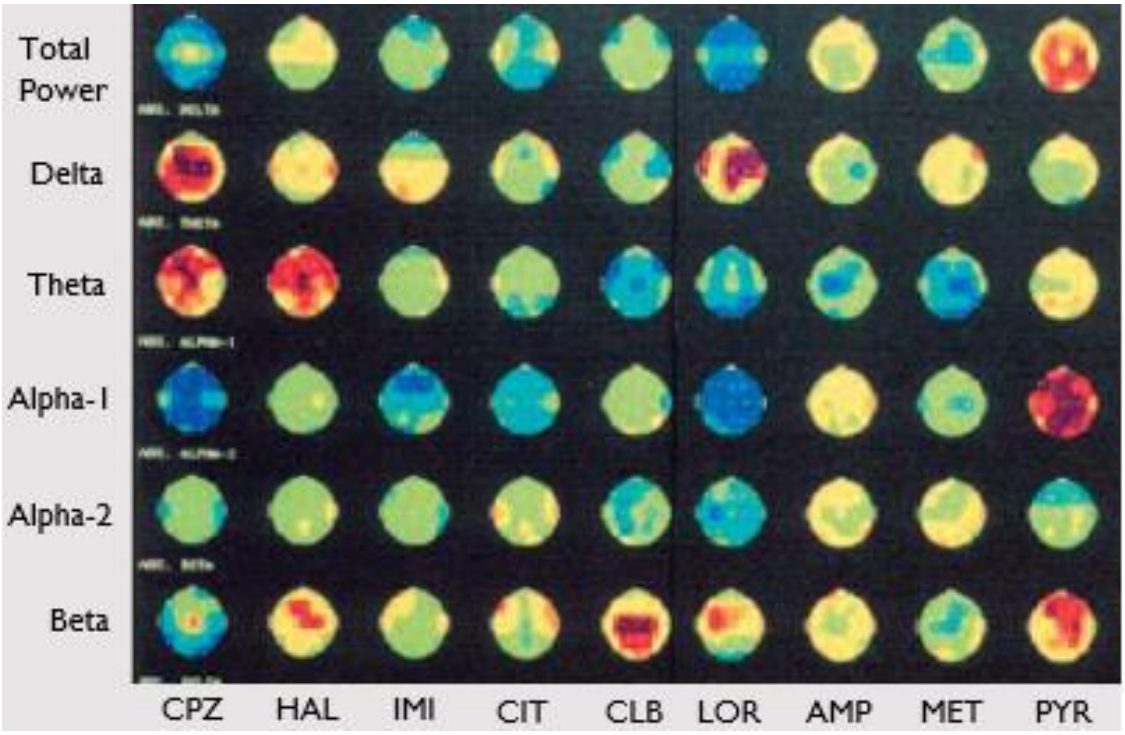

Figure 7. EEG mapping of representative drugs from the major psychopharmacological classes (time of PD peak, around 2h post-dose): chlorpromazine (CPZ); haloperidol (HAL); imipramine (IMI); citalopram (CIT); clobazam (CLB); lorazepam (LOR); amphetamine (AMP); metamphetamine (MET); pyritinol (PYR). Orange, red, and purple colors represent significant increases; Dark green, light blue, and dark blue indicate significant decreases. Adapted from *Saletu et al* [83].

The administration of sedative (e.g., chlorpromazine) and non-sedative (e.g., haloperidol) antipsychotics induces typical EEG changes, such as an increase of alpha power, decrease of beta-1 and increase of beta-2 bands, associated with an increase in delta/theta power (predominantly theta). Total power was found to be either attenuated (sedative neuroleptics) or not changed (non-sedative neuroleptics) [83–85].

Antidepressants, sedative (e.g., imipramine-amitriptyline type) and non-sedative (citalopram), present relatively similar profiles, attenuating total power and specifically alpha power, as well as increasing beta activity [83–85]. The effect of antidepressants on delta/theta activities is still dubious, with reports of slowing [84] and, contrarily, dose-dependent increases[85].

Anxiolytic drugs are essentially represented by benzodiazepines (clobazam, lorazepam, diazepam, etc) and their administration typically induces an increase in beta frequencies associated with a decrease in alpha power [83–85]. For benzodiazepines with a more potent hypnotic effect, an increase in delta/theta activities is expected to be observed [84,85].

Psychostimulants (e.g., amphetamine and methylphenidate) profiles usually present a tendency of this class of drugs towards a decrease of both slow and fast activities as well as an attenuation of total power.

Nootropics and cognition enhancers (such as pyritinol) predominantly induces a decrease in delta and theta power along with an increase in alpha and beta activities and total power [83,84].

5. DISCUSSION AND CONCLUSION

In an era where pharmaceutical development has been suffering from low clinical success rates in the early clinical development of new CNS-active drugs, PD endpoints have significant potential to assist with this problem. Using PD biomarkers in early-phase clinical trials with healthy subjects allows a low PD profile variability setting, enabling robust dose-effect evaluations and PK/PD assessments. Ultimately, PD data can contribute to guide the decision-making process of Phase I studies, providing insights on the potential clinical success of a new drug and guiding "go-no go" decisions.

The integration of functional biomarkers in FIH studies helps to determine if the NME has the expected specific properties, assess safety and tolerability as well as estimate optimal dose and stratify patient population for further clinical trials. To that end, CNS test batteries have already been employed in early-phase clinical trials with adapted designs. These test batteries normally comprise both neurophysiological and neuropsychological tests, thus enabling the evaluation of various functional domains.

Pharmaco-EEG is a neurophysiological biomarker that is not fully established in the CNS-active drugs development but has promising advantages compared to other functional biomarkers. This high temporal resolution method is a direct measure of pharmacological action and target engagement, that can feasibly be integrated into FIH studies with psychoactive drugs. For a consistent use in early-phase drug development, appropriate and well-structured protocols must be strictly followed and pharmaco-EEG must become a standardized assessment. Pharmaco-EEG equipment, procedure and data processing are critical aspects when assessing early-stage CNS studies, in order to obtain robust and reliable data.

The most typical approach in FIH studies is to monitor EEG activity before and after drug administration at various time intervals, either under vigilance controlled or resting conditions. The recording under the resting condition examines spontaneous EEG variations without external intervention, whereas the vigilance-controlled recording condition seeks to keep the individual at a considerably high level of vigilance. Also, studies strongly suggest that a 5-min EEG recording session is optimal to obtain reliable and pharmacosensitive data [77]. Thus, the recommended standard procedure is to measure EEG activity under both vigilance-controlled and resting conditions with a recording time of 5 minutes, so that the effects of drugs in both states are considered [77].

Several recording requirements, such as number and placement of electrodes, and recording environment and timepoints should be strictly followed. Also, a robust spectral analysis and EEG mapping are the usual "go-to" data processing procedures, and these should be properly applied to the EEG signal. Deviations in these conditions may represent a source of variability and data impairment and could be major pitfalls in the success of a clinical trial.

These requirements can be considered pivotal for a reliable QEEG acquisition and processing, with the potential to enable ground-breaking PD results when evaluating novel psychotropic drugs. In 2016*, Jobert et al* issued a standard protocol, based on the previously published IPEG guidelines, for a pharmaco-EEG study (a randomised, double blind, crossover trial aimed at comparing the effect of diazepam and placebo in 16 healthy volunteers [77]). This study presented trustworthy PD findings and could serve as basis for numerous trials protocols employing this promising PD biomarker.

Pharmaco-EEG is an optimal method to streamline drug development and characterize the pharmacology of psychotropic drugs. Demonstrating a "key-lock principle" is one of the main features to understand a novel drugs' PD effects. The pharmaco-EEG profiles of healthy subjects after administration of psychoactive drugs can be correlated to the QEEG measures from mental disorder patients. The changes induced by drugs in healthy subjects (compared to placebo) are sometimes opposite to the differences observed in patients (compared to controls). This fact supports the notion of the "key-lock principle" in the psychopharmacological treatment of mental disorders [83].

PK/PD analysis based on pharmaco-EEG can also be extremely relevant in the assessment of the PD effects of new psychotropic medicines. The relationship between the drugs' serum levels (PK) and total QEEG changes (PD) normally reveals a loop-shaped curve (hysteresis loop). The appearance of the hysteresis loop depends upon the penetrability of the BBB by psychotropic drugs. Thence, the maximum PD effect is expected to be observed on the descending slope of the PK curve (rather than on the rising). This measure constitutes an ideal assessment of the drug capability to penetrate the BBB and consequently modulate the expected target [83,84].

In future FIH clinical trials, assessing the pharmacology of a novel medicine through integration of pharmaco-EEG along with additional CNS test batteries must become a recurrent tool. To achieve such a level of confidence on pharmaco-EEG as a consistent method, collaboration between pharmaceutical industry and academia is required to address standardisation challenges, increase translatability expertise, and improve signal processing and data analysis methodologies.

BIBLIOGRAPHY

1. Kesselheim AS, Hwang TJ, Franklin JM. Two decades of new drug development for central nervous system disorders. *Nat Rev Drug Discov*. 2015;14(12):815-816. doi:10.1038/nrd4793

2. Srinivas N, Maffuid K, Kashuba ADM. Clinical Pharmacokinetics and Pharmacodynamics of Drugs in the Central Nervous System. *Clin Pharmacokinet*. 2018;57(9):1059-1074. doi:10.1007/s40262-018-0632-y

3. de Lange ECM, van den Brink W, Yamamoto Y, de Witte WEA, Wong YC. Novel CNS drug discovery and development approach: model-based integration to predict neuro-pharmacokinetics and pharmacodynamics. *Expert Opin Drug Discov*. 2017;12(12):1207-1218. doi:10.1080/17460441.2017.1380623

4. Wilson FJ, Danjou P. Early Decision-Making in Drug Development: The Potential Role of Pharmaco-EEG and Pharmaco-Sleep. *Neuropsychobiology*. 2016;72(3-4):188-194. doi:10.1159/000382022

5. Daimon T, Hirakawa A, Matsui S. *Dose-Finding Designs for Early-Phase Cancer Clinical Trials*. 1st ed. Tokyo: Springer, Tokyo; 2019. doi:10.1007/978-4-431-55585-8

6. Karakunnel JJ, Bui N, Palaniappan L, et al. Reviewing the role of healthy volunteer studies in drug development. *J Transl Med*. 2018;16(1):1-15. doi:10.1186/s12967-018-1710-5

7. Pasqualetti G, Gori G, Blandizzi C, Del Tacca M. Healthy volunteers and early phases of clinical experimentation. *Eur J Clin Pharmacol*. 2010;66(7):647-653. doi:10.1007/s00228-010-0827-0

8. Committee for Medicinal Products for Human Use. Guideline on strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products. *London, Eur Med Agency*. 2017:1-12. www.ema.europa.eu/contact. Accessed March 10, 2021.

9. The Association of the British Pharmaceutical Industry. Guidelines for Phase 1 Clinical Trials 2018 edition. *Abpi*. 2018:1-49.

10. European Medicines Agency. ICH Topic E8 General considerations for clinical trials. 1997;4(4):25-32.

11. FDA. FDA facts: biomarkers and surrogate endpoints. *US Food Drug Adm*. 2017:1-3. https://www.fda.gov/about-fda/innovation-fda/fda-facts-biomarkers-and-surrogate-endpoints%0Ahttps://www.fda.gov/aboutfda/innovation/ucm512503.htm.

12. NIH Clinical Center: Healthy Volunteers. https://clinicalcenter.nih.gov/recruit/volunteers.html. Accessed April 3, 2021.

13. Emanuel EJ, Bedarida G, Macci K, Gabler NB, Rid A, Wendler D. Quantifying the risks of non-oncology phase I research in healthy volunteers: Meta-analysis of phase I studies. *BMJ*. 2015;350. doi:10.1136/bmj.h3271

14. Young TC, Srinivasan S, Vetter ML, et al. A Systematic Review and Pooled Analysis of Select Safety Parameters Among Normal Healthy Volunteers Taking Placebo in Phase 1 Clinical Trials. *J Clin Pharmacol*. 2017;57(9):1079-1087. doi:10.1002/jcph.913

15. Johnson RA, Rid A, Emanuel E, Wendler D. Risks of phase i research with healthy participants: A systematic review. *Clin Trials*. 2016;13(2):149-160. doi:10.1177/1740774515602868

16. Pinquier JL. 2021. CNS Pharmacodynamics in Phase I: Challenges & Opportunities [Webinar]. https://applauncher.gotowebinar.com/#webinarOver/6887352244421601295/en_US.

17. Kuhlmann J. The applications of biomarkers in early clinical drug development to improve decision-making processes. *Ernst Schering Res Found Workshop*. 2007;(59):29-45. doi:10.1007/978-3-540-49529-1_3

18. Joshi YB, Tarasenko MA, Light GA. *Applications of Neurophysiological Biomarkers in CNS Drug Development: Focus on Psychoses*. Vol 29. 1st ed. Elsevier B.V.; 2019. doi:10.1016/B978-0-12-803161-2.00012-6

19. Macaluso M, Krams M, Savitz J, Drevets WC, Preskorn SH. *New Approaches in Translational Medicine for Phase I Clinical Trials of CNS Drugs*. Vol 29. 1st ed. Elsevier B.V.; 2019. doi:10.1016/B978-0-12-803161-2.00006-0

20. Degroot A. *Biomarker-Guided Drug Development for Better Defined Early Patient Studies and Clinical Trial Efficiency*. Vol 29. 1st ed. Elsevier B.V.; 2019. doi:10.1016/B978-0-12-803161-2.00002-3

21. English BA, Thomas K, Johnstone J, Bazih A, Gertsik L, Ereshefsky L. Use of translational pharmacodynamic biomarkers in early-phase clinical studies for schizophrenia. *Biomark Med*. 2014;8(1):29-49. doi:10.2217/bmm.13.135

22. van Gerven J. *Functional Measurements of Central Nervous System Drug Effects in Early Human Drug Development*. Vol 29. 1st ed. Elsevier B.V.; 2019. doi:10.1016/B978-0-12-803161-2.00004-7

23. Aronson JK. Concentration-effect and dose-response relations in clinical pharmacology. *Br J Clin Pharmacol*. 2007;63(3):255-257. doi:10.1111/j.1365-2125.2007.02871.x

24. Markou A, Chiamulera C, Geyer MA, Tricklebank M, Steckler T. Removing obstacles in neuroscience drug discovery: The future path for animal models. *Neuropsychopharmacology*. 2009;34(1):74-89. doi:10.1038/npp.2008.173

25. Deo AK, Theil FP, Nicolas JM. Confounding parameters in preclinical assessment of blood-brain barrier permeation: An overview with emphasis on species differences and effect of disease states. *Mol Pharm*. 2013;10(5):1581-1595. doi:10.1021/mp300570z

26. Venitz J. Using exposure-response and biomarkers to streamline early drug development. Ernst Schering Res Found Workshop. 2007;(59):47-63. doi: 10.1007/978-3-540-49529-1_4. PMID: 17117714.

27. Gundert-Remy U. 3. Dose-response information to support drug registration. *Eur J Clin Res*. 1994;6(November 1994):325-328.

28. Morgan P, Van Der Graaf PH, Arrowsmith J, et al. Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival. *Drug Discov Today*. 2012;17(9-10):419-424. doi:10.1016/j.drudis.2011.12.020

29. Danhof M, Alvan G, Dahl SG, Kuhlmann J, Paintaud G. Mechanism-based pharmacokinetic-pharmacodynamic modeling - A new classification of biomarkers. *Pharm Res*. 2005;22(9):1432-1437. doi:10.1007/s11095-005-5882-3

30. Frank R, Hargreaves R. Clinical biomarkers in drug discovery and development. *Nat Rev Drug Discov*. 2003;2(7):566-580. doi:10.1038/nrd1130

31. Walker DK. The use of pharmacokinetic and pharmacodynamic data in the assessment of drug safety in early drug development. *Br J Clin Pharmacol*. 2004;58(6):601-608. doi:10.1111/j.1365-2125.2004.02194.x

32. Atkinson AJ, Colburn WA, DeGruttola VG, et al. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clin Pharmacol Ther*. 2001;69(3):89-95. doi:10.1067/mcp.2001.113989

33. Cohen AF, Burggraaf J, Van Gerven JMA, Moerland M, Groeneveld GJ. The use of biomarkers in human pharmacology (Phase I) studies. *Annu Rev Pharmacol Toxicol*. 2015;55(September):55-74. doi:10.1146/annurev-pharmtox-011613-135918

34. Paul SM, Mytelka DS, Dunwiddie CT, et al. How to improve RD productivity: The pharmaceutical industry's grand challenge. *Nat Rev Drug Discov*. 2010;9(3):203-214. doi:10.1038/nrd3078

35. Carter CS, Barch DM, Buchanan RW, et al. Identifying Cognitive Mechanisms Targeted for Treatment Development in Schizophrenia: An Overview of the First Meeting of the Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia Initiative. *Biol Psychiatry*. 2008;64(1):4-10. doi:10.1016/j.biopsych.2008.03.020

36. U.S. Department of Health and Human Services FDA. Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products. *FDA Guid Ind*. 1998;(May):23. https://www.fda.gov/ohrms/dockets/ac/06/briefing/2006-4227B1-02-02-FDA-Appendix1.pdf.

37. Cohen A. Pharmacokinetic and pharmacodynamic data to be derived from early-phase drug development: Designing informative human pharmacology studies. *Clin Pharmacokinet*. 2008;47(6):373-381. doi:10.2165/00003088-200847060-00002

38. De Visser SJ, Van Der Post JP, De Waal PP, Cornet F, Cohen AF, Van Gerven JMA. Biomarkers for the effects of benzodiazepines in healthy volunteers. *Br J Clin Pharmacol*. 2003;55(1):39-50. doi:10.1046/j.1365-2125.2002.t01-10-01714.x

39. Groeneveld GJ, Hay JL, Van Gerven JM. Measuring blood–brain barrier penetration using the NeuroCart, a CNS test battery. *Drug Discov Today Technol*. 2016;20:27-34. doi:10.1016/j.ddtec.2016.07.004

40. Zuiker RGJA, Chen X, Østerberg O, et al. NS11821, a partial subtype-selective GABAA agonist, elicits selective effects on the central nervous system in randomized controlled trial with healthy subjects. *J Psychopharmacol*. 2016;30(3):253-262. doi:10.1177/0269881115620435

41. Te Beek ET, Moerland M, De Boer P, et al. Pharmacokinetics and central nervous system effects of the novel dopamine D2 receptor antagonist JNJ-37822681. *J Psychopharmacol*. 2012;26(8):1119-1127. doi:10.1177/0269881111415733

42. De Haas SL, Franson KL, Schmitt JAJ, et al. The pharmacokinetic and pharmacodynamic effects of SL65.1498, a GABA-A 2,3 selective agonist, in comparison with lorazepam in healthy volunteers. *J Psychopharmacol*. 2009;23(6):625-632. doi:10.1177/0269881108092595

43. Hart EP, Alvarez-Jimenez R, Davidse E, et al. A Computerized Test Battery to Study Pharmacodynamic Effects on the Central Nervous System of Cholinergic Drugs in Early Phase Drug Development. *J Vis Exp*. 2019;(144):1-10. doi:10.3791/56569

44. Center for Human Drug Research. NeuroCart® Measuring a wide range of CNS effects in a pharmacological context. www.CHDR.nl. Accessed October 4, 2021.

45. Nij Bijvank JA, Petzold A, Balk LJ, et al. A standardized protocol for quantification of saccadic eye movements: DEMoNS. *PLoS One*. 2018;13(7):1-19. doi:10.1371/journal.pone.0200695

46. Wilson SJ, Glue P, Ball D, Nutt DJ. Saccadic eye movement parameters in normal subjects. *Electroencephalogr Clin Neurophysiol*. 1993;86(1):69-74. doi:10.1016/0013-4694(93)90068-7

47. Leigh RJ, Kennard C. Using saccades as a research tool in the clinical neurosciences. *Brain*. 2004;127(3):460-477. doi:10.1093/brain/awh035

48. Gijsman HJ, Van Gerven JMA, Verkes RJ, et al. Saccadic peak velocity and EEG as end-point for a serotonergic challenge test. *Hum Psychopharmacol*. 2002;17(2):83-89. doi:10.1002/hup.374

49. Reilly JL, Lencer R, Bishop JR, Keedy S, Sweeney JA. Pharmacological treatment effects on eye movement control. *Brain Cogn*. 2008;68(3):415-435. doi:10.1016/j.bandc.2008.08.026

50. De Haas SL, Schoemaker RC, Van Gerven JMA, Hoever P, Cohen AF, Dingemanse J. Pharmacokinetics, pharmacodynamics and the pharmacokinetic/ pharmacodynamic relationship of zolpidem in healthy subjects. *J Psychopharmacol*. 2010;24(11):1619-1629. doi:10.1177/0269881109106898

51. Muehlan C, Boehler M, Brooks S, Zuiker R, van Gerven J, Dingemanse J. Clinical pharmacology of the dual orexin receptor antagonist ACT-541468 in elderly subjects: Exploration of pharmacokinetics, pharmacodynamics and tolerability following single-dose morning and repeated-dose evening administration. *J Psychopharmacol*. 2020;34(3):326-335. doi:10.1177/0269881119882854

52. Hoever P, De Haas S, Winkler J, et al. Orexin receptor antagonism, a new sleep-promoting paradigm: An ascending single-dose study with almorexant. *Clin Pharmacol Ther*. 2010;87(5):593-600. doi:10.1038/clpt.2010.19

53. Van Der Post J, Schram MT, Schoemaker RC, et al. CNS effects of sumatriptan and rizatriptan in healthy female volunteers. *Cephalalgia*. 2002;22(4):271-281. doi:10.1046/j.1468-2982.2002.00344.x

54. Mets MAJ, Volkerts ER, Olivier B, Verster JC. Effect of hypnotic drugs on body balance and standing steadiness. *Sleep Med Rev*. 2010;14(4):259-267. doi:10.1016/j.smrv.2009.10.008

55. Chen X, De Haas S, De Kam M, Van Gerven J. An overview of the CNS-pharmacodynamic profiles of nonselective and selective GABA agonists. *Adv Pharmacol Sci*. 2012;2012. doi:10.1155/2012/134523

56. Liem-Moolenaar M, de Boer P, Timmers M, et al. Pharmacokinetic-pharmacodynamic relationships of central nervous system effects of scopolamine in healthy subjects. *Br J Clin Pharmacol*. 2011;71(6):886-898. doi:10.1111/j.1365-2125.2011.03936.x

57. Chen X, Broeyer F, de Kam M, Baas J, Cohen A, van Gerven J. Pharmacodynamic response profiles of anxiolytic and sedative drugs. *Br J Clin Pharmacol*. 2017;83(5):1028-1038. doi:10.1111/bcp.13204

58. Van Steveninck AL, Wallnöfer AE, Schoemaker RC, et al. A study of the effects of long-term use on individual sensitivity to temazepam and lorazepam in a clinical population. *Br J Clin Pharmacol*. 1997;44(3):267-275. doi:10.1046/j.1365-2125.1997.t01-1-00580.x

59. Meador KJ, Loring DW, Boyd A, et al. Randomized double-blind comparison of cognitive and EEG effects of lacosamide and carbamazepine. *Epilepsy Behav*. 2016;62:267-275. doi:10.1016/j.yebeh.2016.07.007

60. Gualtieri CT, Johnson LG. Reliability and validity of a computerized neurocognitive test battery, CNS Vital Signs. *Arch Clin Neuropsychol*. 2006;21(7):623-643. doi:10.1016/j.acn.2006.05.007

61. Norris H. The action of sedatives on brain stem oculomotor systems in man. *Neuropharmacology*. 1971;10(2):181-191. doi:10.1016/0028-3908(71)90039-6

62. BOND A, LADER M. The use of analogue scales in rating subjective feelings. *Br J Med Psychol*. 1974;47(3):211-218. doi:10.1111/j.2044-8341.1974.tb02285.x

63. Van Steveninck AL, Schoemaker HC, Pieters MSM, Kroon R, Breimer DD, Cohen AF. A comparison of the sensitivities of adaptive tracking, eye movement analysis, and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. *Clin Pharmacol Ther*. 1991;50(2):172-180. doi:10.1038/clpt.1991.122

64. Izaute M, Bacon E. Specific effects of an amnesic drug: Effect of lorazepam on study time allocation and on judgment of learning. *Neuropsychopharmacology*. 2005;30(1):196-204. doi:10.1038/sj.npp.1300564

65. Wingen M, Langer S, Ramaekers JG. Verbal memory performance during subchronic challenge with a selective serotonergic and a mixed action antidepressant. *Hum Psychopharmacol Clin Exp*. 2006;21(7):473-479. doi:10.1002/HUP.800

66. Jaeger J. Digit symbol substitution test. *J Clin Psychopharmacol*. 2018;38(5):513-519. doi:10.1097/JCP.0000000000000941

67. Beste C, Ziemssen T. Why Cognitive–Cognitive Dual-Task Testing Assessment Should Be Implemented in Studies on Multiple Sclerosis and in Regular Clinical Practice. *Front Neurol*. 2020;11(July). doi:10.3389/fneur.2020.00905

68. Pilli R, Naidu M, Pingali UR, Shobha JC, Reddy AP. A computerized stroop test for the evaluation of psychotropic drugs in healthy participants. *Indian J Psychol Med*. 2013;35(2):180-189. doi:10.4103/0253-7176.116251

69. Micallef-Roll J, Rihet P, Hasbroucq T, Possamaï C, Blin O. Levodopa-induced drowsiness in healthy volunteers: Results of a choice reaction time test combined with a subjective evaluation of sedation. *Clin Neuropharmacol*. 2001;24(2):91-94. doi:10.1097/00002826-200103000-00004

70. Drinkenburg WHIM, Ahnaou A, Ruigt GSF. Pharmaco-EEG Studies in Animals: A History-Based Introduction to Contemporary Translational Applications. *Neuropsychobiology*. 2016;72(3-4):139-150. doi:10.1159/000443175

71. Herrmann WM, Schärer E, Wendt G, Delini-Stula A. Pharmaco-EEG profile of maroxepine: third example to discuss the predictive value of pharmaco-electroencephalography in early human pharmacological evaluations of psychoactive drugs. *Pharmacopsychiatry*. 1991;24(6):214-224. doi:10.1055/S-2007-1014471

72. Fink M. A useful example of pharmaco-electroencephalogram science: Invited commentary on article by Dr Dias Alves. *J Clin Psychopharmacol*. 2018;38(6):552-554. doi:10.1097/JCP.0000000000000974

73. Jobert M, Arns M. Pharmaco-EEG, Pharmaco-Sleep and EEG-Based Personalized Medicine. *Neuropsychobiology*. 2016;72(3-4):137-138. doi:10.1159/000443192

74. Boeijinga PH. Multimodal EEG Recordings, Psychometrics and Behavioural Analysis. *Neuropsychobiology*. 2016;72(3-4):206-218. doi:10.1159/000437434

75. Jobert M, Wilson FJ, Ruigt GSF, Brunovsky M, Prichep LS, Drinkenburg WHIM. Guidelines for the recording and evaluation of pharmaco-eeg data in man: The international pharmaco-EEG society (IPEG): The IPEG pharmaco-EEG guideline committee. *Neuropsychobiology*. 2012;66(4):201-220. doi:10.1159/000343478

76. Wilson FJ, Leiser SC, Ivarsson M, Christensen SR, Bastlund JF. Can pharmaco-electroencephalography help improve survival of central nervous system drugs in early clinical development? *Drug Discov Today*. 2014;19(3):282-288. doi:10.1016/j.drudis.2013.08.001

77. Jobert M, Wilson FJ. Advanced Analysis of Pharmaco-EEG Data in Humans. *Neuropsychobiology*. 2016;72(3-4):165-177. doi:10.1159/000431096

78. Chatrian GE, Lettich E, Nelson PL. Ten percent electrode system for topographic studies of spontaneous and evoked EEG activities. *Am J EEG Technol*. 1985;25(2):83-92. doi:10.1080/00029238.1985.11080163

79. Alder G, Signal N, Rashid U, Olsen S, Niazi IK, Taylor D. Intra- and inter-rater reliability of manual feature extraction methods in movement related cortical potential analysis. *Sensors (Switzerland)*. 2020;20(8). doi:10.3390/s20082427

80. Nottage JF, Horder J. State-of-the-Art Analysis of High-Frequency (Gamma Range) Electroencephalography in Humans. *Neuropsychobiology*. 2016;72(3-4):219-228. doi:10.1159/000382023

81. Muthukumaraswamy SD. High-frequency brain activity and muscle artifacts in MEG/EEG: a review and recommendations. *Front Hum Neurosci*. 2013;7(MAR). doi:10.3389/FNHUM.2013.00138

82. Lelic D, Olesen SS, Graversen C, Brock C, Valeriani M, Drewes AM. Electrophysiology as a tool to unravel the origin of pancreatic pain. *World J Gastrointest Pathophysiol*. 2014;5(1):33. doi:10.4291/WJGP.V5.I1.33

83. Saletu B, Anderer P, Saletu-Zyhlarz G. EEG mapping and tomography in drug evaluation. In: *Medicographia, 32*. ; 2010:190-200.

84. Saletu B, Anderer P, Saletu-Zyhlarz GM, Arnold O, Pascual-Marqui RD. Classification and evaluation of the pharmacodynamics of psychotropic drugs by single-lead pharmaco-EEG, EEG mapping and tomography (LORETA). *Methods Find Exp Clin Pharmacol*. 2002;24(SUPPL. C):97-120.

85. Brienza M, Pulitano P, Mecarelli O. Effects on EEG of Drugs and Toxic Substances. *Clin Electroencephalogr*. 2019:715-729. doi:10.1007/978-3-030-04573-9_45