ALTERNATIVE METHODS

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Preclinical Studies and Go/No-Go Decision Criteria in Early Drug Development

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ABSTRACT

The development of new drugs requires rigorous evaluation at early stages to ensure safety, efficacy, and overall viability. Preclinical studies represent a critical phase in which predefined go/no-go decision criteria are applied to determine whether a candidate should progress toward clinical evaluation. This article reviews the objectives of preclinical studies, the methodologies commonly employed, and the key criteria used to support strategic decision-making in pharmaceutical development.

INTRODUCTION

The drug discovery and development process encompasses multiple stages, ranging from the identification of bioactive compounds to their eventual approval for clinical use. Preclinical studies constitute a critical phase in this process, enabling the characterization of safety, efficacy, and other key parameters of candidate molecules prior to their evaluation in humans. Within this stage, ADME-tox studies—addressing Absorption, Distribution, Metabolism, Excretion, and Toxicity—play a central role in defining the pharmacokinetic and toxicological profiles of drug candidates.

While drug development includes a series of studies mandated by regulatory agencies such as the European Medicines Agency (EMA)[1] and the U.S. Food and Drug Administration (FDA)[2], which must be conducted under strictly regulated conditions, there is an earlier phase that is equally important but less regulated: non-regulatory or non-GLP (Good Laboratory Practice) preclinical research. These studies, which precede or complement formal regulatory preclinical testing, are designed to generate exploratory insights into the biological behavior of compounds. They support the early identification of toxic or genotoxic liabilities, potential drug–drug interactions, reactive metabolite formation, optimal routes of administration, and suitable experimental models.

The value of non-regulatory preclinical research lies in its ability to inform candidate selection, anticipate developmental risks, reduce costs, refine working hypotheses, and ultimately lower the likelihood of failure in later stages. In this context, non-regulatory ADME-tox studies offer a flexible and strategic framework for assessing safety and bioavailability, thereby facilitating informed decision-making and the prioritization of the most promising drug candidates.

At this stage, go/no-go criteria are applied to establish a decision-making workflow that guides whether development should proceed, while optimizing resources and minimizing risk. The assays performed at this point may be conducted internally or outsourced to external providers. Because these studies are not required to comply with Good Laboratory Practice (GLP) standards, they are considerably more cost-effective, allowing multiple candidates to be evaluated in parallel. This approach facilitates the selection of the most promising compound (lead) for advancement to later development stages or for entry into an optimization phase, during which targeted chemical modifications are introduced to improve drug-like properties through medicinal chemistry campaigns[3].

This article examines non-regulatory preclinical studies, with particular emphasis on the most relevant ADME-tox assays, which enable the early identification of risks that may arise in more advanced stages of drug development. Special attention is given to approaches that prioritize in vitro assays over in vivo studies in order to reduce the use of experimental animals, in accordance with the recommendations of Royal Decree RD 1386/2018 on animal experimentation and the principles of the 3Rs (Replacement, Reduction, and Refinement). The article also discusses how this non-regulatory phase interfaces with formal regulatory requirements and how it can be integrated into a more efficient and predictive pharmaceutical development strategy.

In addition, practical recommendations based on the experience of the Preclinical Area at Fundación MEDINA[4] are presented, together with references to companies offering ready-to-use experimental models and customized preclinical studies on demand.

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Overall, non-regulatory preclinical research enables the early characterization of efficacy, pharmacokinetic behavior, and toxicological profiles of drug candidates, thereby optimizing development strategies prior to entry into regulated preclinical phases.

NON-REGULATORY PRECLINICAL TRIALS

In general, the primary objectives of preclinical studies are to assess toxicity, characterize pharmacokinetic parameters, investigate pharmacodynamics and mechanisms of action, identify potential adverse effects in target organs, and establish an initial safe starting dose for subsequent clinical studies.

To achieve these objectives, a combination of in vitro and in vivo studies is conducted in accordance with international regulatory frameworks, including those established by the European Medicines Agency (EMA)[1] and the U.S. Food and Drug Administration (FDA)[2], as well as internationally recognized guidelines issued by the Organisation for Economic Co-operation and Development (OECD)[5] and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)[6].

Although non-regulatory preclinical studies offer greater flexibility in experimental design, aligning them as closely as possible with standardized regulatory guidelines enhances the translational value of the data and improves predictability for subsequent regulatory studies. The main categories of preclinical studies typically considered during this phase are outlined below.

ASSESSING TOXICITY

Toxicity assessment is a fundamental component of preclinical drug development, as it is essential to ensure the safety of candidate compounds prior to their administration in humans. These studies enable the identification of potential adverse effects, the determination of safe dose ranges, and the establishment of critical parameters to inform the design of subsequent clinical trials.

The experimental models employed for toxicity evaluation, arranged according to increasing levels of biological complexity and translational relevance, are summarized in Figure 1.

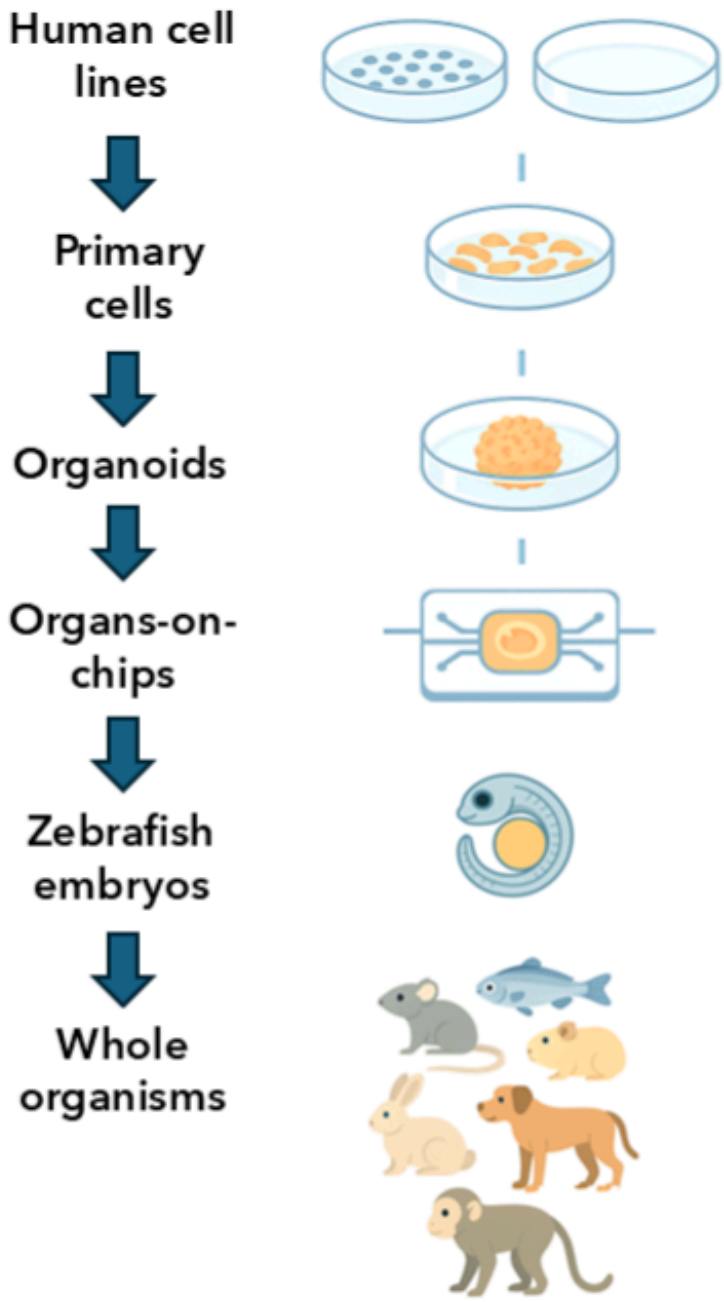

Figure 1: Models used for toxicity testing arranged according to increasing complexity.

The simplest toxicity assessment models consist of in vitro assays using established human cell lines, followed by studies in primary cells, three-dimensional organoids, and organ-on-chip platforms. As model complexity and translational relevance increase, toxicity evaluation progresses to whole-organism systems, including zebrafish embryos and vertebrate models such as zebrafish, mice, rats, guinea pigs, rabbits, dogs, and non-human primates, the latter being subject to particularly stringent ethical and regulatory constraints. Accordingly, following in vitro toxicity screening, early in vivo studies are commonly initiated in zebrafish and/or mice. For regulatory preclinical development, authorities generally require toxicity studies to be conducted in two species: one rodent (typically rat or mouse) and one non-rodent species (most commonly rabbit or dog).

Tip: A robust toxicity-testing strategy at early stages can substantially reduce risks and complications in later phases of development. Of particular note is the zebrafish embryo test known as FET (Fish Embryo Toxicity), which is described in detail in the OECD TG236 guide[7,8]. As zebrafish embryos are not covered by the Royal Decree on animal experimentation (RD1386/2018) during the first five days after fertilisation, they do not require approval by the Authorised Body and the Competent Authority, which facilitates their use. There are several companies that offer on-demand studies in zebrafish, such as ZeClinic[9] and Biobide[10] and other higher models, such as Charles River Laboratories[11] or Vivotecnia[12].

DETERMINE PHARMACOKINETIC AND ADME PARAMETERS

Pharmacokinetics describes the effects of the body on a drug and characterizes the temporal changes in drug concentration following administration. The gold-standard approach for pharmacokinetic assessment involves in vivo studies in animal models, typically mice or rats, in which the compound is administered via oral, intravenous, or intraperitoneal routes. Blood and selected organs are collected at defined time points to determine plasma concentration-time profiles as well as tissue distribution and retention[13]. These analyses are commonly performed using liquid chromatography coupled to mass spectrometry (LC-MS). This approach enables the calculation of key pharmacokinetic parameters, including maximum plasma concentration (Cmax), time to reach maximum concentration (Tmax), elimination half-life (t1/2), drug clearance (CL), and bioavailability (F).

Prior to in vivo pharmacokinetic studies, a range of in vitro assays can be employed to predict metabolic stability, membrane permeability, plasma protein binding, and the potential for drug–drug interactions. These experiments fall under the ADME framework and are valuable for early triaging of compounds with unfavorable properties, such as rapid metabolic degradation, poor absorption, limited target accessibility, or a high likelihood of metabolic interactions with other drugs or xenobiotics. The most informative and widely used ADME assays are summarized in Figure 2.

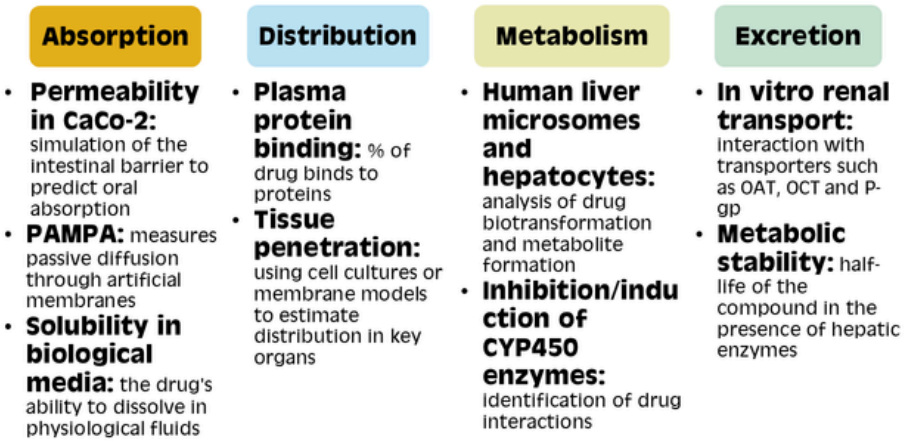

Figure 2: Most relevant ADME assays.

Tip: the most effective approach to ADME evaluation is to follow a stepwise, rational strategy that progresses from assays with the highest predictive value and lowest cost to those that are more resource-intensive. This sequence should be guided by predefined go/no-go criteria, which will be discussed in the next section. Ideally, ADME tests should not be performed in parallel; instead, each stage should be completed before advancing to the next. This allows rapid decision-making at any point in the workflow, enabling development to proceed only when results are favorable and to be halted early when undesirable properties are detected. Various companies offer the possibility of conducting these tests on demand: Eurofins Corporation[14,] Evotec[15,] Fundación MEDINA[4], etc. Others supply ready-to-test models, such as MedTech Barcelona[16].

STUDY PHARMACODYNAMICS AND THE MECHANISM OF ACTION

Pharmacodynamics refers to the study of the biological effects a drug exerts on the body and is therefore closely linked to its mechanism of action. Both in vitro and in vivo assays are required to elucidate this mechanism, characterize the molecular target, and define the key parameters governing the drug–target interaction. Pharmacodynamic evaluation also involves demonstrating the biological activity of the drug on its intended therapeutic target, analysing the downstream molecular and cellular responses, determining the minimum dose capable of eliciting the maximal biological effect, and assessing the efficacy of different dosing regimens based on their pharmacological outcomes.

The following in vitro assays are particularly relevant for characterizing the pharmacodynamic properties of a candidate compound:

Receptor binding: using radioligand binding assays or, more recently, fluorophore-labelled ligand assays to determine whether the drug binds to a specific receptor[17].

Activation/repression of signalling pathways: using techniques such as ELISA, western blot or PCR to detect proteins or genes activated in cells after exposure to the drug.

Functional changes at the cellular level: whether the compound modulates phenomena such as proliferation, apoptosis, necrosis, migration, etc. These can be evaluated with assays such as the MTT test, caspase activation measurement, cellular or subcellular morphological changes using imaging, or more specific assays such as "wound healing" to analyse cell migration in the context of an antitumour drug[18].

To investigate the mechanism of action (i.e., how the drug exerts its biological effects), the following in vitro assays are particularly relevant:

Specific cell models: cell lines that express the drug target either endogenously or through exogenous introduction via gene editing technologies are commonly used. Increasingly, more physiologically relevant and advanced cell models (NAMs) are being incorporated into pharmacodynamic studies, including 3D cultures, tumoroids, organoids, and organ on chip systems. Moreover, induced pluripotent stem cells (iPSCs) derived from both patients and healthy donors are being employed, as they can be differentiated into specific cell types and genetically modified using CRISPR to introduce disease relevant mutations, thereby providing models that more closely recapitulate human pathophysiology[19,20].

Biochemical assays: for enzyme inhibitors or activators, enzymatic activity is quantified in the presence of the drug to determine its effect on catalytic function. In many cases, protein-protein interaction (PPI) assays provide an invaluable complementary approach, as they enable the analysis of physical interactions between two or more proteins, a key factor for elucidating the mechanism of action of numerous therapeutic agents. Among PPI methodologies, energy-transfer–based assays are particularly noteworthy. FRET (Fluorescence Resonance Energy Transfer) detects interactions based on the proximity of fluorophore-labelled proteins, while BRET (Bioluminescence Resonance Energy Transfer) operates on a similar principle but uses a luciferase enzyme as the donor. More advanced systems, such as NanoBRET™ and NanoBiT® (Promega Corporation), allow highly sensitive detection of protein interactions in living cells and have become powerful tools for studying dynamic, real-time protein networks relevant to drug activity[21].

Imaging techniques: high-content imaging (HCI) systems are noteworthy. These are automated microscopy systems for both brightfield and fluorescence that can be used to observe morphological changes, with techniques such as cell painting[22], or to determine the subcellular localisation of proteins[23].

Gene expression studies: using transcriptomics (RNA-seq) to see which genes are regulated, which are overexpressed and which are repressed after treatment.

Tip: before progressing to animal studies, the candidate's effect on the target must be thoroughly characterised, and its specificity assessed whenever possible.

IDENTIFY ADVERSE EFFECTS ON ORGANS AND TISSUES

Before moving on to the direct study of organs at the macro and microscopic levels in an animal model, it is necessary to perform a series of highly specific and relevant in vitro tests, such as those shown in Figure 3.

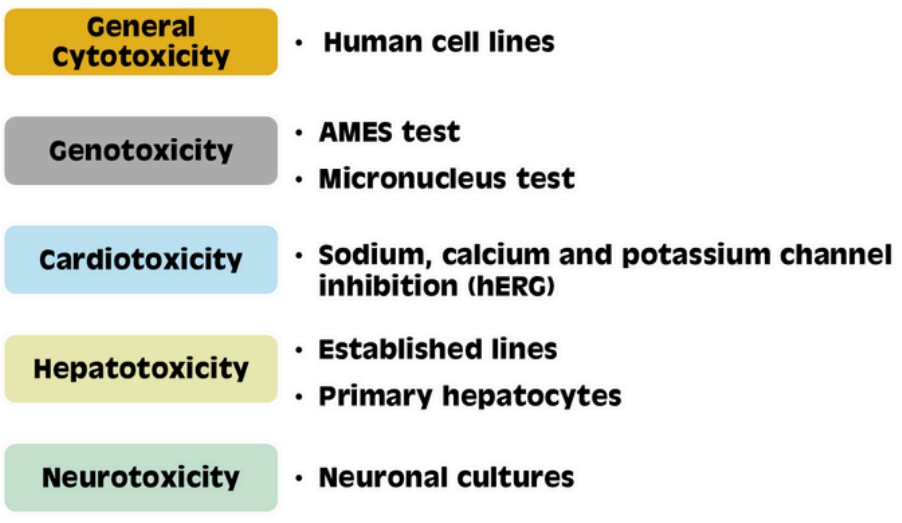

Figure 3: Organ- and tissue-dependent toxicity tests.

General cytotoxicity: the comments made regarding toxicity in the first point would apply. Simpler cell models such as human cell lines or more complex ones such as organs-on-chips can be used[24]. There are many types of tests to measure cytotoxicity, among the most common are the MTT test, LDH or ATP measurement.

Genotoxicity: refers to the ability of a compound or agent to damage genetic material, which can cause mutations, cancer and other diseases. One way to measure whether a compound is genotoxic is through the Ames test, which uses bacteria to determine whether a chemical is mutagenic and is detailed in the OECD's *TG471-Bacterial Reverse Mutation Test guide*[25], and by

the micronucleus test, which measures the percentage of daughter cells during cell division that do not correctly incorporate genetic material due to exposure to genotoxic agents, detailed in the OECD *TG487-In vitro Mammalian Cell Micronucleus Test*[26].

Hepatotoxicity: this assessment is critical because the liver is the principal organ responsible for drug metabolism, and hepatotoxicity can lead to severe adverse outcomes, including acute liver failure. However, predicting hepatotoxicity in vitro remains challenging, as cultured cells often lose a substantial portion of the metabolic enzymes required for xenobiotic biotransformation. The key difficulty, therefore, lies in identifying or developing models that retain the full complement of hepatic metabolic capabilities. Although established hepatic cell lines such as HepG2 are widely used due to their robustness and ease of culture, primary hepatocytes or cells grown in extracellular matrix-based systems generally provide more physiologically relevant models and are better suited for detecting compound-induced liver toxicity[27].

Cardiotoxicity: in particular, the measurement of hERG potassium channel inhibition is one of the preclinical studies required by the FDA and EMA and is mandatory for progression to later stages of development. hERG (*human Ether-à-go-go-Related Gene*) encodes a potassium channel (Kv11.1) essential for cardiac repolarisation. Its inhibition can cause QT interval prolongation (measured as the time between the start of the Q wave and the end of the T wave on an electrocardiogram), which can lead to life-threatening arrhythmias. The ICH has developed a fundamental guideline for addressing the in vitro study of this channel, ICH S7B[28]. The principal in vitro models used for this assessment are cell systems engineered to heterologously express the hERG channel and, more recently, cardiomyocytes derived from induced pluripotent stem cells (iPSCs)[29]. Measurement technologies range from classical patch-clamp electrophysiology to thallium-sensitive fluorescence assays and, more recently, advanced bioluminescence-based platforms.

In vitro neurotoxicity: neurotoxic effects can be evaluated using a variety of neuronal culture models, including established cell lines such as PC12 or SH-SY5Y, primary rodent neurons, or human induced pluripotent stem cells (iPSCs) differentiated into neurons, which offer greater physiological relevance. These systems enable the detection of cytotoxic responses, but a comprehensive neurotoxicity assessment should also examine additional endpoints such as oxidative stress, mitochondrial dysfunction, synaptic activity, and neuroinflammatory responses, thereby providing a more complete profile of the compound's neurological impact.

Tip: before progressing to animal testing, a comprehensive in vitro characterisation must be performed, evaluating the potential toxic effects of the candidate not only on the primary target organ but also on organs that are particularly sensitive to chemical injury, such as the liver, heart, and brain.

ESTABLISHING THE SAFE STARTING DOSE FOR CLINICAL STUDIES

The safe starting dose for clinical studies is predicted using animal models in which the NOAEL (*No Observed Adverse Effect Level*) is calculated, but prior in vitro testing can significantly help by applying the MABEL (*Minimal Anticipated Biological Effect Level*), recommended by the FDA and EMA. The MABEL is calculated by integrating the preclinical results of different studies such as the *in vitro* affinity of the drug to its target, pharmacological potency, estimated bioavailability, and cellular toxicity data[30].

GO/NO-GO CRITERIA IN EARLY DRUG DEVELOPMENT

Currently, approximately 90% of drug candidates that enter the clinical phase ultimately fail to reach the market. Improving this outcome requires highly rigorous preclinical evaluation and the use of increasingly reliable in vitro predictive models, an objective that is becoming more achievable thanks to recent technological advances, including iPSCs, organoids, organs-on-chips, innovative imaging methods, artificial intelligence, and in silico predictive tools.

Go/no-go criteria play a pivotal role in determining whether a compound should progress to the next stage of development. Although primarily grounded in experimental evidence, these criteria also anticipate potential technical, operational, and economic challenges that may arise later. A robust strategy during the preclinical phase can therefore save both time and resources by ensuring that only candidates with a higher probability of success progress to the regulatory preclinical stage. While specific criteria may vary depending on the therapeutic modality and the disease being targeted, they must in all cases be applied sequentially and consistently.

Figure 4 presents a proposed flow chart summarizing the go/no-go criteria for this early stage of drug development, organized according to their relative relevance and implementation cost.

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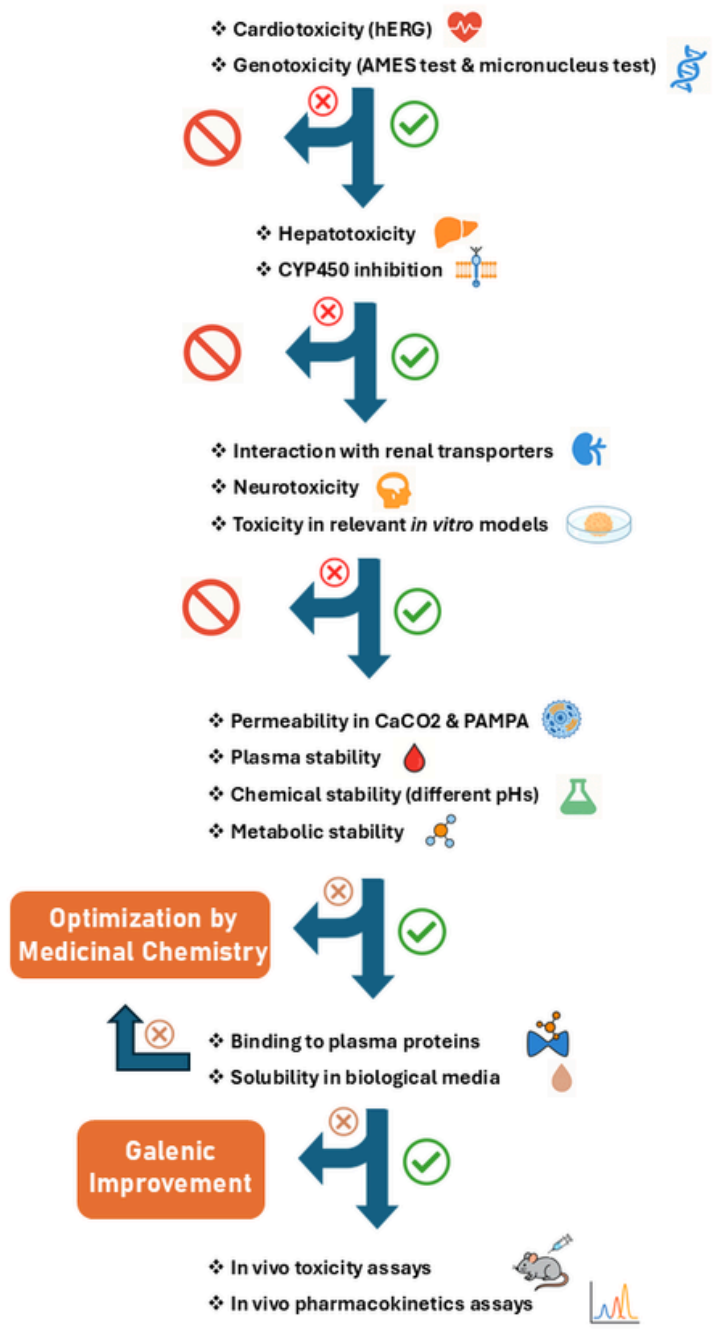

Figure 4: Flow chart with go/no-go criteria for an early phase of drug development.

The initial steps should eliminate candidate compounds that exhibit general or organ specific toxicity, as well as those with potential mutagenic effects. Compounds likely to cause drug–drug interactions, such as inhibitors of cytochrome P450 enzymes, or those capable of altering renal excretion through binding to kidney transporters should also be excluded at this stage.

Subsequent steps should focus on evaluating the compound's potential bioavailability, including its stability under different physiological conditions and its ability to reach the target organ by crossing relevant biological barriers. Candidates that perform poorly in these assays may undergo optimization through medicinal chemistry, in which structural modifications are introduced to address specific liabilities while preserving the molecular scaffold responsible for efficacy and safety. In some cases, where deficiencies can be addressed through appropriate formulation strategies, chemical modification may not be necessary; however, formulation development will eventually be required during later stages of drug development.

Once these preliminary steps have been completed, compounds that meet the established criteria should progress to in vivo studies, beginning with toxicity assessments followed by pharmacokinetic profiling.

To advance to subsequent stages, results must be unambiguous and must satisfy the predefined go/no-go criteria. Some outcomes will clearly warrant discontinuation of development, whereas others may indicate the need either to halt progression temporarily or to optimize the compound chemically or through formulation improvements to enhance its drug-like properties and enable successful administration.

CONCLUSIONS

Preclinical studies and well-defined go/no-go criteria are essential for ensuring the safety and efficacy of drug candidates during early development. Rigorous evaluation at this stage increases the likelihood of clinical success and enables a more efficient allocation of resources. Implementing clear decision criteria, together with robust analytical and predictive tools, is crucial for advancing candidates into the clinical phase with confidence.

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