Available online on 15 Jun, 2025 at https://ijdra.com/index.php/journal
International Journal of Drug Regulatory Affairs
Published by Diva Enterprises Pvt. Ltd., New Delhi
Associated with RAPS & Delhi Pharmaceutical Sciences & Research University
Copyright© 2013-25 IJDRA

Review Article

Open Access

Non-Clinical In-vitro and In-vivo Studies in Drug Development

Abdul Mannan, Tasneem Rasheed*, Zoya Nousheen

Department of Pharmaceutics, Deccan School of Pharmacy, Hyderabad -01

Abstract

The process of discovering and developing drugs is intricate involving multiple stages of critical importance, from basic research to FDA approval. sequence of steps involved, namely Fundamental Investigation, Preliminary Development, Clinical Testing, and FDA Submission/Authorization. Each stage incorporates major scientific and regulatory assessments, including lead identification, toxicity studies, pharmacological profiling, and clinical testing, spanning Phases I–III. Additionally, Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP), Good Clinical Practice (GCP) at different stages. systematic flow indicates the significance of iterative feedback loops between stages to guarantee safety, efficacy, and regulatory compliance, ultimately leading to the introduction of secure and efficient treatment options to the market.

Keywords: Lead Optimization, Target Validation, Identification, Mutagenicity, In-vitro Pharmacology, Toxicity, Drug Discovery and Development, Pre-Clinical Development, metabolic stability

Article Info: Received 22 Apr 2025; Review Completed 28 May 2024; Accepted 30 May 2025

Cite this article as:
Mannan A, Rasheed T, Nousheen Z. Non-Clinical In-vitro and In-vivo Studies in Drug Development. Int. J. Drug Reg. Affairs [Internet]. 2025 Jun 15 [cited 2025 Jun 15]; 13(2):43-51. Available from: http://ijdra.com/index.php/journal/article/view/756

DOI: https://doi.org/10.22270/ijdra.v13i2.756
*Corresponding author

1. Introduction

1.1 Drug Discovery and Development

The process of discovering new drugs includes detection of a compound that can therapeutically treat or cure a disease. (1) This procedure includes identifying potential drug candidates, synthesizing them, characterizing their properties, and conducting screenings and assays to evaluate their therapeutic effectiveness. The drug discovery and development process incurs high costs due to the significant financial resources needed for research and development, along with clinical trial expenses. On average, it takes around 12 to 15 years to move drug from the discovery phase to being available for patients (2) The costs associated with drug development can vary widely, typically ranging from about US$ 897 million to US$ 1.9 billion.

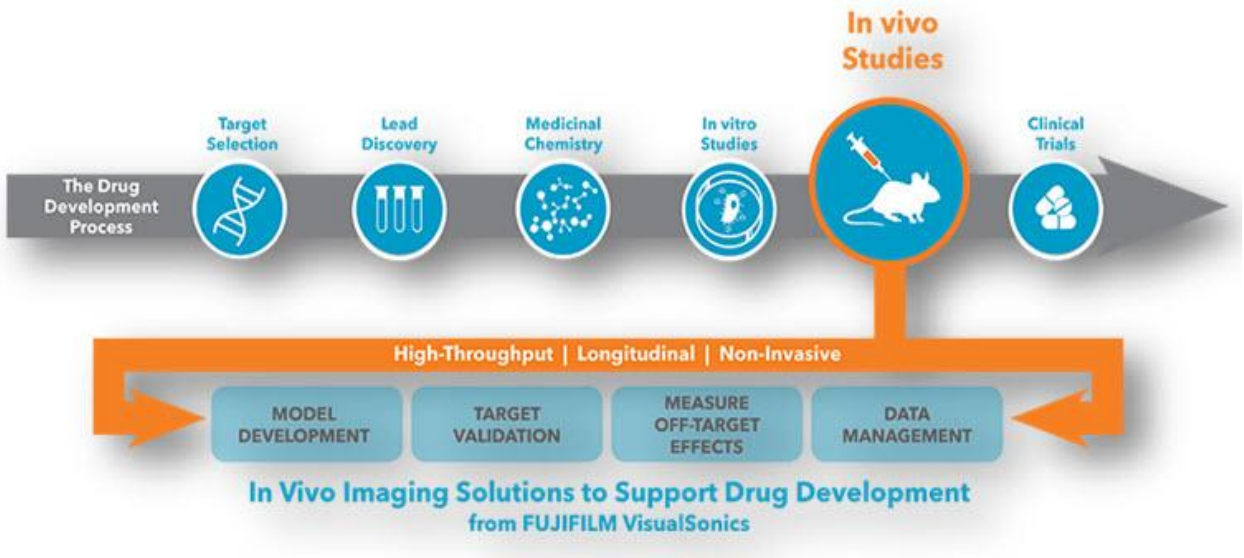

Figure 1. Drug discovery and development stages (6)

1.2 Target Identification

The initial step in drug discovery is pinpointing biological origin of disease and locating possible intervention targets. The process of target identification starts with ascertaining the function of a potential therapeutic target. (2) An optimal target should demonstrate effectiveness, safety, compliance with therapeutic market requirements, possess characteristics suitable for drug development. (3)

A particular target for medication may possess the following traits:

- The target for the drug is a biomolecule that can exist in a standalone or complex form.
- These biomolecules contain specific sites that interact with others.
- The structure of the biomolecule may change when it binds to small molecules and this process is reversible.

1.3 Target Validation

Target validation involves verifying anticipated molecular target, which may be gene, protein, or nucleic acid. (4) This involves showing the operational importance of the determined target in connection with the characteristics of the disease phenotype. Drug must be validated across different cellular models and animal models pertinent to diseases.

Essentially, the target validation process can be outlined in six main steps:

- Identification of a biomolecule
- Developing a rapid-throughput screening method.
- Conducting screening to identify promising candidates.
- Assessment of recognized hits
- Target validation consists of two essential phases:
- *Reproducibility:* Upon discovering a drug target, whether via a particular method or through reproducibility. Approaches used for target validation comprise affinity chromatography, expression cloning, protein analysis, DNA techniques, and reverse transfected cell microarrays. (5,7)

Introducing diversity to the ligand target-environment:

This could entail the genetic alteration of target genes in vitro, including gene knockdown through shRNA, siRNA, or miRNA, gene knockout utilizing CRISPR, or gene knockout through viral introduction of mutant genes. Furthermore, antibodies that bind strongly to the target can be employed to block additional interactions. (8)

1.4 Identification of Lead

After identifying and validating a disease-related molecular target in disease models, compounds are discovered that interact with intact animals or cell-based disease models, providing insights into the organism's integrated response, which can help forecast the potential effects of new medications in patients. The selected compounds are referred to as "leads." A chemical lead is defined as a synthetic molecule that is stable and viable, demonstrating activity in assays.

The features of a chemical lead include:

- Structure-activity relationship (SAR)
- Drug ability considerations
- In vitro evaluation

Evaluating a lead molecule to a drug is crucial; it possess the ability to bind to a specific target and have pharmacokinetic profile concerning ADME. For instance, if the objective is to inhibit a protein that activates gene expression, assay can incorporate a readout to determine whether compound decreases gene's expression. (9)

1.5 Lead Optimization

Lead optimization refers to methodology of designing a drug candidate after identifying an initial lead compound. This process entails the synthesis and characterization of potential drug. Molecules undergo chemical modifications and are then characterized to produce compounds with desirable properties to qualify as drugs. Leads are evaluated based on pharmacodynamic properties in both in vitro and in vivo settings, along with physiochemical and pharmacokinetic properties, as well as toxicological considerations. This process necessitates the simultaneous enhancement of various parameters and is often a lengthy and expensive phase, typically representing the most significant bottleneck in drug discovery. (10)

Compounds identified through hit-to-lead high throughput screening are put through lead optimization to identify the most promising candidates. The aim of lead optimization is to maintain the beneficial features while correcting any deficiencies in their structure. High throughput screenings for DMPK become essential in the lead optimization process, helping to understand and predict in vivo pharmacokinetics. To create new drugs with enhanced efficacy and safety profiles, structural modifications are made during the optimization phase. Automated screening technologies are crucial in drug discovery laboratories. Mass spectrometry is employed to identify and quantify metabolites. MALDI imaging is an important method for evaluating drug and their metabolites within tissue architecture. NMR Fragment-based Screening (FBS) is method used for discovering, refining lead molecules during focused screening efforts. (11)

1.6 Pre-Clinical Development

The pre-clinical development consists of several key elements, including extensive production techniques, safety assessments involving animals, tests for cancer-causing potential, investigations into methods of drug delivery, research on elimination and metabolic processes, experiments in drug formulation, and studies to determine effective dosage ranges in animal models.

Additionally, preclinical trials must receive approval from the relevant regulatory bodies. These authorities are responsible for ensuring that trials are conducted ethically and safely, approving only to drugs confirmed to be both safe and effective.

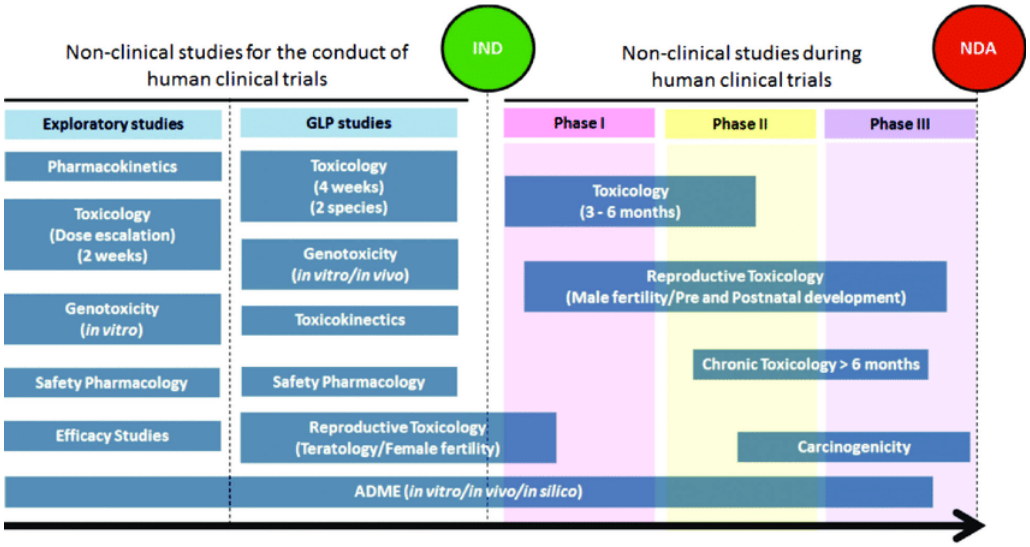

Figure 2. Steps for Non-Clinical studies in drug development process (12)

There are two main methods for conducting preclinical trials:

- Pharmacology in general
- The study of toxicology

Pharmacokinetics and pharmacodynamics are the main topics of pharmacology. Investigating potential negative pharmacological effects with suitable animal models and keeping an eye on them in toxicological investigations are essential. Pharmacokinetic studies are essential for figuring out safety and efficacy measures that have to do with metabolism, excretion, distribution, and absorption. These studies offer information on absorption rates for different routes of administration.

The drug's harmful effects can be evaluated using both in-vitro and in-vivo toxicological investigations. Direct effects on cell proliferation and phenotypic can be assessed in vitro, and toxicological effects can be assessed both qualitatively and quantitatively in vivo. (13)

2. In-Vitro Pharmacology

Drug candidates undergo mechanism-of-action tests in vitro employing a range of time- and cost-effective methods to determine the next phase of the drug development process.

When assessing the potency of most cytotoxic drugs, monolayer cultures are the simplest and most "controllable" models. It has a significant impact on the spread of tumor cells and multidrug resistance. (14,15)

Cell lines with certain biological traits that align with the reasoning behind compound design are suggested. To be more precise, the legitimacy of the data should be guaranteed by using validated cell lines.

Human umbilical vein endothelial cells that have been immortalized and express integrin subunits consistent with an endothelial origin, provide valuable in vitro models for studying molecular mechanisms underlying endothelial cell proliferation and migration during tumor metastasis. (16,17)

2.1 Mutagenecity

Changes in the genetic material of cells that happen spontaneously by chemical or physical processes are referred to as mutagenesis., leading to permanent and heritable differences in successive generations compared to their ancestors. Substances that cause harmful effects by interacting with genetic material, specifically deoxyribonucleic acid (DNA), are termed genotoxic. (18)

2.2 Mutagenecity Testing

Chemical or physical influences can lead to a permanent change in the genetic material (mutation) within cells, resulting in fatal or inheritable defects. To detect these substances before they cause damage, mutagenicity tests have been created, which include genetic tests and screening methods. A chemical's capacity to cause genotoxicity can be evaluated using various genetic endpoints. There are two main types of endpoints, gene mutations, chromosomal alterations, both somatic and inheritable abnormalities are caused by them. (19)

a) Test for Gene Mutations in Bacteria-Ames Test

The most commonly employed for evaluation of the mutagenic characteristics of substances is the Salmonella typhimurium histidine (his) reversion system. (20) Mutations in the histidine operon brought on by mutagen exposure may lead strains to move from a histidine-dependent to a histidine-independent growth pattern. Using a metabolic activity fraction made from rat liver homogenate to replicate an in vivo environment allows researchers to examine how metabolism activity affects the carcinogenic effects of substances.

b) Test for Chromosomal Aberrations in Mammalian Cells

The chromosome aberration assay in cell cultures serves as a valuable and sensitive method for identifying genotoxic substances. Chromosomal damage is assessed through microscopic analysis of chromosomes during the mitotic metaphase stage (F1). Experiments are conducted both with and without additional metabolic activation. (21) Suitable tests involve cultured Chinese Hamster cells and

are designed to evaluate substances whether or not metabolic activity is present.

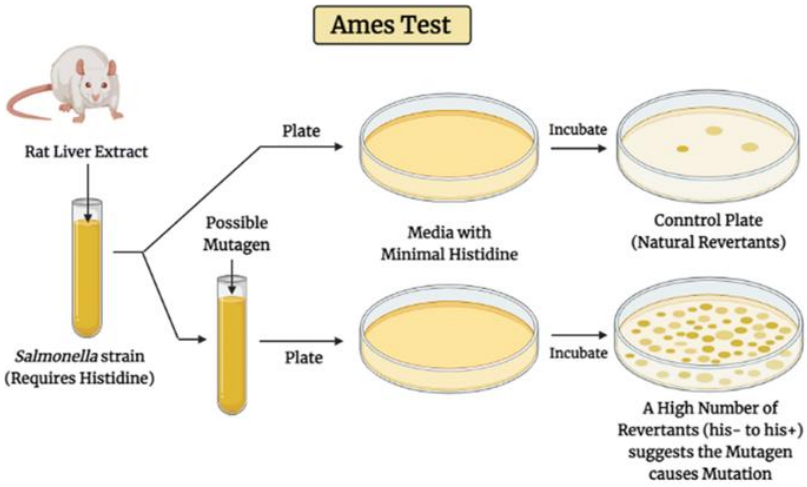

Figure 3. Salmonella typhimurium Ames test (22)

If chromosome mutations occur in the growth or DNA synthesis phase of the cell cycle; if they occur in mitosis phase, they can manifest as chromatid-type aberrations. Chromatid type aberrations refer to modifications in individual chromatids or the breakage and re-joining of chromatids from different chromosomes.

c) Micronucleus Assay in In-Vitro and In-Vivo

The micronucleus test assesses chromosome or mitotic apparatus damage caused by chemicals. (23) Following telophase, these fragments may be excluded from the daughter cell nuclei, leading to the development of one or more cytoplasmic micronuclei. The test improved to detect aneugens and clastogens in both in vitro and in vivo settings. Regulatory agencies require the mutagenic in vivo micronucleus testing, which involves microscopic examination of cytological materials taken from the bone marrow of animals, specifically examining polychromatic erythrocytes.

Pharmacokinetic properties of a compounds and their metabolites, and the healing of any ensuing lesions are all considered in this in vivo experiment. A cultured cell in vitro micronucleus assay established. (24) Consequently, this assay become commonly used method for screening mutagens. Both with and without a metabolic activation system, CHO cell cultures are exposed to the test chemical. After being exposed to cytochalasin B (F3), the treated cells are allowed to develop binucleated cells, and the number of micronuclei within these binucleated cells is measured using light microscopy as a measure of the mutagenic response.

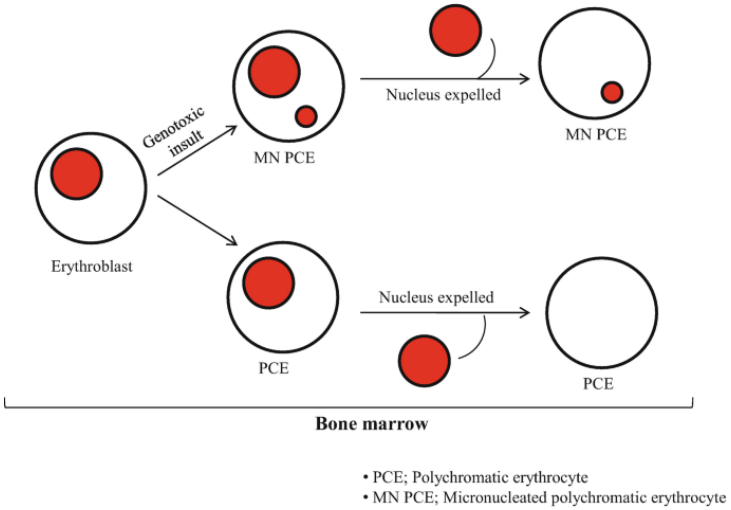

Figure 4. In vivo micronucleus assay (25)

Table 1. Tests and End Point

Mutagenic Process	End–Points	Test
Lesions of pre-mutagenic	DNA and Chemical interaction	Additive DNA
Destruction DNA	Repair & Destruction DNA	Comet Assay
Gene mutation fixed	Gene mutations possess a substitution base pair and a frameshift	Ames test In vivo genetic assay

DNA alterations	DNA breakage Chromosome aberrations	Micronucleus assay Chromosome aberration assay

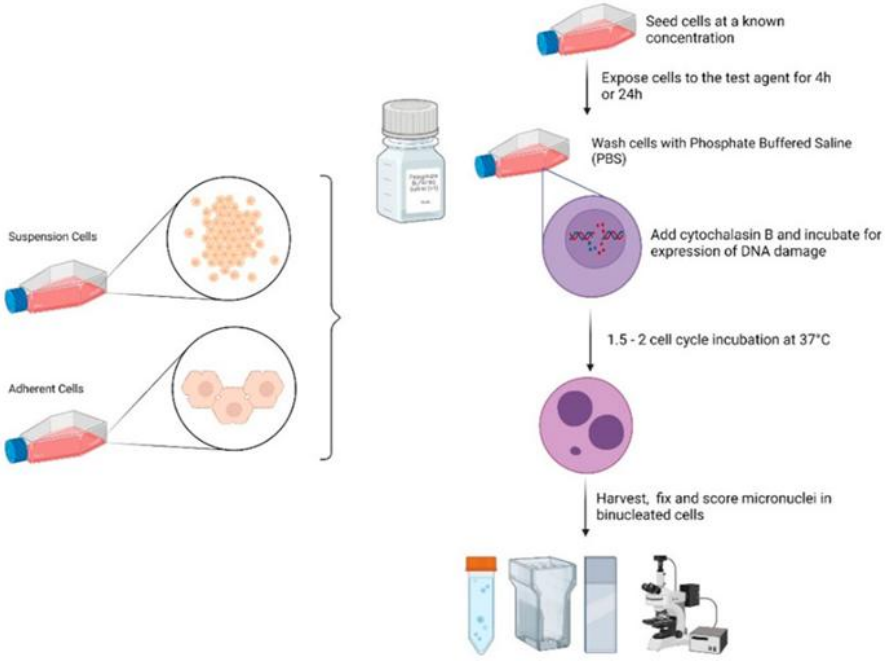

Figure 5. In vitro micronucleus assay (27)

2.3 Toxicity Models

Toxicity refers to "the degree to which substance can be harmful to humans or animals." Models of toxicity have been developed to assess the negative effects of substances on the human body. There are different types of models of toxicity: in silico, in vivo, and in vitro models. (27)

a) In Vivo Toxicity Model

In vivo tests involve conducting experiments with living organisms as subjects. A key benefit of in vivo models is that the in vivo model of toxicity encompasses the entire organism, capturing all physiological responses, biochemical interactions, by offering insights into how a drug disperses within the organism and its potential effects on non-target organs. However, there are certain drawbacks to existing in vivo models of toxicity, because of the notable differences in humans and animal species. (28) The inability to accurately predict drug toxicity using in vivo models can largely attributed to the differences between humans and animals. Furthermore, there are limited opportunities for enhancing in vivo models because they are nearing their maximum capacity for toxicity evaluation. (29)

b) In Vitro Toxicity Model

In vitro testing entails utilizing a specific component from a certain organism to study it within a controlled environment, significantly reducing the impact of surrounding internal or external factors. The different models of invitro toxicity are available, Models for cell-based toxicity are more inclined to consider the known metabolic networks within the cell compared to other in vitro models. (30)

Intricacy for newly developed cell-based in vitro models of toxicity now rivals that of various organs, and in certain instances, and provide greater accuracy for traditional in vivo model of toxicity. (31)

Many in vitro toxicity models using cell cultures for organs lack the variety of cell lines for specific target. Therefore, substance might not occur toxicity in one-line cell but could still harmful to other cell lines present in the target organ.

Moreover, substance that is harmful to a particular cell line may not have the same effect on the entire organ, as other cells within that organ can adjust and makeup for disturbance. Although all process in the human body are interconnected, in vitro models of toxicity primarily focus in biomolecules associated with specific cellular pathways. The results can differ depending on the cell population size (for example, this is evident in organ-on-a-chip models). Investigations using in vitro model of toxicity are conducted over a short, defined timeframe and conclude after a set period rather than being observed continuously. As a result, a substance can induce toxicity in cell at one moment, but damage might be repaired later, leading ultimately to non-toxic outcomes. (32)

2.4 Assessment of Metabolic Stability

Early on in the drug development process, research on metabolic stability of novel chemical entities (NCE) is crucial. (33) Generally, drug metabolism investigations rely on both in vitro cellular/sub-cellular models and in vivo animal experiments. (34) In vivo studies using animal models offer the most insights to pharmacokinetics properties of NCEs. Notably, for possible medication candidates, information gathered from in vivo experiments provides a thorough pharmacokinetic profile. Experiments with biological systems, however, are often expensive, long process, and inappropriate for testing a large number of chemicals. (35)

By incubating a chemical with suitable metabolic models, metabolic stability evaluation can be carried out. The generated incubation mixes are then subjected to chromatographic assessment (such as HPLC-MS/MS). (36)

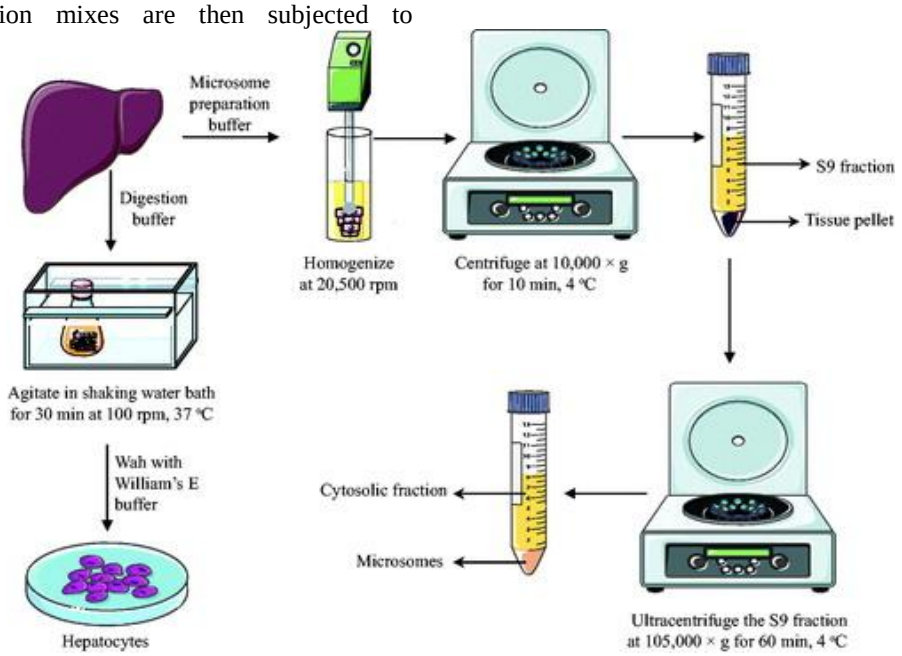

Figure 6. Preparation of invitro metabolic stability system used for metabolic stability studies (37)

Commonly used in vitro systems for metabolic stability include microsomes and hepatocytes. Microsomes are commonly utilized to evaluate phase I metabolism mediated by CYP enzymes. Some of the key benefits of using microsomes include their accessibility, availability of various species models, the simplicity of the procedure, the minimal quantity of sample required for the study, and the ability to store microsomes for extended periods. (38)

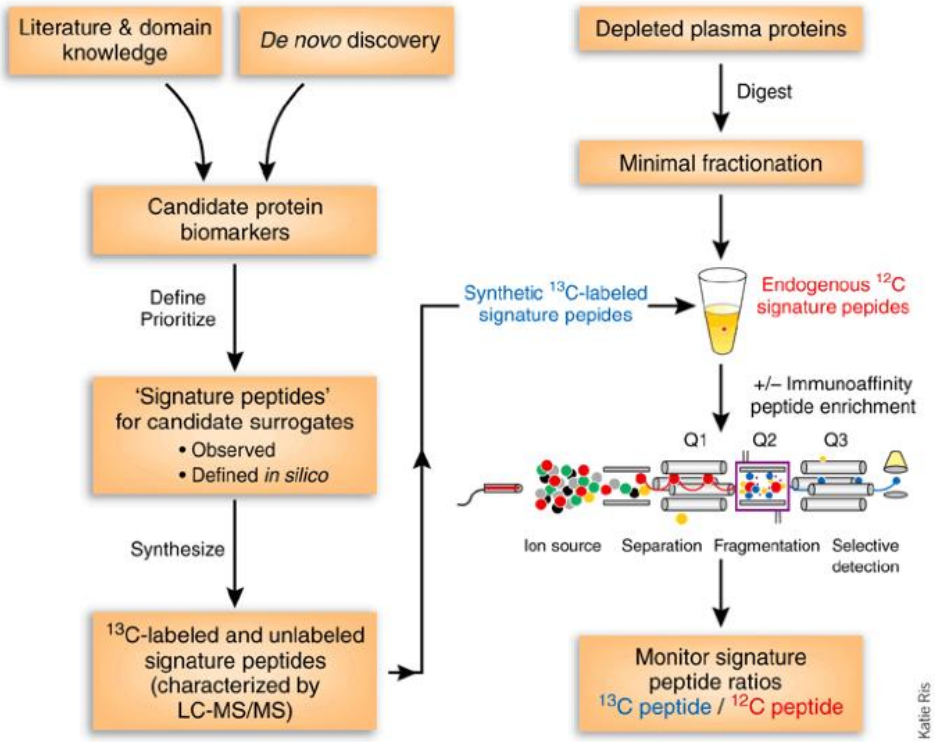

Figure 7. Process flow for candidate biomarker verification (39)

2.5 Study of Distribution, Metabolism and Pharmacokinetics (DMPK) Of New Substances

The effectiveness of a drug candidate is greatly influenced by its kinetic characteristics. It is possible to define the pharmacokinetic profile of a compound to establish a suitable dosage which ensures patient adherence to treatment and enables proper evaluation of results from safety, effectiveness. Typically, adverse reactions associated with substances that lead to the cessation of

their development connected to prolonged systemic exposure to drug, the formation of toxic metabolites, risk of drug-drug interactions. (40)

Pharmaceutical companies have integrated DMPK studies into the initial phases of development. Performing DMPK studies during pre-clinical phases is vital. Additionally, DMPK evaluations conducted early in drug development provide essential insights into a molecule's that could improve its DMPK attributes. (41)

2.6 Biomarkers

A dynamic biological entity that offers information (such as a physiological indicator, protein, metabolite, cellular, biochemical, molecular, genetic, or unique post-translational alteration) is called a biomarker which has a scope to quantified, analyzed as a representation of biological functions, disease process progression, pharmacological effects of a treatment. Application of biomarkers from the initial discovery phase through to late-stage clinical drug development is essential for evaluating both efficacy and safety for animal models and for assessment of human safety during development.

Every phase of drug development involves unique series of biomarkers. For biomarkers to hold value, they need to be reliable, reproducible, and easily measurable. The identification of a biomarker must be clinically significant and offer tangible advantages to the patient in preclinical research, examples of biomarkers include serum chemistries, expression of cell surface proteins, pharmacokinetic and pharmacodynamic measurements of drugs, phenotyping of drug-metabolizing isoenzymes, serum transaminases, genomic expression profiles, as well as imaging that assesses drug distribution or receptor occupancy.

In late- stage drug development, biomarkers are utilized to assess the dose-response relationship to determine optimal regimen for achieving desired pharmacological effect. (42)

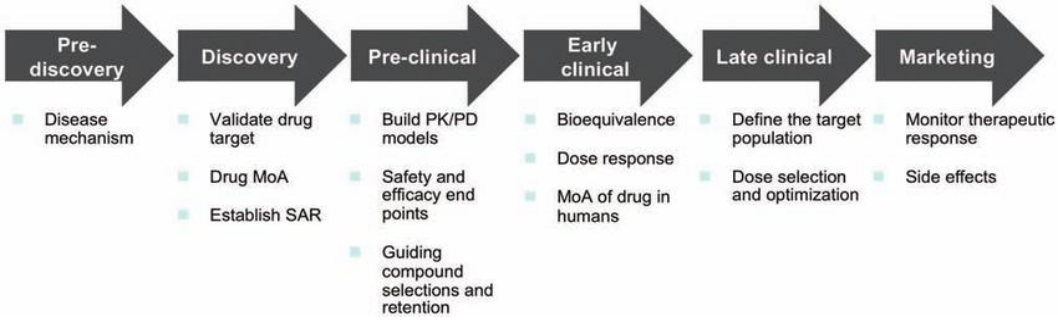

Figure 8. Biomarkers use in Non clinical and clinical studies (43)

2.7 CYP Screening

Cytochrome P450 (CYP450) enzymes are predominantly found in the liver and intestines and are responsible for oxidizing most medications for metabolism. The activity of CYP450 enzymes can be either increased or decreased by various drugs and substances, leading to interactions that may result in toxicity or diminished therapeutic effects. Changes in ADME characteristics during inflammation can frequently be traced back to alterations in drug metabolism due to enzymatic changes. (44)

A significant element of drug metabolism involves the interaction between the drug and a protein, frequently a cytochrome (CYP), which modifies its chemical structure. It is advantageous to predict these interactions during the preclinical phase. Various computational tools are available that aim to effectively forecast these drug-target interactions. Below is a broad yet non-exhaustive overview of these tools: Computer-aided drug design (CADD). These comprise structure-based (protein target-based), ligand-based, and hybrid approaches (proteochemometric). Chemoinformatics techniques, such as quantitative structure-activity relationships (QSAR), also utilize established binding affinity data of drug substrates to different metabolizing enzymes to predict additional drug-enzyme interactions.

Table 2. Non-Clinical Assays of ADME /PK

GLP IN VIVO	NON GLP IN VIVO	IN VITRO
Toxicokinetics	Toxicokinetic	Physical/Chemical Properties
Metabolisation	Linearity	Hepatic Clearance
Route of Elimination	Metabolisation	Permeability

3. Chemistry, Manufacturing and Control

The chemistry, manufacturing, and controls (CMC) of an active ingredient or final product are integral to the successful execution of non-clinical and clinical studies for a candidate, as well as to the precise interpretation and connection of results gathered at every phase of the discovery and development process. It is recommended that the manufacturing process conforms to Good Manufacturing Practices (GMP) to guarantee the quality, safety, and efficacy of pharmaceutical products, while also ensuring consistency and reproducibility across different batches. (45)

CMC of final product provides vital information that verifies its identity and quality throughout the manufacturing process, submitting CMC data alongside the application for product registration is regarded as one of the requirements established by regulatory authorities. (46)

Biodisponibility	Routes of Elimination	Physiological Characteristics
Biological Fluids Quantification	Biodisponibility	Metabolic Stability

4. Investigational New Drug (IND)

A clinical study sponsor submits a request for an Investigational New Drug Application (IND) to obtain the FDA's approval to test an experimental drug or biological product on human participants. Clinical trials are often conducted to collect data on safety and efficacy to support marketing applications for drugs and biologic products. In addition to obtaining approval from the pertinent regulatory agencies, the study protocol and informed consent documents that participants must sign prior to joining a clinical trial also need to be sanctioned by an Institutional or Independent Review Board (IRB) or Ethical Advisory Board. An IRB is an independent committee comprised of physicians, community representatives, and others that ensures a clinical trial complies with ethical standards and safeguards the rights of participants. (47) These regulations and laws define the responsibilities and obligations of entities such as sponsors, clinical investigators, and institutional review boards. Additionally, various guidance documents and standard operating procedures are available to clarify the policies and processes related to the IND process. (48)

Content of an initial IND

- Cover Sheet (Form FDA 1571)
- Table of Contents
- Introductory Statement & General investigational plan
- Investigator's Brochure
- Protocols
- Chemistry, Manufacturing & Control Information
- Previous Human Experience with the Investigational Drug
- Additional Information

5. Conclusion

Drug discovery and development is a rigorous, stepwise journey that combines scientific innovation with strict regulatory control. It starts with basic research and moves through preclinical and clinical stages before finally achieving regulatory approval. Each step has a specific purpose-from the identification of lead compounds to the verification of their safety and efficacy in humans. Ongoing interaction between investigation, testing, and regulation confirms that only those drugs most potent and safest attain patients. The entire pathway indicates the intricacies and significance of each stage in advancing new treatment from the lab bench to the bedside.

Acknowledgements

I would like to express my sincere gratitude to the editorial team and reviewers of International Journal of Drug Regulatory Affairs for their valuable feedback and support throughout the review and publication process. Their constructive comments have significantly enhanced the quality of article.

Financial Disclosure statement:

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Reference

1. Giersiefen H, Hilgenfeld R, Hillisch A. Modern Methods of Drug Discovery. Institute of Molecular Biotechnology, Beutenbergster. Germany 2003;1:109-155. doi: 10.4172/1948-5964.1000025
2. Drews J. Drug discovery: a historical perspective. Science 2000;287:1960-1963. doi: 10.4172/1948-5964.1000025
3. Wang S, Sim TB, Kim YS, Chang YT. Tools for target identication and validation. Curr Opin Chem Biol. 2004;8: 371-777. doi: 10.4172/1948-5964.1000025
4. Imming P, Sinning C, Meyer A. Drugs, their targets and the nature and number of drug targets. Nature Reviews Drug Discovery. 2006;5:821-834. doi: 10.22270/ajprd.v7i6.616
5. Croston G. The utility of target-based discovery. Expert Opinion on Drug Discovery. 2017;12(5):427-429. doi: 10.22270/ajprd.v7i6.616
6. Malviya R, Srivastava M, Bansal V, Sharma PK. Drug design, discovery and development and their safety or efficacy on human body. ResearchGate [Internet]. 2021 [cited 2025 Jun 15]. Available from: https://www.researchgate.net/publication/356643651....
7. Henning SW, Beste G. Loss-of-function strategies in drug target validation. Current Drug Discovery Technology. 2002;17–21. Doi: 10.22270/ajprd.v7i6.616
8. John GH, Martyn NB, Bristol-Myers S. High throughput screening for lead discovery. Burgers Medicinal Chemistry and Drug Discovery, 6th edition, Drug Discovery and Drug Development, Wiley Press. 2002;2:37-70. Doi: 10.22270/ajprd.v7i6.616
9. Patidar AK, Selvam G, Jeyakandan M, Mobiya AK, Bagherwal A, Sanadya G, Mehta R. Lead Discovery and lead optimization: A useful strategy in molecular modification of lead compound in analog design. International journal of drug design and discovery. 2011; 2(2):458-463. Doi: 10.22270/ajprd.v7i6.616
10. Huber W. A new strategy for improved secondary screening and lead optimization using high-resolution SPR characterization of compound– target interactions. J Mol. Recognition. 2005;18:273–281. Doi: 10.22270/ajprd.v7i6.616
11. Lofas S. Optimizing the hit-to-lead process using SPR analysis. Assay of Drug Development Technologies. 2004; 2:407-416. Doi: 10.22270/ajprd.v7i6.616
12. Peixoto MP, Vieira ML, Nunes AS, Araújo SL, Monteiro G. Non-clinical studies in the process of new drug development - Part II: Good laboratory practice, metabolism, pharmacokinetics, safety and dose translation to clinical studies [Internet]. 2016 [cited 2025 Jun 15]. Available from:

https://www.researchgate.net/publication/311567021
13. Friedman LM, Furberg CD, Demets DL. Fundamentals of clinical trials. 4th edition. New York: Springer Science and Business Media LLC; 2010. doi:10.21873/anticanres.13485
14. Van Beijnum JR, Giovannetti E, Poel D, Nowak-Sliwinska P and Griffioen AW. miRNAs: Micro-managers of anticancer combination therapies. Angiogenesis. 2017; 20(2):269-285. doi: 10.1007/s10456-017-9545-x
15. Phillips RM. Targeting the hypoxic fraction of tumours using hypoxia-activated prodrugs. Cancer Chemother Pharmacol.2016;77(3):441-457. Doi: 10.1007/s00280-015-2920-7
16. Rhim JS, Tsai WP, Chen ZQ, Chen Z, Van Waes C, Burger AM and Lautenberger JA. A human vascular endothelial cell model to study angiogenesis and tumorigenesis. Carcinogenesis. 1998;19(4):673-681.
17. Filipović L, Aranđelović S, Krivokuća A, Janković R, Dojčinović B and Radulović S. Trans-platinum(II)/(IV) complexes with acetylpyridine ligands as antivascular agents in vitro: Cytotoxic and antiangiogenic potential. Anticancer Agents Med Chem. 2016;16(12):1628-1639.
18. Galloway S.M.. Mut. Res. 1994;312:195-322.
19. Madle S., Korte A. and Ball R. Mut. Res. 1987;182:187-192.
20. Maron D.M. and Ames B.N. Mut. Res.1983;111:173-215.
21. Galloway S.M. Env. Mol. Mutagen. 2000;35:191-201.
22. Sinha S, Roy A, Ghosh M. Unravelling the molecular mechanism of mutagenic factors impacting human health [Internet]. ResearchGate; 2021 [cited 2025 Jun 15]. Available from: https://www.researchgate.net/publication/354008517
23. Schmid W. Agents Actions.1973;3:77-78.
24. Kirsch-Volders M., Sofuni T., Aardema M. et al. Mut. Res. 2003;540:153-163.
25. Koch MA, Waldmann H. Protein structure similarity clustering and natural product structure as guides to compound library design. In: High-content screening: Science, techniques and applications [Internet]. New York, NY: Springer; 2019 [cited 2025 Jun 15]. p. 137–53. Available from: https://link.springer.com/protocol/10.1007/978-1-4939-9646-9_7
26. Taft D, Perry M, La Rocco S, Smith B. Regulatory toxicology and the promise of new approach methodologies (NAMs). Front Toxicol. [Internet]. 2023 [cited 2025 Jun 15];5:1171960. Available from: https://www.frontiersin.org/articles/10.3389/ftox.2023.1171960/full
27. Guarino RA, editor. New Drug Approval Process: Accelerating Global Registrations. New York: Marcel Dekker, Inc;2004. Doi: 10.17305/bjbms.2019.4378
28. Darwin C, Wallace J. The Origin of Species. Wordsworth Editions Ltd; 1998 Doi: 10.17305/bjbms.2019.4378
29. Suzanne Clancy. Genetic mutation. Nat Educ. 2008;1:187. Doi: http://dx.doi.org/10.17305/bjbms.2019.4378
30. Oltvai ZN, Barabási AL, Jeong H, Tombor B, Albert R. The large-scale organization of metabolic networks. Nature.2000;407:651-4. Doi: 10.1038/35036627
31. Jakasa I, Kezic S. Evaluation of in-vivo animal and in-vitro models for prediction of dermal absorption in man. Hum Exp Toxicol. 2008 Apr;27(4):281-8. doi: 10.1177/0960327107085826.
32. Cressey D. DNA repair sleuths win chemistry Nobel. Nature. 2015 Oct 15;526(7573):307-8. doi: 10.1038/nature.2015.18515.
33. Lai Y, Chu X, Di L, Gao W, Guo Y, Liu X, Lu C, Mao J, Shen H, Tang H, Xia CQ, Zhang L, Ding X. Recent advances in the translation of drug metabolism and pharmacokinetics science for drug discovery and development. Acta Pharm Sin B. 2022 Jun;12(6):2751-2777. doi: 10.1016/j.apsb.2022.03.009.
34. Wang L, Chiang C, Liang H, Wu H, Feng W, Quinney SK, Li J, Li L. How to Choose In Vitro Systems to Predict In Vivo Drug Clearance: A System Pharmacology Perspective. Biomed Res Int. 2015;2015:857327. doi: 10.1155/2015/857327.
35. Plant N. Strategies for using in vitro screens in drug metabolism. Drug Discov Today. 2004 Apr 1;9(7):328-36. doi: 10.1016/s1359-6446(03)03019-8.
36. Ma S, Chowdhury SK, Alton KB. Application of mass spectrometry for metabolite identification. Curr Drug Metab. 2006 Jun;7(5):503-23. doi: 10.2174/138920006777697891.
37. U.S. FDA and the drug development process. Drug Dev Ind Pharm [Internet]. US FDA;2021 [cited 2025 Jun 15]; 47(10):1627–35. Available from: https://www.tandfonline.com/doi/full/10.1080/03602532.2021.1970178
38. Gómez-Lechón MJ, Donato MT, Castell JV, Jover R. Human hepatocytes in primary culture: the choice to investigate drug metabolism in man. Curr Drug Metab. 2004 Oct;5(5):443-62. doi: 10.2174/1389200043335414..
39. Service RF. A growing role for computational biology in drug development. Nat Biotechnol [Internet]. 2004 [cited 2025 Jun 15];22(10):1235–7. Available from: https://www.nature.com/articles/nbt1235
40. Singh SS. Preclinical pharmacokinetics: an approach towards safer and efficacious drugs. Curr Drug Metab. 2006;7:165–182. doi: 10.2174/138920006775541552.
41. Ducharme J, Dudley AJ, Thompson RA. Pharmacokinetic issue in drug discovery. In: Rang HP (Editor), Drug discovery and development. Philadelphia: Churchill Living-stone Elsevier. 2006;141–161.
42. Lee, J. W. et al. Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement. Pharmaceutical research. 2006;23:312-28. Doi: 10.1007/s11095-005-9045-3.
43. European Pharmaceutical Review. Drug development and approval processes in Europe [Internet]. ResearchGate [cited 2025 Jun 15]. Available from: https://www.researchgate.net/publication/235946588
44. Ducharme J, Dudley AJ, Thompson RA. Pharmacokinetic issue in drug discovery. In: Rang HP (Editor), Drug discovery and development. Philadelphia: Churchill Living-stone Elsevier; 2006. P. 141–161.
45. International Conference on Harmonisation (ICH). Q7: Good manufacturing practice guide for active pharmaceutical ingredients; 2000.
46. International Conference on Harmonisation (ICH). M4Q(R1): The common technical document for the registration of pharmaceuticals for human use: quality overall summary of module 2 and module 3; 2002.
47. Types of clinical trials [Internet]. Wikipedia [cited 2025 Jun 16]. Available from: https://en.m.wikipedia.org/wiki/Clinical_trial
48. U.S. Food and Drug Administration (FDA). Investigational new drug applications (INDs) – CBER regulated products [Internet]. US FDA; 2025 Mar 27 [cited 2025 Jun 16]. Available from: https://www.fda.gov/vaccines-blood-biologics/development-approval-process-cber/investigational-new-drug-applications-inds-cber-regulated-products