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PRECLINICAL TOXICITY TESTING AND ADVERSE DRUG REACTION MONITORING IN DRUG DISCOVERY PROCESS

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ABSTRACT

The process of drug discovery and development involves a synchronous activities of both preclinical toxicity testing methods and adverse drug reaction monitoring at clinical set ups. In clinical phases of drug development, pharmacovigilance guarantee the safety of pharmaceutical usage by identifying and evaluating adverse drug reactions (ADRs). An appropriate and well-structured ADR monitoring could help patient safety by determining the degree and causation of ADRs by certain drugs. Recently, the ADR detection and assessment are being improved by new instruments, algorithms, scientific and regulatory developments. Early detection and proactive management of ADRs can help to mitigate potential risks to patient safety and ensure the success of drug development endeavours.

KEYWORDS: ADR Detection, Clinical trials, Drug Discovery, Pharmacovigilance, Preclinical toxicity studies.

INTRODUCTION

The emergent drug development process is like a journey where scientists work to discover and create new medicines. Drug distribution is a costly, high-risk endeavour that often takes ten to fifteen years to finish.[1] They start by identifying targets, then they test and refine potential drugs in the lab before moving on to clinical trials in people. Eventually, if a drug is found to be safe and effective, it gets approved by regulators and can be used to treat patients. It's a step-by-step process that aims to find new treatments to help improve people's health. The whole medication development process is expensive and time-consuming, from discovery to sale. Strict procedures are followed during

clinical studies that guarantee gathering of adverse event data and the safety of study participants.[2] For medication development and patient safety, early identification and precise prediction of adverse drug reactions (ADRs) are therefore essential.[3] Pharmacology, pharmacovigilance, and pharmacoinformatics are three scientific fields that have been tackling the issue of ADRs from various angles.[3] Identification of genetic and phenotypic predispositions of patients susceptible to higher risks of ADRs could provide a comprehensive understanding of how drugs interact with the biological systems and could help in the implementation of medication-related decision support system.[3]

DRUG DISCOVERY AND DEVELOPMENT PROCESS

The Method: Years, Disappointments, and Uncertainty Abound

The US Food and Drug Administration addressed these difficulties on the "critical path" of drug development elsewhere.[4] The market eventually sees less than 10% of novel drugs that start clinical trials, and many more are unsuccessful in the preclinical phases of research. The first step in developing a new medication, particularly for conditions like Alzheimer's disease (AD), often involves identifying a biological target, such as a receptor, enzyme, protein, or gene that plays a role in the dysfunctional biological processes associated with the disease. This discovery marks the beginning of the creation of a completely new medication—one that works differently from existing treatments and aims to address therapeutic needs not covered by current options.[5]

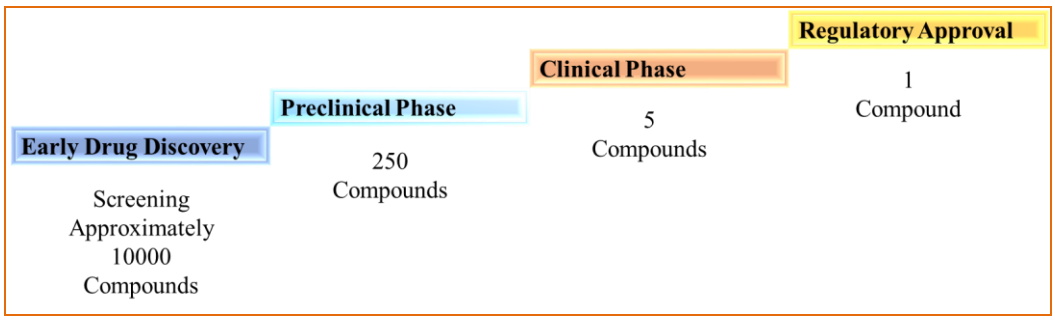

Fig 1: Different Phases of Drug discovery and development process.

While some new medications may not require modifications to existing prescriptions, others represent incremental improvements over current treatments. These improved medications offer benefits such as increased potency, enhanced safety, better tolerability, or greater convenience for patients. Even though they may not revolutionize treatment, these incremental advancements contribute to overall progress in managing conditions like AD.[5]

DISCOVERY AND TARGET IDENTIFICATION

Early chemical starting points have been found in naturally occurring substances in humans, animals, and plants, but lead compounds are more frequently found in random or receptor-targeted high-throughput screening or in targeted chemical synthesis aimed at binding to the structures of known receptors and enzymes.[6] Identify a potential therapeutic target based on disease understanding, molecular pathways or biological mechanisms. Conduct target validation studies to confirm the relevance of the target in disease pathogenesis. Identification of targets is the initial step in the drug development process. A target is an object that, upon binding with an endogenous ligand or medication, alters its behaviour or function. Drugs primarily target enzymes, receptors, transport proteins, DNA, and RNA at the molecular level.[7] The pre-clinical drug development method is predicated on the idea that altering the target or targets of interest would improve a disease state. One of the main objectives of the human genome data project was to open the door for novel approaches to illness diagnosis, treatment, and prevention since it has the potential to identify targets.[8]

IN SILICO TESTING

In silico is an idiom that means "performed on computer or via computer simulation." It was developed as a comparison to the well-known terms in vivo and in vitro. Computational toxicology, often known as in silico toxicity, is a field with enormous promise and rapid advancement. It is challenging to characterize in silico toxicology precisely because in silico components are now a substantial part of almost all toxicological research and risk assessment. "Integrating modern computing and information technology with molecular biology to improve agency prioritization of data requirements and risk assessment of chemicals" is how the US Environmental Protection Agency (US EPA) describes in silico toxicology.[9] More broadly, "anything we can do with a computer in toxicology" is how Hartung and Hoffmann (2009) described in silico techniques. "There are few tests that would not fall into this category, as most make use of computer-based planning and/or analysis." They identified nine main types of in silico techniques, of which I will focus on the use of what the European Union (EU) refers to as "non-testing methods" and their use in chemical control and toxicity testing.[10]

Using computer methods to simulate and predict potential adverse drug reactions based on chemical structure and pharmacological properties is a helpful approach. High-throughput screening methods in labs are used to systematically test various drug combinations to find effective ones. However, testing each potential combination takes a lot of time and money, especially as the number of drugs increases.[11]

To tackle this challenge, researchers have developed systematic computer-based methods, known as in silico methodologies, to study potential medication combinations. These methods calculate drug similarity based on different kinds of drug information, such as chemical structure, target proteins, ATC code, and adverse effects. While these algorithms are good at predicting medication combinations, they can't predict the actual effects of the drug combinations on the body and need additional annotated data.[11] As an alternative approach, some computer methods analyse drug-induced gene expression profiles to predict potential drug combinations based on network-based analysis. These methods provide another way to forecast possible drug combinations and their effects.[11]

The creation of in silico toxicology (IST) protocols, which are designed to provide a systematic approach to utilize in silico methodologies, can support and promote the use of IST approaches to predict organ toxicity. In addition to providing a way to enable a more transparent examination of the data, this standardization procedure encourages acceptance of the methodologies and the accompanying forecasts by end users, colleagues, partners, and regulators. Based on the experience of a cross-industry cooperation consisting of several companies, protocols that explain the integration of in silico methodologies with current experimental data to identify probable Geno toxicants and skin sensitizers have been produced.[12,13]

An IST protocol comprises the following elements and describes the in-silico prediction procedure in a standardized and well-defined manner[14] - recommendations on the generation of predictions and the evaluation of pertinent experimental data; indications on the effectiveness of the in-silico analysis to generate results, including expert review; recommendations on the reporting styles for disseminating the findings and associated uncertainty identification of adverse effects or mechanisms to predict alongside the corresponding experimental data and/or in silico methodologies and approaches to use.

LEAD DISCOVERY AND OPTIMIZATION

Cooperative computational and experimental research can be used to explore lead generation and optimization.[15] There are two methods for producing leads: *de novo* design in conjunction with the ligand-growing program BIOMB (Building Biochemical and Organic Models)[16] and virtual screening with the use of the Glide docking software.[17] Another well-liked option is fragment-based design, which entails docking and connecting several tiny molecules in a binding site.[18] While compounds via virtual screening of commercial catalogs are usually purchased, desirable compounds from *de novo* design usually need synthesis. In both scenarios, starting with a high-resolution crystal structure of the target protein complexed with a ligand is the recommended method. It is not recommended to begin from an apo structure, even when the ligand has been removed, as side chains may have been rearranged to partly cover the empty binding site.[15]

It is thought that when biomolecule-inhibitor binding grows, so does inhibitory potency. For lead optimization, then, precise biomolecule-ligand binding affinity prediction is crucial from a computational standpoint. Although there are several methods, the most rigorous ones have the potential to be the most accurate.[19]

Use high-throughput screening (HTS), computational modelling, or other techniques to identify chemical or biological compounds that interact with the target. Optimize lead compounds through medicinal chemistry, structure-activity relationship studies, or other approaches to improve potency, selectivity, and pharmacokinetic properties. The creation of novel pharmacologically active candidates with potential for therapeutic use is linked to lead molecule discovery. A better fit in the target site is necessary to improve the structure affinity relationship with higher efficiency, potency, efficacy, selectivity, thermodynamic and kinetic parameters, and pharmaceutical properties. This is the first step in the process of determining chemical structure and modification. With regard to the structural, functional, and metabolic details of innovative lead compounds, computational drug design is essential. Identification of the target site, hit identification, and hit-to-lead-to-candidate optimization are all involved. Preclinical and clinical studies are used after these stages. Lead optimization enhances the performance of the generated lead molecule in terms of toxicity, thermodynamic and pharmacokinetic characteristics, binding affinity, structural alteration, and target to lead interaction.[20]

PRECLINICAL STUDIES

It is obvious that preclinical research, which is the initial phase of the process, is essential to the creation of new drugs. Regretfully, a significant number of these preclinical investigations carried out on animals that suggested a potential therapeutic impact do not correspond to comparable outcomes in investigations involving humans. The majority of preclinical studies' inadequate design, execution, and reporting are primarily to blame for this problem.[21-24] As a result, the National Institute of Neurological Diseases demands that these studies be reported with more rigor in order to increase public understanding of appropriate preclinical study design and conduct, as well as appropriate study result interpretation.[23]

Preclinical pharmacology and toxicology play a crucial role in the translation of laboratory and clinical data, and they are fundamental components of the drug discovery and development process. A candidate molecule, or New Chemical Entity (NCE), is put through a series of iterative processes in the complicated drug development process with the goal of optimizing its pharmacological and drug-like qualities while reducing the likelihood of toxicities and side effects. Compound discovery, lead optimization, pharmaceutical profiling, the use of animal models to predict safety and

efficacy, and toxicological evaluation as they relate to the regulatory criteria for Phase I trial commencement are some of the key ideas covered in this document. Additionally, commentary is given on the present difficulties with translational medicine and how these relate to the successful advancement of candidates.[25]

Preclinical toxicity studies assess the recently screened chemicals in at least two distinct animal species to ascertain their safety. Preclinical toxicity studies can be divided into four categories: acute, subacute, sub-chronic, and chronic toxicity studies, depending on the length of the research, its goals, and its time and duration. The toxicity studies may be divided into single-dose (acute toxicity study) and repeated-dose (sub-acute, sub-chronic, and chronic toxicity studies) categories based on the quantity of doses given. Comparably, toxicity studies may be categorized into two groups according to the location in which animals were exposed to interventional drugs: systemic toxicity studies, which include acute, sub-acute, chronic, sub-chronic, and reproductive toxicity studies, and local toxicity studies, which include dermal and ocular toxicity.[26]

IN VITRO TESTING

When evaluating the cells involved and the mediators released during the acute phase of a response, as well as when identifying the medicine responsible for the resolution, in vitro diagnostic tests might be helpful.[27] Based on certain investigations, in vitro stimulation may be necessary as a supplemental diagnostic test.[28-30]

Drug hypersensitivity responses (DHRs) are a serious problem for inpatient and outpatient care alike. The assessment of these individuals is difficult as in vivo studies, particularly drug provocation tests, can be costly, time-consuming, and possibly dangerous due to their inadequate sensitivity. Several in vitro techniques are already available, and they may be broadly categorized into two groups: those that aid in characterizing the reaction's active phase and those that aid in determining the substance that is causing the problem.[27] The sensitivity and specificity of in vitro assays vary based on the medication and clinical phenotype.[31] Notwithstanding the significant adverse effects that are directly connected to immune-mediated hypersensitivity and autoimmune reactions, there are currently no established in vivo or in vitro approaches for evaluating a drug's sensitizing potential during the pre-clinical phase. The effector phase of immediate-type drug allergic responses is the primary focus of available in vitro diagnostics, such as the CAST-ELISA®.[32] This depends on the BASO-Test® and the existence of certain IgE antibodies according to the basophils' activation similar.[33]

Despite the limitation that findings from in vitro experiments may not always perfectly reflect what happens in whole organisms, these methods are still crucial for ADR monitoring. They provide valuable preliminary data on the toxicity of drugs, helping to identify potential risks and inform further investigation.[34] Moreover, in vitro methods reduce the need for test organisms, which aligns with the ethical principle of minimizing animal usage in research. They also offer a faster, more cost-effective, and reliable means of toxicity testing compared to traditional in vivo studies. This expedites the process of ADR monitoring and allows for more efficient evaluation of drug safety.[34] Overall, in vitro toxicity research using tissue or cell culture techniques is an essential component of ADR monitoring. It complements in vivo studies by providing preliminary data on drug toxicity, contributing to the reduction of animal usage, and facilitating faster and more cost- effective toxicity assessment.[34]

IN VITRO TOXICITY TESTING

In the context of ADR monitoring, in vitro toxicity research using tissue or cell culture is valuable for several reasons. By utilizing tissues or cells cultured from organisms, researchers can simulate the effects of drugs on specific parts of the body or biological processes in a controlled environment. This allows for the identification and examination of potential adverse effects at the cellular level before advancing to in vivo studies.[34]

Key instruments for reducing the attrition of new drug candidates as they move through the research and development phase are in vitro toxicity screening techniques. When it comes to enhancing the translation of test data to anticipate unfavourable consequences in humans, recent developments in the creation of physiologically appropriate in vitro model systems are showing promise.[35] Across seven of our locations in North America and Europe, we provide an extensive spectrum of in vitro toxicology tests that cover a broad variety of services along the in vitro toxicity assay continuum, such as[35]:

- Evaluating tests for important biochemical and biomarker toxicity endpoints testing for certain bad outcome pathways using validated model systems in accordance with the OECD Test Guidelines.
- Sophisticated 3D tissue models intended to evaluate the vulnerabilities of certain target organs.
- Assays combined as a component of a comprehensive toxicity evaluation plan
- Unique mechanistic tests created to pinpoint new negative outcome pathways.
- We are pioneers in the creation, validation, and adoption of many animal-free models, and many of our assays serve as direct substitutes for screens or tests conducted on animals.

IN VIVO TESTING

A screening technique for chromosomal abnormalities caused by medications, food additives, and other substances has been evaluated in vivo. It is far easier, faster, and more dependable than the conventional approach.[36] Because of kinetic factors, the in vitro findings correlate better with rodent parenteral (ip or iv) LD50 values than with oral LD50 values. The in vitro results exhibit a strong correlation with LD50 values for specific sets of unrelated chemicals as well as for some groups of related chemicals (such as metal salts and antitumor drugs).[37]

There is general consensus that animal experimentation needs to be reduced, improved, or eliminated if possible. Instead of using animal research for screening, a variety of in vitro models are employed. Traditionally, studies of harmful health effects seen at high doses in suitable animal models (i.e., toxicological endpoints) have been the basis for toxicology testing in order to establish a no-observed-effect level (NOEL) or no-observed-adverse- effect level (NOAEL).[38]

A variety of in vitro cytotoxicity assays have been developed to evaluate the acute toxicity of chemical substances. So yet, there aren't many in vitro methods in use. The first approved in vitro testing methods were for genotoxicity testing, which comprised tests for (e.g., phototoxicity, corrosivity) and bacterial reverse mutation utilizing *Salmonella typhimurium* strains.[38]

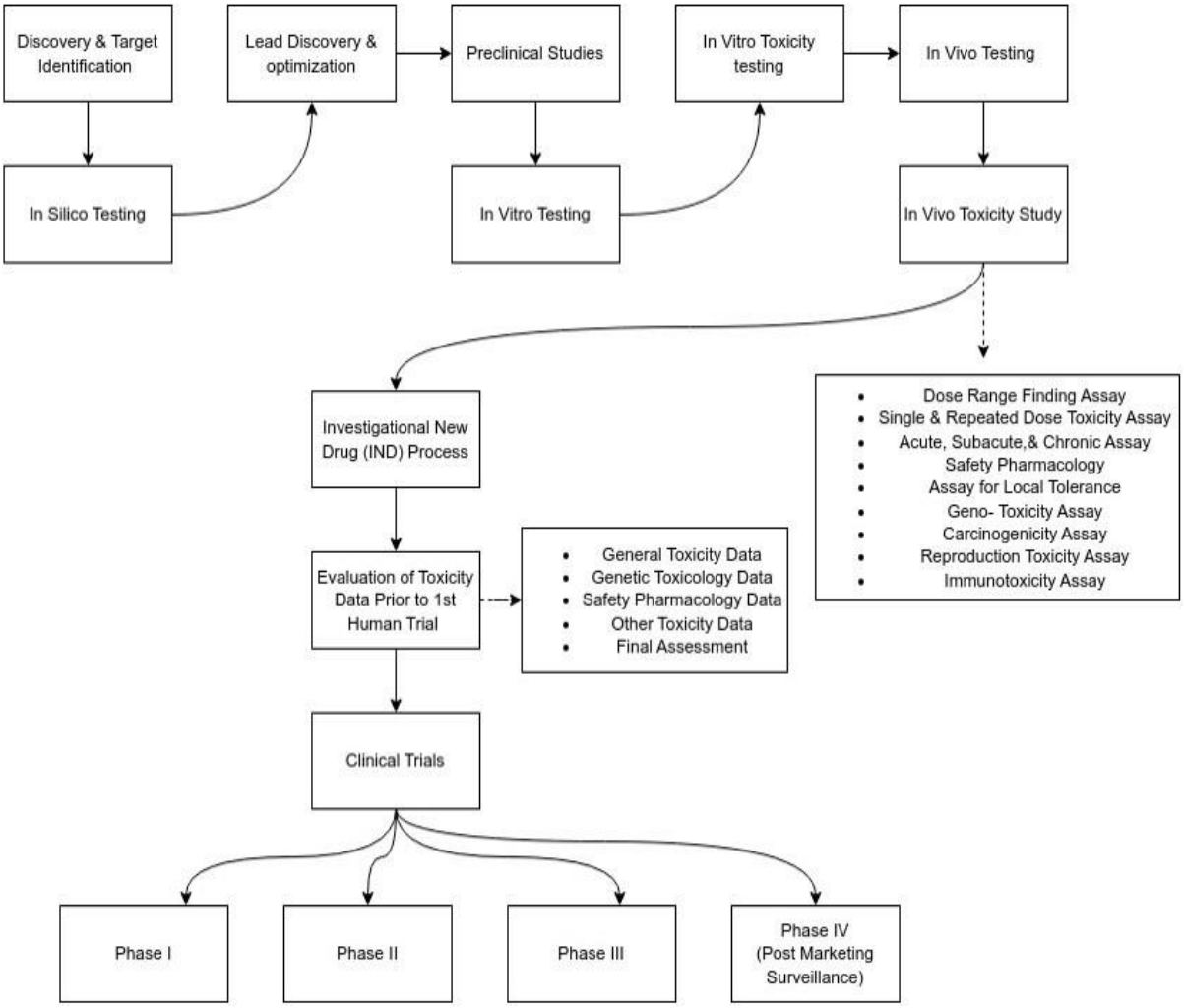

Fig 2: Summary of Drug development process.

IN VIVO TOXICITY STUDY

Utilizing animal models to assess toxicity and potential adverse effects of experimental drugs. In vivo toxicology is the study of the potentially harmful effects of chemicals on a live creature, such as a lab animal. Numerous in vivo toxicity tests are carried out, including the assay for determining the dosage range, the assays for single and repeated doses, the acute, sub chronic, and chronic phases of toxicity, the assays for local tolerance, the genotoxicity, the carcinogenicity, and the reproductive toxicity.[38] The need for reliable in vitro assays is highlighted by the generally low sensitivity of standard in vivo toxicity tests to immunotoxicities, inter-species variability in the structure and function of the immune system, the high cost and relatively low throughput of in vivo tests, and ethical concerns regarding animal use.[39]

It is common knowledge that toxicity results from administering something in excess. Pharmacodynamics and pharmacokinetics are aspects of pharmacotoxicology. It focuses specifically with how a medicine affects the body and how the body reacts to it as an antigen. A variety of factors need to be taken into account while researching a drug's toxicity. The primary determinants of toxicity are the dosage and mode of drug delivery.[38]

Fig 3: Schematic illustration of toxicity data required in in-vivo study.

Toxicology research is crucial to the creation and approval of medications. Pre-clinical toxicology research is primarily concerned with evaluating the safety of medications. The ability of a treatment to be both safe and effective is critical to its development. One of the main justifications for pausing medication development at any stage of the procedure is toxicology (safety). Anticipating human safety, examining the relationship between drug exposure and toxicity in animal models, elucidating potentially hazardous target organs and toxic reactions, and providing reliable toxicokinetic (TK) data for future (human) clinical drug trials are among its objectives. The main specific features of the research include tests for carcinogenic toxicity, genetic toxicity, reproductive toxicity, chronic or sub chronic toxicity, acute toxicity, etc.[40] Pre-clinical toxicity tests from Creative Bio- array cover a range of dosing scenarios, from single-dose to multiple-dose, acute to chronic. Additionally, we provide a variety of medication delivery methods, including topical, intramuscular, intraperitoneal, intravenous, and oral. Additionally, a wide variety of animal models are available, such as those of non-human primates, rodents, rabbits, and miniature pigs. Lastly, the complete range of toxicological services offered by Creative Bio-array is accessible, encompassing chemistry, pathology, histopathology, haematology, ophthalmology, urinalysis, bioanalysis, TK analysis, and statistics analysis.[40]

The in-vivo toxicological investigation uses a number of crucial tests. One of these is a dose-range discovery test, which evaluates the toxicity of different dosages over a three to seven-day period. The assay measures several parameters, including the therapeutic index (TI), minimum toxic dose, maximum tolerated dose (MTD), median lethal dose (LD50), toxic dose (TD), minimal toxic dose (MTD), and no-observable-adverse-effect level (NOAEL). Subacute and sub-chronic repeat-dose toxicity experiments, which last between 14 and 30 days and more than 30 days, respectively, come next. Dose escalation studies and investigational new drug (IND) enabling studies are also available. Toxicokinetic (TK) evaluations are carried out last.[40]

Dose range finding assay

The Dose-Range Finding Assay from Creative Bio-array offers quick and precise toxicological and TK analysis findings for each and every novel therapeutic candidate. In this instance, a medication is given at various research dosages and is delivered as such in order to thoroughly evaluate the therapeutic index and the highest tolerated or viable dose.[40]

Single and Repeat-Dose Toxicity Assay

To establish the dosage range for toxicological and TK analysis, single-dose dose range discovery investigations are often carried out prior to multiple-dose toxicology studies. For repeated dosage toxicity studies, the length of the chronic dose is determined by the size, duration, and therapeutic indications. Two distinct animals must participate in the procedure, and one of them must not be a rodent.[40]

Acute, Sub-chronic and Chronic Assay

The maximum tolerated dosage level for a single dose is determined by acute dose toxicity, which encompasses all the harmful health consequences brought on by a single dose exposure.[40] Determining the levels to test for sub-chronic toxicity is the goal of sub-acute toxicity research. Treatment with the chemical under examination for an extended length of time permits the recording of hazardous effects, which become apparent after a latent period. The majority of the time, only one biological species is researched (dogs or rats). Ten of them are placed in groups, and each of the three doses is examined.[41]

All harmful health consequences brought on by means of repeated dosing at reduced levels over a longer period of time are referred to as sub-chronic dosage toxicity.[40] Adverse pharmacological responses resulting from extended exposure to particular toxins or stresses are referred to as chronic drug toxicity. It is typically taken into account in conjunction with the direct evaluation of lethality. On the other hand, it also discussed several sub-lethal aspects, such behavioural shifts, slower development, and decreased reproduction.[40]

Safety pharmacology

In order to support drug research and investigate the results of medications on the central nervous system, respiratory system, and cardiovascular system, Creative Bi-array offers animal safety pharmacological experiment services.[40] When a medicine is inside or above the therapeutic range, safety pharmacology mainly monitors any negative effects on the central nervous system, cardiovascular system, and respiratory system. If necessary, more safety pharmacology research may be conducted, including observations of the autonomic nervous system, digestive system, urinary system, and other organs and tissues. Finding unanticipated pharmacological effects connected to clinical safety, assessing adverse responses or pathological consequences shown in toxicological testing or clinical trials, and investigating the mechanism of adverse reactions are the goals and significance of safety pharmacology research.[40]

Assay for local tolerance

Studies on local tolerance are done to evaluate possible side effects at the administration site. In order to ascertain whether there is any irritation or other negative effects at the injection site, they are often given parenterally. The route of administration should not change from clinical to undertake local tolerance tests on animal species undergoing pre-clinical evaluation. An evaluation of local tolerance may be included in further toxicity research.[40]

Genotoxicity Assay

Screening procedures that incorporate mode of action information are becoming more and more beneficial for the biological evaluation of possible human dangers, as DNA-reactive chemicals have the ability to initiate carcinogenic processes. Then, in the context of adverse outcome pathways, actionable mechanisms (i.e., physiologically crucial events) may be assessed to identify potential molecular initiating events that result in a positive test result. When appropriate, we also include in vivo genetic toxicity research, even though the majority of our work is done in vitro.[40]

Carcinogenicity Assay

The potential for long-term exposure to IND to cause cancer is evaluated through research on carcinogenicity. Unless there is a reason for worry, completed carcinogenicity investigations are often not required prior to clinical trials. Based on the client's project and the ICH document, our knowledgeable specialists will assess if carcinogenicity studies are necessary. They will do this by looking at the intended animal usage, experimental procedures, and medication doses.[40]

Reproduction Toxicity Assay

For the purpose of conducting safety evaluations in accordance with globally accepted norms, Creative Bio array offers comprehensive planning for reproductive toxicology programs as well as bespoke study designs (including ICH and OECD). To assess novel products over the whole reproductive spectrum, we carry out research on male and female fertility, developmental toxicity (embryo-foetal development), prenatal and postnatal development, and multigenerational studies.[40]

Immunotoxicity Assay

Potential immunogenicity assessment is one facet of immunotoxicology examination. Numerous medications developed using biotechnology aim to either activate or inhibit the immune system. As such, they might impact both humoral immunity and cell-mediated immunity. Irritation may be the cause of the inflammatory reaction at the injection site. It is crucial to understand, nonetheless, that straightforward injection trauma and particular toxic effects brought on by the formulation carrier might potentially result in toxic alterations at the injection site.[40]

INVESTIGATIONAL NEW DRUG (IND) PROCESS

A medication is tested in people for the first time following preclinical testing in a study known as a first-in-human (FIH) experiment. These trials aid in identifying the safe dosage range for additional clinical development. Researchers must show that the substance is adequately safe for initial human use before starting FIH studies.[42] First-in-human trials, sometimes referred to as phase 1 studies, are investigations that come after regulatory submission in the nations where testing will take place. This form is known as the Investigational New Drug (IND) application in the United States.[42]

For the numerous smaller organizations that conduct a large portion of today's cutting edge biopharmaceutical research, the exploratory IND offers substantial advantages. A small business or academic researcher can save limited funds by using it to test possible chemicals for a fraction of the price of the conventional IND method. It provides a way to compare experimental molecules more quickly, making it possible to determine which one is the most promising for further research and development. It enables proof of concept demonstrations more quickly, which is crucial for businesses seeking investors to assist them advance their technologies to clinical development. Lastly, the exploratory IND lowers the entrance barrier into the market for smaller research-based businesses, which ought to result in the

development of more novel treatments.[43]

Research conducted under an exploratory IND can take many forms, ranging from repeat- dose studies employing pharmacologic exposures to single, sub pharmacologic doses (micro dose studies). A micro dose research evaluates compounds at sub pharmacologic doses, which are less than 1/100th of the dose required to produce a pharmacologic effect according to animal testing. When done with extreme sensitivity, micro dose exploratory IND studies can provide useful information about a drug candidate, such as (a) the pharmacokinetic characteristics of the drug.[44]

A thorough IND application is contingent upon the type of drug, therapeutic indication, and the whole clinical strategy. Toxicology investigations with supporting DMPK and bioanalytical data, safety pharmacology studies, and genetic toxicology studies are commonly included in IND-enabling testing. Studies on abuse liability, immunotoxicity, and phototoxicity are added as needed.[42]

Preclinical testing aims to prove that a chemical is pharmacologically active and fairly safe, which validates commercial development. Toxicology data in particular will support [42]:

1. Determine the toxicity to organs
2. Examine the connection to drug exposure
3. Assess the consequences that are both on- and off-target.
4. Determine the significance for humans.
5. Create safety indicators that the clinic can keep an eye on.

EVALUATION OF TOXICITY DATA PRIOR TO FIRST-IN-HUMAN TRIALS

First-in-human (FIH) studies are necessary for drug development in order to provide preliminary data on tolerability, pharmacokinetics/dynamics, and fundamental aspects of drug safety.[45] Investigators, local review committees, the appropriate national drug regulatory authority (DRA), and ethical committees rigorously examine each of these study protocols. The UK-conducted TeGenero study in 2006[46], led to significant modifications in the design[47], first in the UK with the Duff committee report of 2006[48] and later in 2007 with updated EU guidelines.[49]

Preclinical toxicity studies' applicability to humans is reviewed as part of the risk assessment of FIH investigations conducted in Europe.[49] Research with nonhuman primates (NHPs) may be more relevant than studies involving rats and dogs, and if no untoward incidents are reported, this may be comforting. The risk evaluation also takes into account whether substances with a comparable structure and/or mechanism have ever been given to humans.[50] The types of toxicity data that you will want before you may plan and carry out FIH studies are summarized below for your IND submission.[42]

General Toxicology Data

The idea of a maximum tolerated dose (MTD), which is the point at which an organism's compromised state prevents a particular toxicity or activity from being linked to a test drug, was developed by mammalian toxicologists.[51] Single dosage toxicity tests to identify secondary pharmaceutical effects and harmful consequences. These investigations serve as yet another crucial bridge to your pivotal GLP research, aiding in the definition, characterization, and determination of the MTD as well as the intrinsic toxicity of the tested chemical.[42] In repeat dosage trials, the maximum dose level needs to be restricted to a realistic value, such as one that results in noticeable but mild toxicity (for instance, current

guidelines frequently suggest consequences like a 10% decrease in body weight increase). In repeat dose experiments, choosing the high dosage with the intention of producing overt/significant systemic toxicity (i.e., pain, anguish, suffering) or fatality has no scientific basis or usefulness.[52]

Genetic Toxicology Data

Studies on genetic toxicology assess a drug's capacity to induce chromosomal damage or mutations in order to forecast its carcinogenic potential.[42] Over the past two decades, molecular biology has made unprecedented strides that have led to a vast database of genetic sequence information, a remarkable array of effective new technologies for monitoring genetic sequences, genetic variation, and global functional gene expression, as well as a dramatic increase in our understanding of gene structure and function. Because of these developments, toxicology now has a distinct subdiscipline called "toxicogenomics." Toxogenomics is defined as the "study of the relationship between the adverse biological effects of exogenous agents and the structure and activity of the genome (the cellular complement of genes).[53]"

Safety Pharmacology Data

Regulatory agencies that approve the use of drugs in people include the US Food and Drug Administration (FDA), Health Canada, the European Medicines Agency (EMEA), and the Japanese Pharmaceutical Manufacturers Association (JPMA). Therefore, a crucial step in the drug discovery and development process is persuading the authorities that a medication is both safe and effective.[54] Determining a drug's possible adverse pharmacodynamic effects on the central nervous, cardiovascular, and respiratory systems as well as doing further testing to assess other organ systems comprise the framework of a Safety Pharmacology "core battery" program.[55]

Other Toxicity Data

Published in 2006, the ICH S8 immunotoxicity testing guideline for human pharmaceuticals was designed to offer direction for evaluating the immunotoxicity potential of low-molecular-weight medications that do not aim to modify the immune system. Immunotoxicity testing procedures are often designed on a case-by-case basis for medications that aim to modify the immune system, since the sort of testing required will depend on the targets, targeted patient group, and mechanisms of action of the test chemical.[56] The "S10 Photosafety Evaluation of Pharmaceuticals" advice is now available for business use, according to the Food and Drug Administration (FDA). The advice aims to standardize photosafety assessments in order to assist human clinical studies and pharmaceutical marketing authorization. It also recommends worldwide standards for photosafety evaluation.[57] A drug's or a class of drugs' abuse liability is its tendency to be abused and result in negative public health effects. While a drug's pharmacological qualities—that is, its capacity to create psychoactive effects linked to a risk of abuse and/or addiction, often referred to as abuse potential - account for a large portion of what defines a drug's abuse liability. The abuse liability of individual products can vary, even if they contain the same generic medication. This can be attributed to factors including the product's precise composition, intended purpose, and market penetration.[58]

Final Assessment

Upon completion of all these toxicity tests and data collection and analysis, the research teams will have solid scientific justification to decide if the novel medication is safe enough to proceed to Phase I clinical trials. Furthermore, in the event that it proceeds, all of the patients included in those studies will benefit from the continued use of the toxicity data for the duration of the human clinical trials.[59]

CLINICAL TRIALS

Every country has laws requiring pharmaceutical corporations to conduct clinical trials, which test new medications on humans before they are released into the general public. Typically, a control group and a representative sample of the few thousand patients for whom the medicine is intended are chosen by the makers or their representatives. A placebo or an additional medication that is currently on the market for the conditions may be given to the control group. In general, clinical trials provide valuable insights about a drug's effectiveness and possible side effects. Clinical trials, also called clinical studies, are intended to assist in the process of determining the safe and effective administration of novel treatments to humans. An structured research study aimed at enhancing a patient's quality of life by exploring novel approaches to illness or disease prevention, detection, diagnosis, or treatment is known as a clinical trial.[60] Four Phases of clinical Trials have been discussed subsequently.

Phase 1

Finding the agent's ideal dosage and toxicity profile for additional research are the primary goals of phase 1 trials.[61] The latter is made more difficult by the often weak anti-tumor activity seen in individuals receiving treatment in early phase studies who had very advanced disease stages.[61] Therefore, it is standard procedure to establish the appropriate dosage using the maximum tolerated dose (MTD).[61] Dose-limiting toxicity (DLT), or toxicity-severity that restricts the ability to treat a patient at the prescribed dose, is what determines the MTD itself. Any grade 3–4 non-haematological or grade 4 haematological toxicity that occurs during the first cycle of therapy and is at least potentially attributable to the treatment is considered DLT.[61] Certain modifications to this criteria, such febrile neutropenia, neutropenia grade 4 lasting longer than seven days, or aberrant laboratory findings classified as a DLT only when clinical symptoms are present, have gained widespread acceptance.[61]

However, because to the unique toxicity profiles of molecularly targeted therapies (MTA), the traditional definition of DLT for cytotoxic drugs presents questions for phase 1 studies.[62] Usually phase 1 trial prefer to conducts in healthy volunteers but in few cases where the disease is incurable or severe like AIDS and cancer are experimented in severely ill patients or patients have lower chances of recovering from the previous diseases. These trials are conducted with respect to special arrangement in pharmaceutical companies and these types of trails are required special contract from the responsible organization. Phase 1 trails prime intention is to study the tolerability and safety of a particular drug of decided dosage although with time they will increase. The volunteers are observed and watched overnight. 60-100 volunteers are considered for phase 1 trial. The anxiety is normal in volunteers it may be ignored but the consideration towards their safety should be accurate by following the guideline. As the women go through more hormonal changes they may not be considered as volunteers. The volunteers should take rest until the experiment completed on him. Finally, the aim safety and tolerability will be studied.[63]

A phase I trial is intended to ascertain the MTD for a particular dosage regimen, specify the human toxicity profile, pinpoint dose-limiting toxicities, and investigate the pharmacokinetics of a medication. Patients who have experienced a recurrence and have used all conventional and approved therapy are eligible to enroll in phase I investigations. The dose is typically increased in subsequent cohorts of three to six patients once a modest number of patients (15–30) are enrolled, until a dose-limiting hazard is reliably noted.[64]

Phase 2

Phase II studies are conducted on bigger groups (20–300) and are intended to evaluate the medicine's efficacy in addition to carrying out phase I safety evaluations in a larger number of patients and volunteers. Phase I trials validate the first safety of a test drug. Phase II trials are often when a new drug's development process falters. Certain studies evaluate both toxicity and effectiveness, combining phase I and phase II testing. A medication's safety and effectiveness in a particular patient population are shown in certain phase II trials, which are structured like case series. A placebo or standard medication is given to certain participants in other phase II trials, which are structured as randomized clinical trials. Phase II studies are typically single blind, meaning that while the patient is blind, the doctor and medical personnel are aware of whether the medicine or placebo is being given.[65] The priority of this phase is to study the efficacy of a drug substance but not aimed to get information about risk profile of drug. Placebo are preferred for studying the efficacy by comparing with the active drug substance with the response and body language of volunteers.[63]

Phase 3

Phase III studies are for novel chemotherapeutic drugs that have demonstrated efficacy in phase I and II trials. Phase III trials are randomized studies that compare novel drugs (standard therapy plus new agent versus standard therapy alone) in individuals who have not received prior treatment. When creating treatment plans, it's also important to take into account the drug's mode of action, pharmacokinetics, toxicities, possible interactions with other medications, and mechanisms of drug resistance.[64] Efficacy and safety are the prime aim of phase 3 to achieve by conducting the trials. Mostly, this phase conducted in hospitals, where all the patients which are admitted due to certain diseases and linked with the treatment of particular test compound considered as volunteers. The randomized technique applied here, where the standard drug and test compound given to the patient without the knowledge of the patient. The volunteer's number may vary from 300-3000 in these trials with respect to need.[63]

Phase 4: Post-market surveillance

Phase IV of drug evaluation, which focuses on studying medications in real-world usage, is essential because information gathered from earlier phases (I through III) is insufficient for making final conclusions about a drug's clinical value post-marketing.[66] Differences in patient characteristics, treatment settings, and indications between routine use and controlled scientific studies can alter the nature and impact of adverse drug reactions (ADRs).[67] While Phase III primarily addresses rare but serious ADRs, Phase IV encompasses broader questions. Unlike Phase III, which typically employs controlled, randomized, double-blind studies, Phase IV requires diverse study designs tailored to specific questions.[68] Established methodologies for Phase IV studies include spontaneous reports, stimulated spontaneous reports, comprehensive observation studies, Phase IV intervention studies, case-control studies, prescription event record linkage, and data bank comparisons.[69]

ADR Monitoring

The World Health Organization (WHO) defines an ADR as any unpleasant, unintentional, or undesirable side effect of a medication that happens at dosages used in humans for prevention, diagnosis, or treatment.[2] It is widely acknowledged that ADRs are an important reason for hospital admissions and a substantial financial burden on healthcare facilities. Hospital-based ADR monitoring and reporting initiatives seek to determine and measure the hazards connected to using medications given in a medical facility.[70] Any new medication must have its safety profile confirmed by a clinical

study and safety database before being released into the market. Increasing patient awareness of ADRs is imperative, as the problem is widely acknowledged in both developed and developing nations.[71]

Among the main causes of morbidity and death in the medical field are ADRs. According to a 2000 Institute of Medicine research, medical mistakes cause 44,000–98,000 fatalities in the United States each year. Out of this, an estimated 7000 fatalities are attributed to ADRs. Based on a forty-year analysis of 39 research on the American pharmaceutical system, 106,000 deaths were attributed to ADRs in 1994 and over 2 million experienced severe adverse effects.[72] Monitoring ADRs in the emergent drug development process helps make sure new medicines are safe.[71]

An ADR refers to any harmful or unintended response to a medication, occurring at normal doses during normal use. These reactions can range from mild, such as nausea or headache, to severe, including allergic reactions or organ damage. ADRs may result from various factors, such as individual patient characteristics, drug interactions, or dosage errors. Identifying and reporting ADRs is crucial for ensuring medication safety and optimizing patient care.[73]

ADR monitoring is integral to every stage of the drug development process. Its systematic implementation enhances patient safety, supports evidence-based decision-making, and fosters trust in the pharmaceutical industry. By prioritizing ADR monitoring, stakeholders can mitigate risks, optimize drug therapies, and ultimately improve healthcare outcomes for individuals worldwide. ADRs have a substantial cost to healthcare, society, and the economy since they can put a patient's life in danger, force them to discontinue taking a beneficial medication, need further medical interventions, increase the need for healthcare services, and demand lengthy hospital stays.[74] So, the foundation of post market monitoring is the identification and reporting of potential adverse drug reactions in clinical practice.[75] Information on side effects that occur after a medicine is marketed can be found in databases of medical records or electronic claims, as well as information gathered from prospective post marketing studies.[76]

CONCLUSION

Starting from *in silico* testing to the bed side usage, the discovery and development of New Drugs is indeed a challenging process. It requires meticulous efforts and huge research expense. The success rate of finding new molecules are also very less. Hence for drugs which land in the market, longeivity is always an essence. Apart from therapeutic efficacy, patient safety is the ultimate goal of any medication usage. Rigorous monitoring of drug use is therefore essential that comes through vigilant ADR monitoring and reporting undertaken by pharmacovigilance programs.

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