Preclinical Trials

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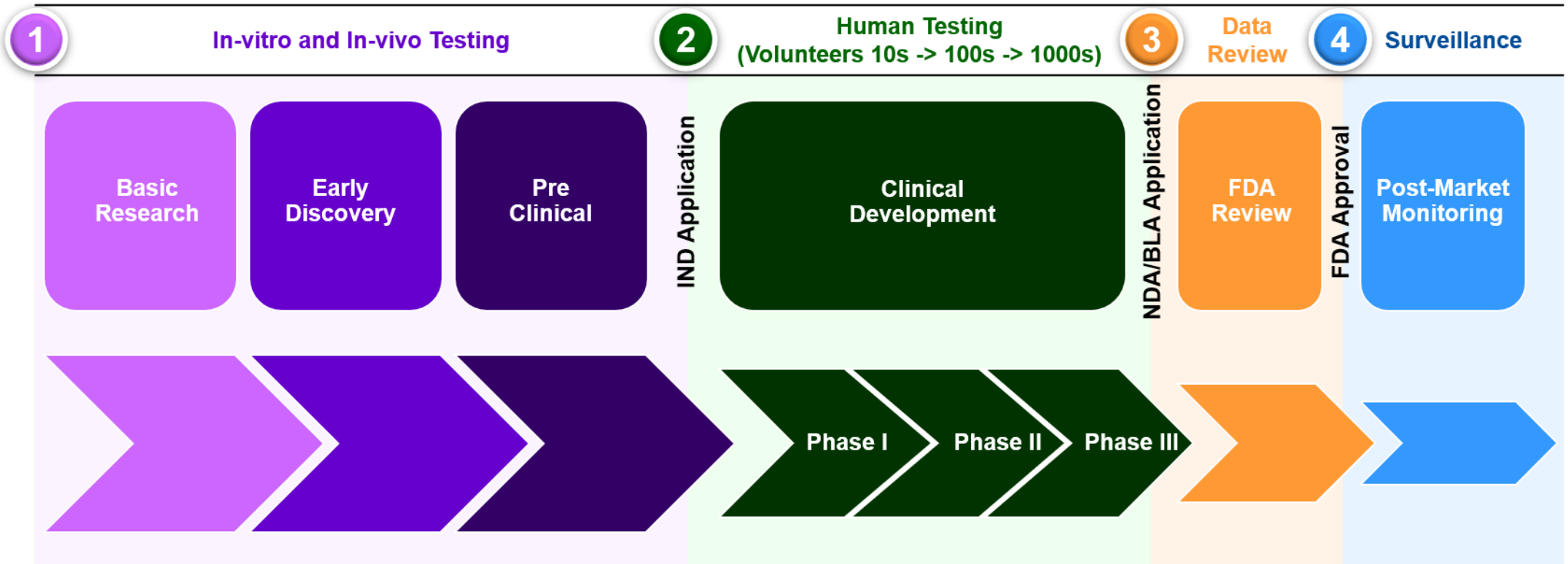
1
In-vitro and In-vivo Testing
2
Human Testing
(Volunteers 10s -> 100s -> 1000s)
3
Data
Review
4
Surveillance
Basic
Research
Early
Discovery
Pre
Clinical
IND Application
Clinical
Development
NDA/BLA Application
FDA
Review
FDA Approval
Post-Market
Monitoring
Phase I
Phase II
Phase III


1. Discovery and Development
2. Preclinical Research
3. Clinical Development
4. FDA Review
5. Post-market Monitoring
DRUG DEVELOPMENT

Drug development process

process can be divided into three main phase

- **1.Drug discovery phase**:- During which the candidate molecules are chosen on the basis of their pharmacological properties
- **2.Preclinical phase** :- During which a wide range of animals studies are performed examples ..pharmacokinetic, pharmacodynamics, toxicity studies.
- **3.Clinical trial phase** :- During which the lead compound is evaluated for efficacy, safety and adverse effects in the human volunteers and patients

- **Preclinical trials**,

also known as non-clinical trials are the laboratory tests of a new drug, device or medical treatment on animal subjects.

- The main aim of preclinical studies is to see whether the drug or the treatment really works and whether it is safe to test on humans.
- Thus, the main goal of a preclinical research is to collect sufficient data and establish the safety profile of the drug or the treatment under question.
- And to fulfill this objective, various types of studies are carried out in a preclinical trial.

Preclinical trials

- Preclinical studies are conducted to define pharmacological and toxicological effects not only prior to initiation of human studies but throughout clinical development.
- Both in vitro and in vivo studies can contribute to this characterization.
- Experiments are generally performed on rodent like mouse, rat, guinea pig, hamster, rabbit.

Preclinical Study CRO: From Proof-of-Concept (POC) to IND Submission

- Preclinical research services involve several studies critical to asses drug efficacy and safety in animal models and complete Investigational New Drug (IND) filing studies.
- Successful completion of the drug IND package is the first milestone towards clinical development.

Preclinical Study CRO: From Proof-of-Concept (POC) to IND Submission

- Many drugs are rejected during the preclinical stage due to subpar treatment effects or adverse outcomes including toxicity.
- If these studies are not appropriately designed, your medication can be dismissed by the regulatory authorities in error.
- Hence, it is imperative to look for a preclinical CRO with competent personnel who have relevant scientific expertise as well as regulatory awareness.

Preclinical Study CRO: From Proof-of-Concept (POC) to IND Submission

- Before a drug could be released in the market, a set of studies are conducted to predict its safety and efficacy in humans.
- These clinical studies are often conducted following intensive animal studies under the supervision of pharmaceutical scientists and experts.
- This animal testing must follow the ethics and regulations set for animal use in preclinical research.
- First in man study is designed based on the preclinical studies including first safe dose in phase I study

In Vivo, In Vitro & Ex Vivo Assays

- These three types of studies are conducted on the whole, living organisms or cells, including animals and humans; or using non-living organisms or tissue extract.
- In vivo, preclinical research examples are the development of new drugs using mice, rat, and dog models.
- In vitro is research conducted in a laboratory.

Ex vivo

- ***Ex vivo*** (Latin: "out of the living") means that which takes place outside an organism. In science, *ex vivo* refers to experimentation or measurements done in or on tissue from an organism in an external environment with minimal alteration of natural conditions.
- *Ex vivo* conditions allow experimentation on an organism's cells or tissues under more controlled conditions than is possible in *in vivo* experiments (in the intact organism), at the expense of altering the "natural" environment.

Ex vivo

- A primary advantage of using *ex vivo* tissues is the ability to perform tests or measurements that would otherwise not be possible or ethical in living subjects. Tissues may be removed in many ways, including in part, as whole organs, or as larger organ systems.
- Examples of ex vivo research assays are finding effective cancer treatment agents; measurements of tissue properties (physical, thermal, electrical, and optical); and realistic modeling for new surgical procedures.
- In an ex vivo assay, a cell is always used as the basis for small explant cultures that provide a dynamic, controlled, and sterile environment.

In Silico Assays

- In silico assays are test systems or biological experiments performed on a computer or via computer simulation.
- These are expected to become increasingly popular with the ongoing improvements in computational power, and behavioral understanding of molecular dynamics and cell biology.

Drug Delivery

- New drug delivery methods include oral, topical, membrane, intravenous, and inhalation.
- Drug delivery systems are used for targeted delivery or controlled release of new drugs. Physiological barriers in animal or human bodies may prevent drugs from reaching the targeted area or releasing when they should.
- The goal is to prevent the drug from interacting with healthy tissues while still being effective.

Drug Delivery

- Oral: Oral delivery of medications is reliable, cost-effective, and convenient for patients.
- Oral drug delivery may not monitor precise dosages to the desired area but is ideal for prophylactic vaccinations and nutritional regimens.
- Delayed action, stomach enzyme destruction, absorption inconsistencies, or patients with gastrointestinal issues or upset can occur, and patients must be conscious during administration.

Drug Delivery

- Topical: Topical drug delivery involves ointments, creams, lotions, or transdermal patches that deliver a drug by absorption into the body.
- Topical delivery is more useful for patient skin or muscular conditions — it is preferred by patients due to non-invasive delivery and their ability to self-administer the medicine.

Drug Delivery

- Parenteral (IM, SC or LP Membrane): Parenteral drug delivery utilizes bodily membranes, including intramuscular (IM), intraperitoneal (IP), or subcutaneous or (SC).
- It is often used for unconscious patients and avoids epithelial barriers that are difficult for drugs to cross.
- Parenteral (Intravenous): Intravenous injection is one of the fastest drug delivery absorption methods. IV injection ensures entire doses of drugs enter the bloodstream, and it is more effective than IM, SC, or LP membrane methods.

Drug Delivery

- Parenteral **(Inhalation)**: Inhalation drug delivery gets the drug rapidly absorbed into the mucosal lungs, nasal passages, throat, or mouth.
- Problems with inhalation delivery include difficulty delivering the optimum dosage due to small mucosal surface areas and patient discomfort. Pulmonary inhalation drug delivery uses fine drug powders or macromolecular drug solutions.
- Lung fluids resemble blood, so they can absorb small particles easily and deliver them into the bloodstream.

Formulation Optimization & Improving Bioavailability

- Formulation optimization is ongoing throughout pre-clinical and clinical stages. It ensures drugs are delivered to the proper place at the right time and in the right concentration.
- Optimization may include overcoming solubility or permeability issues or creating modified-release medicines to protect patients from adverse events. Bioavailability ensures consistent drug delivery without loss of dosage requirements or drug waste due to insufficient absorption.

Dose Range Finding

- Dose range finding uses trials of different drug doses for safety and efficacy.
- Placebo groups and groups that receive different drug doses (low, medium, or high) are tested using gathered maximum tolerable dose (MTD) information

Steps in Doing a Pre-Clinical Trial

Step One:

Get an idea for a drug target

- Drugs usually act on either cellular or genetic chemicals in the body, known as targets, which are believed to be associated with disease. Scientists use a variety of techniques to identify and isolate individual targets to learn more about their functions and how they influence disease.
- Compounds are then identified that have various interactions with the drug targets that might be helpful in treatment of a specific disease

Finding the Right Target Is Not Easy Parkinson’s Disease Example

Parkinson's disease: a disease which causes deterioration of the central nervous system over a period of time. This disease often impairs the patient’s movement, speech, and other functions.

How is Parkinson's treated? Where should the focus be?

- Tremors or shaking occurs when cells in one part of brain die. These cells communicate using a chemical called dopamine.
- Drugs that replace dopamine work only for a few years.
- Other Parkinson's symptoms (depression, sleep disorder, digestive problems, loss of brain function) have other causes.
- Another sign of Parkinson's disease: many cells have deposits of a protein, synuclein.
- Four drug companies are developing drugs to counter synuclein, even though nobody knows if it is a cause or a consequence of Parkinson's.
- Synuclein could be like a tombstone—a marker, not a cause of cell death.

Steps in Doing a Pre-Clinical Trial

Step Two:

Develop a Bioassay A Bioassay is a “live” system that can be used to measure drug effect

- It may be a culture of cells or organs or a whole animal. For example:
- Zebra-fish embryos - you can see effects of drugs on bone density, blood vessel growth and many other systems of the zebra-fish.

Steps in Doing a Pre-Clinical Trial

- **Step Three**:

Screen the drug in the Bioassay

- This is the actual test of the drug on the chosen bioassay. This will determine if the drug is SAFE and if it is EFFECTIVE in the bioassay (BEFORE it is ever tested on humans!)

Steps in Doing a Pre-Clinical Trial

Step Four

- Establish what dosage amount of the drug is safe and what dosage amount of the drug is toxic
- Most drugs have a toxic level or an amount at which the drug will become harmful instead of helpful.

Step Five:

- Application is made to the Food and Drug Administration (FDA) as an Investigational New Drug (IND)
- IND must show how the drug:
- Is manufactured.
- Appears (color, solubility, melting point, particle size, moisture content).
- Formulated (pills, liquid, etc. + inactive ingredients).
- Will be analyzed for purity, concentration, stability.
- Will be tested for safety (this will be the basis for allowing first use in humans).

THE TYPES OF STUDIES INCLUDED IN PRECLINICAL TRIALS

- 1.Screening Test
- 2. Tests on isolated organs, bacterial cultures
- 3. Tests on animal models of human disease
- 4. General observational test
- 5. Confirmatory tests and analogous activities
- 6. Mechanism of action
- 7. Systemic pharmacology
- 8. Quantitative test
- 9. Pharmacokinetics
- 10. Toxicity test

1.Screening test

- It’s a simple and rapidly performed initial screening test to determine the presence or absence of a particular pharmacodynamic activity in the new drug.
- E.g. determination of analgesic o r pain relieving activity in the new drug.

2.Tests on isolated organs and bacterial cultures

- These are few preliminary tests to determine specific activity in the new drug like anti-histaminic, anti-bacterial, anti-secretory, vasodilatation etc.
- Healthy organs isolated from dead animals or bacteria l cultures are used for these preliminary tests.

3.Tests on animal models

- Animal models like rat, pig, mouse, hamster, and rabbit are used to determine the actual effects of the drug in a live organism.
- After successful results in initial stages, higher animals like cat s, dogs, and monkeys are used f or preclinical trials

4.General observational test

- The drug under the trial is injected in tripling doses to a small group of mice which are then observed for any hidden effects.

5.Confirmatory tests and analogous activities

- Compounds which yield a desirable result are carried for ward in the trial for more complex tests.
- Other activities like antipyretic and anti-inflammatory are further determined for an elaborate examination of the drug properties.

6.Mechanism of action

- Experiments are conducted to determine the mechanism of the action of the drug.
- E.g. if the drug is an anti-hypertensive drug, whether it is an alpha or beta blocker, ACE inhibitor or calcium channel blocker

Pharmacodynamics studies

- In search for an antihypertensive activity of lead compound , the study can be undertaken on dogs, cats, rats, to find out systolic – diastolic blood pressure change and other cardiac effects like ECG changes, inotropic- chronoscopic effect, cardiac output and total peripheral resistance.
- Once the lead compound exhibits promising results then the studies can be further made at cellular level.

Pharmacodynamics studies

- Effect on vascular and other muscles can be evaluated in vitro on isolated arteries/vein ,heart or ileum of rat or quinea pig
- An evidence for its receptor activity can be gathered in vitro on cultured cells.
- Depending on the results, the studies can be further extended to molecular level to find out receptor affinity and selectively by performing in vitro receptor binding studies on cell membrane fractional from organs or culture cells.
- The graded response assay are then performed to find out ED50 of the drugs.

7.Systemic pharmacology

- Besides determination of the action of the drug, its effects on individual and major organ systems like nervous, cardiovascular, respiratory, and renal are also examined.
- This can give a clue about any possible side-effects of the drug on any major organ system

8.Quantitative test

- It includes examination of the dose-response relationship, maximal effects, and comparative efficacy of the new drug with the existing drug, thus establishing the market value of the drug.

9.Pharmacokinetics

- It involves the study of the movement of the drug substance in the body of the living organism which includes the processes of absorption, distribution, metabolism, localization in tissues, and excretion from the body.
- They help to know the safe dose and preferred route of administration for the drug

PHARMACOKINETIC STUDIES

- After performing toxicological studies , the promising test compound is subjected to pharmacokinetic studies in several species of animals like rats, dogs, and sometimes monkeys .
- Beside studying its absorption, distribution, metabolism and excretion, these studies also establish its relative bioavailability after its oral or parental administration
- Its elimination half life (t1/2) is also estimated from the pharmacokinetic data.

10.Toxicity test

- Both short-term or acute and long-term or chronic toxicity testing are carried out to determine the toxic effects of the drug and mortality in animal models

Toxicological studies

- **Acute toxicity** :- The aim is to find the acute dose that is lethal to 50% of the animal (LD50). The studies is done at least on two animals species and the drug is given in graded dose to several group of animal by at least two routes, one of which should be the proposed route to be used in human beings.
- **Sub acute toxicity** :-The aim is to identify the target organ to drug toxicity the three doses are used on two animals species .the animals are maintained at the maximum tolerated doses for a minimum period of four weeks to a maximum of three months so as to allow for the development of pathological change. Finally the animals are killed and subjected to histopathological studies.
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Toxicological studies

- **Chronic toxicity**:- Goal are the same as for sub acute toxicity, usually two animals species (one rodent and one non rodent) are used. The duration of study may range from one to two years. These studies may also run simultaneously with clinical trial, to cut short the time factor.
- **Special toxicity**:- Now a days toxicological data on teratogenicity(including the effects on reproductive functions),mutagenicity and carcinogenicity have become mandatory after the unfortunate episode of thalidomide disaster in 1961 which left more than 10,000 newborn congenitally deformed and crippled due to phocomelia

Effect on reproductive performance

- studies are carried out on rats treated with the test drugs before and after mating period .effects of drugs on mating behavior, fertility, parturition and lactation are noticed, including perinatal and postnatal effect if any

Teratogenicity

Such study are carried out in two animals species (rats and rabbits) to assess the effect of drugs on organogenesis . The drugs is given after the mating, during the period of organogenesis .fetus is then examined for any skeletal or birth defects

Carcinogenicity

Malignant and benign tumors occur spontaneously and can also be induced by drugs sometime as are results of mutation. Such studies can still be performed on at least two animals species, by giving same dose as used for chronic toxicity, for two year with assessment of hematological finding.

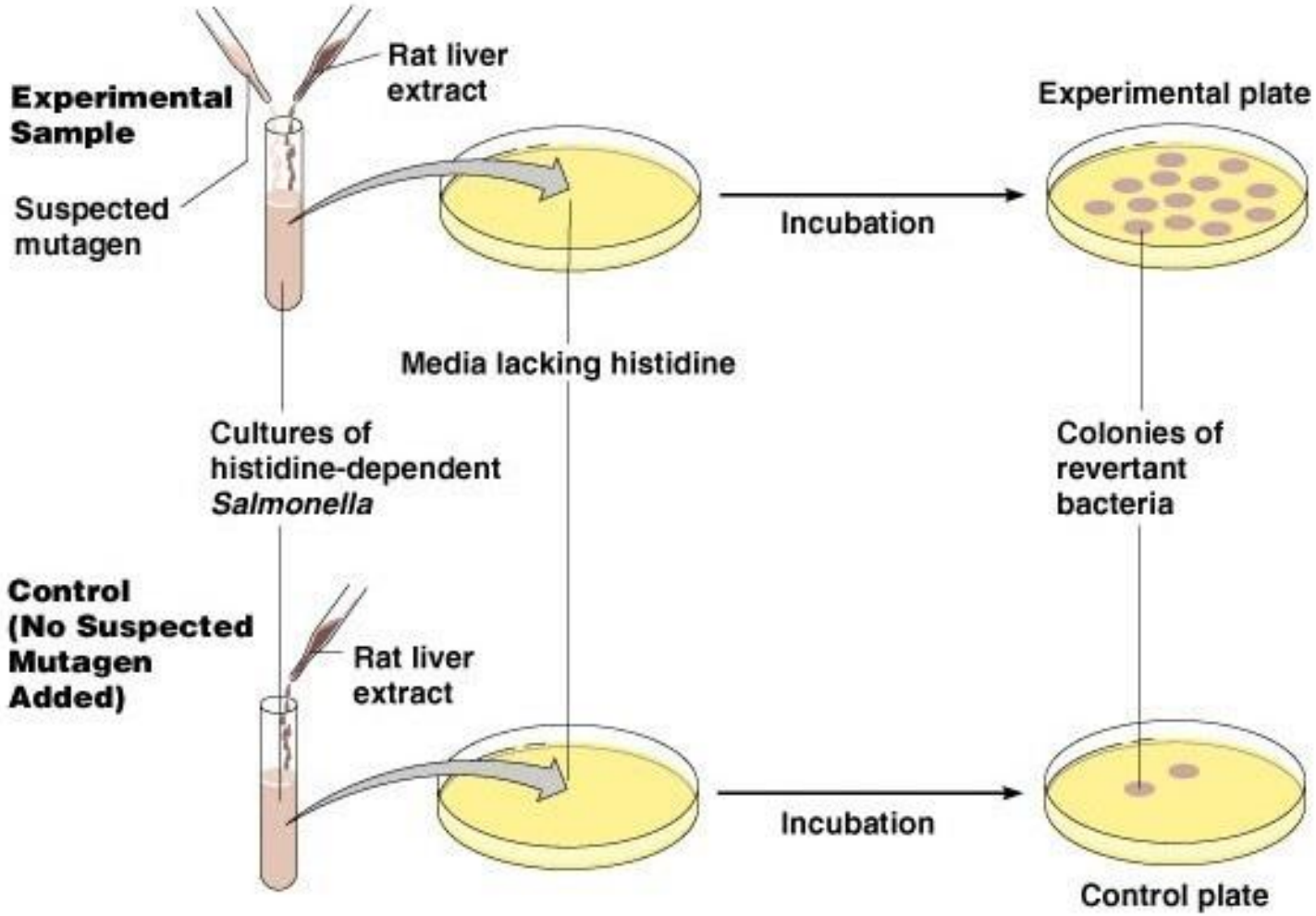
Mutagenicity

when a mutation occur in reproductive cells (spermatozoa or ova), then a hereditary defect occur which may appear in the first generation progeny of the individual., AMES TEST is conducted

Ames test (Salmonella typhimurium reverse mutation **assay)** -

is a widely employed method that uses bacteria to **test** whether a given chemical can cause mutations in the DNA of the **test** organism. More formally, it is a biological **assay** to assess the mutagenic potential of chemical compounds

AMES TEST


Experimental Sample
Rat liver extract
Suspected mutagen
Experimental plate
Incubation
Media lacking histidine
Cultures of histidine-dependent *Salmonella*
Colonies of revertant bacteria
Control (No Suspected Mutagen Added)
Rat liver extract
Incubation
Control plate

Clinical trial phase(human trials)

- Clinical trials mean a systematic study of a new drug in human subject to generate data for discovery or verifying the clinical Claim or pharmacological and adverse effect with an aim to determine the safety and efficacy of the drug.
- when the new compound passes the clinical pharmacological screening, the manufacturer may file a “preclinical new drug” or “investigational new drug” application (IND application) to an authorized drug control body of the respective country.

Clinical trial phase

- The IND application must contain the following

1.The chemical structure, its sources, its manufacturing data with details of its purity

2.The preclinical data about pharmacodynamics, Pharmacokinetics, and toxicological studies with ED50 and LD50 data.

3.Specification of dosage forms in which its has to be administered to human beings.

4.

Clinical trial phase

Detailed description of investigation protocol to be undertaken (including the dose and route of administration)

5.The name and qualifications of each investigator and the facilities available to them,

6.An agreement from the sponsor to submit annual progress reports regularly.

7. A certification that “informed consent” will be obtained from human volunteer and that “ethics of research in human begins” will be strictly followed. Note :- only when the approval is given by the regulatory body, the drugs can be administered to the men for clinical trials.

PHASES OF CLINICAL TRIALS

- Phase one
- Phase Two
- Phase Three
- Phase Four
- .

Phase one:

It is the phase of clinical pharmacological evolution of new drug and is performed on a small number (25-100) of healthy volunteers. If the drugs is expected to have significant toxicity(as in the case of anticancer drugs or drugs to be used in AIDS therapy), the volunteers with the particular disease are used rather than healthy volunteers.

Objective :-

1.To check for safety(i.e. whether the drug affect any cardiovascular, hepatic or renal function adversely) and to check its tolerability (i.e. doses the drugs produce any unpleasant symptom like headache ,nausea, vomiting.

2. To determine whether human and animals show significant pharmacokinetic differences.

3. To determine the pharmacokinetic of the drug in human so as to decide whether the deficiency in drugs effects, if any, is a results of its lack of absorption or its faster elimination.

4.To detect any predictable toxicity

Phase Two

- In this phase, the drug is studied for the first time in patients with target disease, to determine its efficacy (i.e., proof of claims)
- the main purpose of phase 2 trails is to gather evidence that the drug has the effects as suggested by preclinical trials hence the end point is decided
- These trials is divided into early and late phase
- **In early phase 2**:-A small number of patients (up to 200) are studied in details to observe the potential therapeutically benefits and side effects. Its is usually a single blind design where only the subject does not not known whether he is taking an inert placebo(if used) or the new drugs(under trial).
- **In late phase 2**:- It is conducted on a large number of patient (200-400) in controlled double blind manner, where the investigator is also ignorant (besides the subject) whether he is prescribing a placebo, or a positive control medicine or new drugs under trail

Phase Three

- These are large- scale multicenter (heterogeneous population) randomized double- blind trials in patients(1000- 5000 plus) to further establish the safety and efficacy. These are designed to minimize error in the information gathered in phase 1 and phase 2 trials Therefore these trials are made using double-blind cross- over designs
- NEW DRUGS APPLICATION:- once the phase 3 trails are completed satisfactorily the sponsors are file a “new drug application” The new drug application contains thousand of page and includes complete detailed monograph of the product, the result trails If the documentation is acceptable and is in compliance with the regulations, the drugs control authorities can allow the drugs to enter the markets with “new drugs status”

Phase Four

- Once the approval is obtained to market the drugs, phase 4 of the trails begins.it is the post- licensing phase – field trails.
- phase four has no fixed duration as it is the surveillance phase during the post –marketing clinical use of the drugs.
- The performance of the drugs is monitored for several years immediately after marketing, to discover relatively rare side effect (e.g. congenital effects).
- During the “new drug status “ period ,the manufacturer is expected to report any new information about the drugs concerning its safety. Such periodic safety update report (PSUR) is to be submitted every six months for first 2 years and manually for next 2 years.