CHAPTER 1

DRUG DISCOVERY

1.1 INTRODUCTION

The drug discovery is very important process. At the time of invention of new molecule there is necessity of related fields like medicine, biotechnology, chemistry, pharmacology.

In previous histories of drug discovery, more drugs were discovered by serendipity or in process of identification of active constant in traditional plants or medicinal plants. Later according to classical pharmacology, substance which has desirable therapeutic effect is identified. Chemical libraries of synthesis small molecules, natural products and extracts were screened.

Later in reverse pharmacology, sequencing of human genomes which allowed rapid cloning and synthesis of large quantities of purified proteins are identified. High throughput screening is very useful method for this process to screening of large amount of compounds. These methods are very useful against isolated biological targets which are hypothesized to disease modifying process. In recent identifications also so many newer inventions are able to known about biological molecules at the atomic level.

Modern drug discovery involves the identification of screening hits, optimization techniques to increase affinity, bioavailability and also to increase half life of drugs to decreasing of dose at the same techniques for reduction of side effects.

Prior to clinical trails only the compound should fulfill all requirements, and then only it will begin the process of drugs developments.

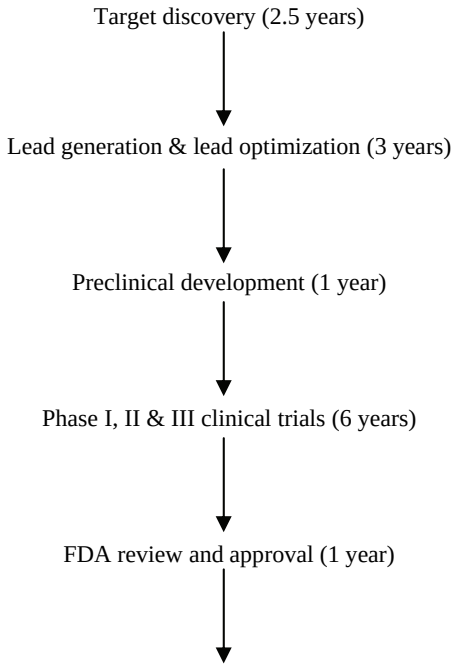
Computer aid drug design is a very important process. It is a very useful thing in new drug discovery. After understanding of all these biological systems and advances in technologies only then it is possible to a new drug discovery. But this new drug discovery is still a lengthy, expensive and also inefficient process.

1.2 DRUG DISCOVERY PROCESS

Drug design also sometimes referred to as rational drugs design, is the inventive process of finding new medications based on the knowledge of biological target. In the most basic sense drug design involves design of small molecules that are complementary in shape and charge to the bimolecular target to which they interact and therefore will bind to it.

Basic considerations in drug design: The drug is most commonly an organic small molecule which activates or inhibits the function of a bimolecular such as a protein which in turn results in therapeutic benefit to the patient.

Table 1.1 Time involved in drug discovery.



During the last 50 years the philosophy of valuable drugs discovery has evolved from one that was mostly around chemistry to one that has more biological approach to treat a disease. These changes were not only driven by strategic imperative but were enabled also by the significant changes in technology that has occurred during the past half century.

1.2.1 Steps in Drug Discoveries

The advent of molecular biology, coupled with advances in screening and synthetic chemistry technologies has allowed a combination of both knowledge around the receptor and random screening to be used for drug discovery.

Target identification

Once a thematic area has been identified, the next stage is to identify a suitable drug target. Example: Receptor, Enzyme or Nucleic acid.

Many early drugs such as the morphine just happen to interact with a molecule target in the human body. As this involves coincidence more than design, the detection of drug targets was very much a hit and miss affair.

By using **Genomics and Proteomics** many proteins were found to be drug targets and still research is going on them to find further new targets.

Target specificity and selectivity between species

- The more selective a drug is for its target, the less chance there is that it will interact with different targets and have undesirable side effects.

  Example: Penicillin targets an enzyme involved in bacterial cell wall biosynthesis.

- Target specificity & selectivity within the body: Enzyme inhibitors should inhibit only the target enzyme and not some other enzyme.
- Receptor agonists and antagonists should only show selectivity for a particular receptor (Example: An adrenergic receptor) or even a particular receptor subtype. (Example : β_2 adrenergic receptors)
- Targeting drugs to specific organs and tissues.

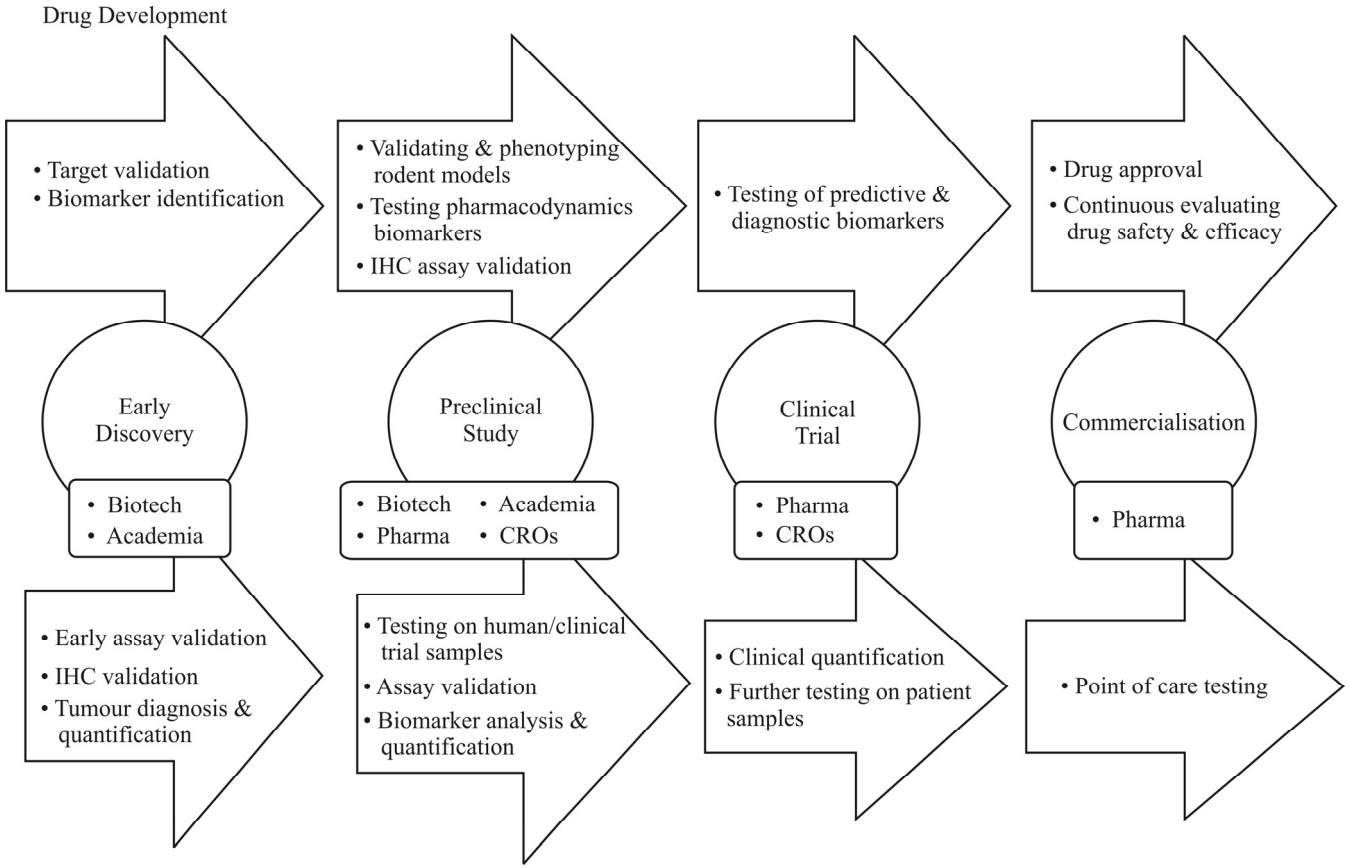
  Example: The adrenergic receptors in heart are predominantly β_1, where as those in the lung are β_2.

- Multi target drug:

  Example: Olanzapine binds to more receptors like Serotonin, Dopamine, Muscarine, Noradrenalin's and Histamine.

- This kind of profile would normally be unacceptable in schizophrenia, probably because it blocks both Serotonin and Dopamine receptors. Drugs which interact with a range of targets are called promiscuous ligand or dirty drugs.

Table 1.2 Steps in drug development.



Structural properties of drug molecules: Also involves in target specificity.

Physical properties of drug molecules may be categorized

- Physicochemical properties
- Shape (Geometric, Steric, Conformation, Topological)
- Stereo chemical properties
- Electronic properties

Physico-chemical properties of drug molecules are

- Role and structure of water on drug structure
- Solubility properties of drug molecules
- Partition coefficient of drug molecule
- Surface activity effects of drug molecule.
- The clinical molecular interface: Bio availability and drug hydration etc.

1.2.2 Lead

A lead is a compound from a series of related compounds, which has some of a desired biological activity. This molecule can be characterized and modified to produce another molecule with a better profile.

The lead seeking methods: (used to selection of lead molecules**)**

- Lead compound identification by serendipity.
- Lead compound identification from existing drugs.
- Lead compound identification by endogenous sources.
- Lead compound identification by exogenous sources.
- Lead compound identification by rational drug design.
- Lead compound identification by combinatorial chemistry with high throughput screening.
- Lead compound identification through genomics and proteomics.
- Pharmacogenomics and the future of lead compound discovery.

(a) ***Lead compound identification by serendipity:*** Serendipity actual meaning is unexpected discovery by accident. If the scientist is working for any known action, sometimes may chances of invention of other things, for example Alexander Fleming is working for examination of systemic fluids, while doing that experiment accidentally he observed the *Penicillin notatum*. This is one of famous example of serendipity reaction. This serendipity has important role in psychotic disorders, hallocinogenation. So examples of drugs discovered by serendipity are

aniline purple, penicillin, lysergic acid, diethylamide, meprobamate, chlorpramazine

(b) ***Lead compound identification from existing drugs:*** Many companies use established drugs from their competitor as lead compounds in order to design a drug disparaged, they can after implement, forms as better drug than original drug.

Example: Modern penicillin is more selective, more potent and more capable than original penicillin's.

(c) ***Lead compound identification from endogenous sources:***

- Endogenous source means natural ligand for receptors. Example: Histamine was used as original lead compound in the development of H_2 Histamine Antagonist, (Cimetidine).
- Natural substrates for enzymes. Example: Enkephalins have been used as lead comp for the design of Enkephalinase inhibitors.
- Enzyme products as lead compounds. Example: Design of carboxypeptidase inhibitor.
- Natural compounds as lead compounds, allosteric site of GABA.

(d) ***Lead compound identification from exogenous sources:***

- **Plant kingdom:** Plants have always been a rich source of lead compound. Example: Morphine, Cocaine, Digitalis, Quinine, Tubocurairne, Nicotine, Muscarine. Many of these lead compounds are useful drugs in themselves.
- Others have been the basis for synthetic drugs. Example: Local anaesthetics developed from cocaine.
- Clinically useful drugs which have recently been isolated from plants include anticancer agent paclitaxol from few trees Taxol, and the antimalarial agent Artemisinin from Chinese plant.

(e) ***Lead compound identification from Micro organism source:***

Example: Cephalosporin, Tetracycline, Amino glycosides, Rifampcin, Chloramphenicol & Vancomycin etc.

(f) ***Lead compound identification from Marine sources:*** Antitumor agents derived from marine sources include Discodermolide, Bryostatin, Dolaostatins and Cephalostatins etc.

(g) ***Lead compound identification from Animal sources:*** Antibiotics polypeptides known as the magainins were extracted from skin of the African clawed frog *Xenopus laevis* etc.

(h) ***Lead compound identification from Venoms and toxins:*** Teprotide, a peptide isolated from the venom of the Brazilian viper was a lead compound for the development of the Antihypertensive agent's Cilazapril and Captopril etc.

(i) ***Lead compound identification by combinatorial chemistry:*** Lead compound identification by combinatorial chemistry with high through put screening. A key to success in drug discovery by screening is the availability of a large and structurally diverse library of compound. .

(j) ***HTS Assays:*** HTS (High Throughput Screening) have been developed and perfected over the past 10-20 years it includes

- *Micro plate activity assay:* Assay is in solution in a well, the result of the assay, such as enzyme inhibitors is linked to some observable, such as colour change to enable identification of bio availability.
- *Gel diffusion assay:* Biological target is mixed in soft agar and spread on the surface of the film, after allowing the compound to diffusion, an appropriate developing agent is sprayed on the agar surface and areas in which bioactivity has occurred will show up as distinct zones.
- *Affinity selection assays:* Compound library is applied to a protein target receptor, all compounds that do not bind are removed, and compounds that do bind are then identified. Of these micro plate assays are probably the most widely used. Screening of combinatorial libraries in 96 or even 384 well micro plates is time & cost efficient.
- Using modern robotic techniques, it is possible to perform more than 1,00,000 bio assays per wheel in a micro plate system.

(k) ***Lead compound identification through genomics and proteomics:***

- Taking **Genomics** one step further for the purpose of drug discovery will require linking specific proteins to those specific genes which helps in treating specific diseases and in development of new drug.
- *Proteomics and lead compound discovery:* Proteomics is the molecular biology discipline that seeks to elucidate the structure and function profiles of all proteins encoded with in a specific genome.
- DNA microarray technology is a powerful technique with which to monitor the relative abundance of a specific mRNA in an individual cell and to correlate this with a specific protein.

(l) ***Bioinformatics and cheminformatics in lead compound discovery:***

- Bioinformatics and cheminformatics will apply knowledge discovery and pattern recognition algorithms to the genome wide and proteome wide experimental data, there by facilitating drug design.
- Pharmacogenomics represents a new conceptual approach to target identification and drug development. Pharmacogenomics and the future of lead compound discovery. Conventional drug design attempts to discovery drugs to treat particular diseases, Pharmacogenomics attempts to design individualized drugs to treat particular people with particular diseases. Single Nucleotide Polymorphism (SNP) is crucial to the task of individualized drug design.

1.2.3 Synthesis of Lead Compound

- Organic synthesis is preparation of complicated organic molecules from other, simplex, organic compounds. Because of the ability of carbon atoms to form chains, multiple bonds and rings an almost unimaginably large number of organic compounds can be conceived and created.
- In planning a synthetic route for the preparation of desired molecule the organic chemist devices a synthetic tree an outline of multiple available routes to get to the target molecule from a available starting materials. An organic synthesis may be either linear or convergent. A linear synthesis constructs the target molecule from a single starting material and progresses in a sequential step by step fashion.

Some lead optimization methods

- Variation of substituent.
- Extension of the structure.
- Chain extension or contraction.
- Ring expansion or contraction.
- Ring variations.
- Ring fusions.
- Isosteres and Bioisosteres.
- Simplification of structure.
- Conformational blockers.
- Drug design by NMR.
- Structure based drug design and molecular modelling.
- The elements of lack and inspiration.

1.2.4 Preclinical Development

- Pre-clinical development is a stage that explains before clinical trials (testing in humans) during which important safety and pharmacology data are collected. Most regulatory decisions on whether a new drug can be approved for marketing. Most regulatory toxicity studies request in a rodent (Example: Rats) and non rodents (Example: Dogs).
- Choice of animal species based on the similarities of its metabolism to humans or the applicability of desired pharmacological properties to humans. It is not possible or ethical to use animals in large numbers, to compensate for the same it is assumed that increasing the dose and prolonging the duration of exposure will improve both sensitivity and productivity of the tests.
- Main goals of preclinical studies are to determine a drugs Pharmacodynamics, Pharmacokinetics & toxicity through animals testing. This data allow researchers to estimate a safe starting dose of the drug for clinical trials in humans.

Types of preclinical studies

- *In vitro* studies
- *In vivo* studies
- *Ex vivo* studies

In vitro studies: *In vitro* studies are done for testing of a drug or chemicals effect on a specific isolated tissue or organ maintaining its body functions in laboratories, also called test tube experiments.

Examples: Langendroff's Heart Preparation, Ileum Preparation, Rectus Abdominal Muscle Preparation.

In vivo studies: In Latin meaning is "in the living" it indicates the use of a whole organism or animals (for an experiment) purpose of model is chosen because it is believed to be appropriate to the condition being investigated and is thought likely to respond in the same way as human to the proposed treatment for the character being investigated.

These studies used to measure

- Therapeutic potential
- Toxicity potential
- Pharmaceutical properties & metabolic pathway
- Mechanism and specification.

In vivo **are preferable than** ***In vitro*** **because**

- Greater similarity to human studies when compared to *in vitro*.
- Drug effects modified by physiological mechanism can be calculated.

- Absorption, Distribution, Metabolism and Excretion also calculated.
- Most animal systems are similar to human systems.
- Effects of drug are studies on complete system rather than tissues and organs.
- Drugs acting on Central Nervous System, Cardio Vascular System, Gastro Intestinal System and other systems are studied.
- Results easier to interpret and extrapolate.

Some of **examples of *in vivo*** studies is

- Non invasive methods – rat tail cuff method.
- Invasive methods – BP recording in anaesthetized dog or cat.

Ex vivo studies: Experiment is performed *in vivo* and then analyzed *in vitro*.

General requirements for conducting preclinical studies

- Toxicity studies should comply with Good Laboratory Practice (GLP).
- Standard Operating Procedures (SOPs) should be followed.
- All documents belonging to each study including its approved protocol, raw data, draft report and histology slides and paraffin tissue blocks should be preserved for a minimum of 5 years after marketing of drug.
- ***Animal toxicity studies****:* Toxicity studies also preformed to assess systemic exposure achieved in animals and its relationship to dose level and the time course of toxicity studies. Some toxicity studies like
  - Systemic toxicity studies
  - Male fertility studies
  - Female reproductive & development toxicity studies
  - Teratogenicity studies
  - Prenatal studies
  - Local toxicity
  - Genotoxicity and carcinogenicity

1.2.5 FDA Requirements for Preclinical Studies

It is essential to ensure the quality and reliability of safety studies and this can be achieved by adhering to Good Laboratory Practices (GLP). The purpose of GLP is to obtain data on properties and safety of these substances with respect to human health and environment, to promote development of quality test data, such comparable data from the basis of mutual acceptance across organizations or countries, confidence in and reliability of data from different countries will prevent duplication tests, save time, energy and resources.

- For every 5000 drug compounds that enter preclinical tests in the United States, only about 5 will eventually be considered acceptable to test in humans.
- Of those final 5 drugs only how many out of 5 drugs may actually receive approval for use in patient care.
- Under FDA requirements, a sponsor must first submit data showing that the drug is reasonably safe for use in initial, small scale clinical studies.
- Depending on whether the compound has been studies or marketed previously, the sponsor may have several options for fulfilling these requirements.
- Compiling existing non clinical data from past *in vitro* laboratory or animal studies on the compound.
- Compiling data from previous clinical testing or markets of the drug in the United States or another country whose population is relevant to the US population.
- Undertaking new preclinical studies designed to provide the evidence necessary to support the safety of administering the compound to humans.
- At the pre-clinical stage, the FDA will generally ask, at a minimum that sponsors.
- Develop a pharmacological profile of the drug.
- Determine the acute toxicity of the drug at least 2 species of animals.
- Conduct short term toxicity studies ranging from 2 weeks to 3 months depending of the proposed duration of use of the substance in the proposed clinical studies.
- Organization of Economic Cooperation & Development (OECD) framed guidelines known as Good Laboratory Practices (GLP).
- GLP gives guidelines for animal test facilities, housing the animals, responsibilities & duties of personnel conducting the animal studies, equipment, quality control etc.
- In India, the Committee for the Purpose of Control and Supervision for Experiments on Animals (CPCSEA) ensures that the animal facilities are well maintained and experiments are conducted as per internationally accepted norms.
- An Institutional Animal Ethical Committee (IAEC) must be established by an institution (or group of organization) which has an approved code of ethical conduct.
- Final report shall be prepared for each non clinical/pre clinical laboratory study and shall include:

- Names and address of facility performing the study and the dates on which the study was initiated & completed.
- Objectives and procedures stated in approved protocol, including any changes in original protocol.
- Statistical methods employed for analyzing the data.
- The test and control articles identified by name, chemical abstracts no. or code number, strength, purity and composition or other appropriate characteristics. Stability of test and control articles under the conditions of administration.
- A description of the methods used.
- A description of the test system used where applicable the final report shall include the no. of animals used, sex, body weight range, source of supplies, species, strain and sub strain, age and procedure used of identification.
- A description of the dosage, dosage regimen, rate of administration and duration.
- The description of all circumstances that may have affected the quality or integrity of the data.
- The name of study director, the name of the other scientists or other professionals and the names of all supervisory personnel involved in the study.
- A description of transformation calculations or operations performed on the data, a summary and analysis of data, statement of conclusions drawn from analysis.
- The signed and data reports of each of the individual's scientists or other professional involved in the study.
- The location where all specimens, raw data and the final report are to be stored.
- A statement prepared and signed by quality assurance unit & the final report signed and dated by study director.

Drug discovery and drug development is being revolutionized due to changes in technology. Technologies like genomics, proteomics high throughput screening and structure based design have enabled the process of discover to evolve into a system where new lead molecules can be rapidly found against novel and difficult targets. FDA's role in the development of a new drug begins when the drug's sponsor (usually the manufacturer or potential markers) having screened the new molecules for pharmacological activity and acute toxicity potential in animals, wants to test its diagnostically or therapeutic potential in humans. At that point the molecule changes in legal study under the

federal food, drug and cosmetic act. Before the sponsor proceeds to study a new drug in human, approval has to be obtained by IND.

Applications

- IND application (Investigational New Drug application) is to provide the data showing that it is reasonable to begin tests of a new drug on humans.
- IND application is result of successful preclinical development programme and it is also the vehicle through which a sponsor advances to next stage of drug development known as clinical trials.
- IND application categories: Commercial Research.

There are 3 types of IND application.

- Investigator IND application.
- Emergency use IND application.
- Treatment IND application.

The IND application must contain information in 3 bridge areas.

- Animal pharmacology and toxicology studies.
- Manufacturing information.
- Clinical protocol & investigator information.

Sponsor files the IND application in form 1571 to the FDA for review once successful series of preclinical studies are completed.

Along with IND application the sponsor submits the statement of the investigator in form 1572. Once the IND application is submitted, the sponsor must wait 30 calendar days before initiating any clinical trials. If the sponsor hears nothing from CDER (Center for Drug Evaluation & Research) then on day 31 after submission of IND application, the study may proceed as submitted. The CDER is a division of FDA that reviews "New Drug Applications" to ensure that drug are safe and effective. After medical review, chemical reviewers, pharmacological toxicology review, statistical analysis, safety review only they promoted to clinical investigation.

1.2.6 Protocol Design

The following topics should be considered in the preparation and review of animals care and protocols:

- Rationale and purpose of the proposed use of animals.
- Justification of the spices and number of animals requested. Whenever possible, the number of animals requested should be justified spastically.

- Availability or appropriateness of the use of less-invasive procedures, the other spices, isolated organ preparation, cell or tissue culture, or computer simulation (see appendix A alternatives).
- Adequacy of training and experience of personnel in procedures used.
- Usual housing and husbandry requirements.
- Appropriate sedation, analgesia, and anaesthesia (scales of pain or invasiveness might aid in the preparation and review of protocols; see appendix A, “anaesthesia, pain and surgery”).
- Unnecessary duplication of experiments.
- Conduct of multiple major operative procedures.
- Criteria and process for timely intervention, removal of animals from a study, or euthanasia if painful or stressful outcomes are anticipated.
- Post procedure Care.
- Method of euthanasia or disposition of animal.
- Safety of working environment for personal.

Occasionally, protocols include procedure that have not been previous study or that have potential to cause pain or distress that cannot be reliably controlled such procedure might include physical restraint, multiple major survival surgery, food or fluid restriction , user edge ones , use of death has an end point , use of noxious stimuli, skin or corneal irritancy testing, allowance of excessive tumor burden, intracardiac or orbital - sinus blood sample, or the use of abnormal environmental conditions. Relevant objective information regarding the procedure and the purpose of the study should be sought from the literature, veterinarians, investigators, and other knowledgeable about the effects of animals. If the little is known regarding a specific procedure, limited pilot studies design to assess the effects of the procedure on animals, conducted under IACUC oversight, might be appropriate. General guidelines for evolution of some of those methods are provided in this section, but they might not apply in all instances.

1.2.7 Clinical Development

Clinical trial or study is any investigation in human subjects intended to discover or verify the clinical, pharmacological and the Pharmacodynamic effects of an investigational product and or identify any adverse reactions to investigational products or study ADME of an investigational product with the object of ascertaining its safety and/or efficacy.

Phases of clinical trials

Phase I: Human Pharmacology.

Phase II: Therapeutic Exploration.

Phase III: Therapeutic Confirmation.

Phase IV: Post Marketing Studies.

Phase I: Human Pharmacology

1st stage of testing in human subjects. Normally (20-80) group of healthy volunteers will be selected to participate in these studies. This phase includes trail designed to assess the safety, tolerability, Pharmacokinetic & Pharmacodynamic of drug.

Phase II: Therapeutic Exploration

Once the initial safety of study drug has been conformed in phase I trials, phase II trials are performed to assess how well the drug works (efficacy) require large group (200-300) of patient volunteers. Most of development processes are failed in phase II only because of toxic effects (in 90% cases).

Phase III: Therapeutic Confirmation

They are performed after preliminary evidence suggestions effectiveness of the drug has been obtained in phase II. They are intended together additional information about effectiveness and safety that is needed to evaluate the overall benefit. Risk relationship of drug. Usually include several hundred to thousand patients. Data obtained from phase III is the major component of new drug application. It determines dosage schedule.

Phase IV: Post Marketing Studies

Those studies performed with drugs that have been granted marketing authorization.

1.2.8 Ethics in Preclinical Research

Monitoring the use of Animals Institutional animal care and use committee was present.

The responsible administrative official at each institution must appoint an IACUC, also referred to as “the committee” to oversee and evaluate the institution’s animal program, procedures, and facilities to ensure that they are consistent with the recommendations is this guide, the AWRs [Animal Welfare and Regulations], and the PHS policy. It is the institution’s responsibility to provide suitable orientation, background materials, access to appropriate resources, and, if necessary, specific training to assist IACUC members in understanding and evaluating issues brought before the committee.

Committee membership should include the following:

- A doctor of veterinary medicine, who is certified (see American College Of Laboratory Animal Medicine, ACLAM, appendix B) or has training

or experienced in laboratory animal science and medicine or in the use of spices in question.

- At least one practicing scientist experienced in research involving animals.
- At least one public member to represent general community interests in the proper care and use of animals. Public members should not be laboratory animal users, be affiliated with the institution, or be members of the immediate family of a person who is affiliated with the institution.

The size of the institution and the nature and extent of the research, testing, and educational programs will determine the number of members of committee and their terms of appointment. Additional information about committee composition can be found in the PHS policy and the AWRS.

The committee responsible for oversight and evolution of the animal care and use program and its components described in this guide. In functions include inspection of facilities; evolution of programs animal activity areas; submissions of reports to responsible institutional officials; review of proposed uses if animals in research testing or educational (i.e., protocols); and establishment of a mechanism for receipt and review of corners involving the care and use if animals at the institutions.

The IACUC must meets as often as necessary to fulfill its responsibilities, but it should meet at least ones every 6 months. Records of committee meetings and results of deliberations should be maintained. The committee should review the animal-care program and inspect the animal facilities, activity areas at least once every 6 months. After review and inspection, a written report, signed by a majority of the IACUC, should be made to the responsible administrative officials of the institution on the status of the animal care and use program and other activities as stated herein and as required by federal, state, or local regulations and policies, protocols should be reviewed in accord with the AWRS, the PHS policy, U.S. government principles for utilization and care of vertebrate animals used in testing, research, and training (IRAC 1985; see appendix D), and this guide (see foot note,p.2).

1.3 HIGH THROUGHPUT SCREENING

Traditional drug discovery research (TDDR) has been revolutionized and every day new assay and techniques are being developed to make DDR a success and to cut down the loss incurred by the failing molecule in clinical trials.

This revolution considerably reduces the time and expenses involved in DDR by setting the process on fast tract. New drug discovery0020 (NDD) for oval therapeutic targets is thus an amalgamated process of various steps in

today's modern pharmaceutical research. This process is used for developing new leads for the new targets with improved Pharmacokinetics/ Pharmacodynamic or finding utility for novel compounds obtained from diverse resources.

In the domain of drug research target identification, purification and assay development constitute the initial step. Then they screened for identify targets. It needs huge investment of manpower, time and money.

It has so many steps

- Like centrifugation, phase extraction, filtration, precipitation and subsequent signal amplification and detection, evaluation of few hundred compounds might take weeks and months. Conventional drug discovery program has been called slow process.

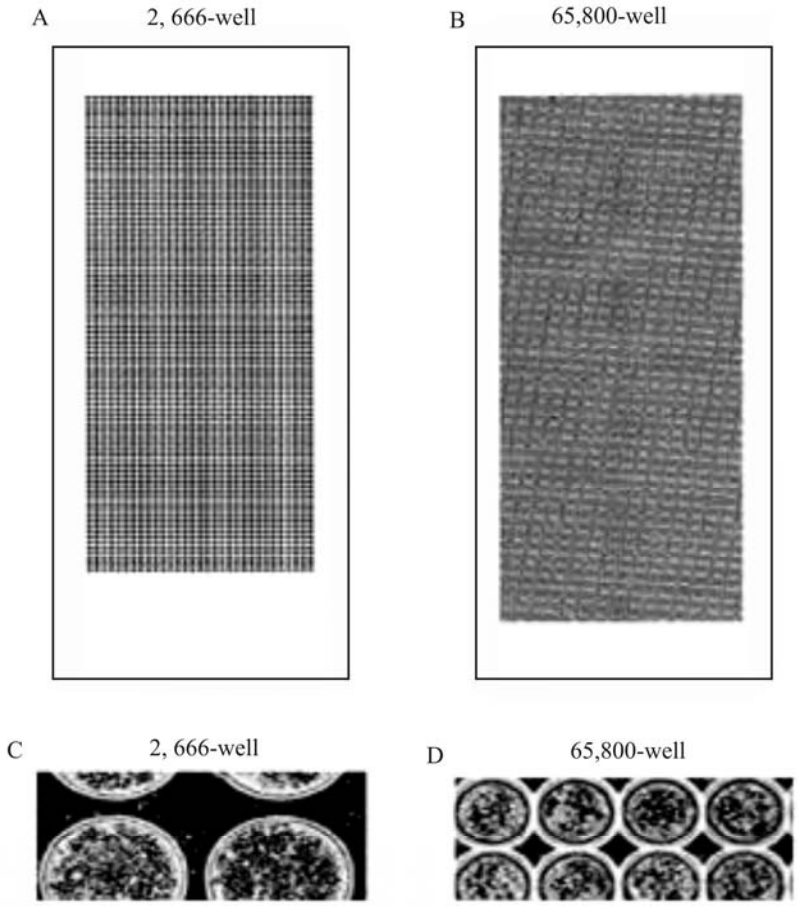


Fig. 1.1 Wells of highthroughput screening.

- The last 2 decades have seen astonishing innovation in technology that have helped the manual low speed screening to evolve into an automated, microprocessor controlled robotic process called "HIGH THROUGH PUT SCREENING". This recent process is synergy of chemistry, biology, engineering and informatics.

  HTS has helped to speed up conventional languid process and now over 50,000-1,00,000 compounds can be screened per week.
- Further advancements are making it possible to screen 10,000-1,00,000 compounds within 24 hours. This process is called "ULTRA HIGH THROUGHPUT SCREENING" (uHTS).
- HTS of large no. of test compounds in a lesser time is also reality now.
- It is also useful in other areas as drug synthesis; toxicity screening, drug metabolism and pharmacokinetics (DM&PK) are helping the process to achieve its ultimate speed in NDD.

1.3.1 *In vitro* Matrix-Ligand Interactions Studies

They are two types

1. Heterogeneous assays.
2. Homogeneous assays.

These Assays are carried to evaluate

- Protein-Protein interactions.
- Receptor-ligand interaction.
- Enzyme-ligand interactions.

Heterogeneous assays include

Nonradio active assay: ELISA (Enzyme linked immunosorbent assays).

Radioactive assay: Such as filtration, adsorption, Precipitation and Radio immune Assay (RIA).

Heterogeneous assays are laborious and require multiple steps like addition, incubation, washing, transfer, filtration and etc., mainly final reading based on color produced or the remaining radioactivity measurement. In contrast heterogeneous assay, homogeneous assays offer a unique advantages by drastically reducing the number of steps involved in analysis and by integrating or sequencing them.

Homogeneous assays include

Nonradioactive assays:

- Chromogenic assays.

- Absorbance-based assay.
- Fluorescence- based assay.
- Luminescence-based assay.
- Bead-based assay.

Radioactive assays:

- Scintillation proximity assay (SPA).
- Scintillation plate assay.

1.3.2 HTS Binding Assay

In HTS the interaction of ligand with the biological compartment is elucidated by luminescence based binding assay. In this manner several thousands of compounds from chemical library can be assessed for their binding in a few days period.

Various florescence techniques

- Fluorescence anisotropy (FA).
- Fluorescence correlation spectroscopy (FCS).
- Fluorescence Intensity Assay (FIA).
- Fluorescence life time Imaging Microscopy (FLIM).
- Fluorescence Resonance energy Transfer (FRET).
- Total Internal reflection Fluorescence (TIRE).
- Time resolved resonance Anisotropy (TRRA).

And NANO based techniques are there

- Scintillation proximity assay (SPA).
- Amplified Luminescence proximity Homogeneous assay (ALPHA).

Fluroscence techniques in drug screening: The recently developed fluorescence based assays are good enough to elucidate the molecular mechanism of receptor function and signal transduction process, as well as for applications in field of screening for novel therapeutic compounds and homogeneous assay there by used. Fluorescent labels are their inherent property of shifting the emission wavelength from the exciter wavelengths to differentiate minute information regarding binding.

(i) ***Fluorescence Intensity Assay (FIA):*** Flurogenic assay is production of property of fluorescence from non fluorescence reagent. And quench is decrease in fluorescence intensity up to cleavage of substrate.

(ii) ***Fluorescence Anisotropy (FA):*** It is suitable to detect receptor ligand binding reactions used to conventional biophysical laboratories. Low

polarization observed when ligand is free in solution. Plane polarized light to excite the fluroprobe and emission is recorded perpendicular and parallel to plane of polarization.

(iii) ***In Vivo Imaging of Drug Action (Near Infrared Imaging):*** It is a newly added method to evaluate the drug action and quantification without killing the animal. Rapid development in this technology is accepted to revolutionize *in vivo* studies that warrant the killing of experimental animals. Fundamental of technique is using molecules capable of emitting light in higher wavelength near IR region there by, it gains the capability of crossing biological membrane and to be detected by sensitive camera turned for near IR region.

1.4 PHARMACOGENOMICS, PROTEOMICS AND ARRAY TECHNOLOGY

1.4.1 Pharmacogenomics

Pharmacogenomics is one of the important branches in new drug development process. In these approaches newly developed drugs leads to advent of "Personalized medicine" in which drugs for each individual's unique genetic makeup. Individual variability in drug efficacy and drug safety is a major challenge in current clinical practice, drug development and drug regulation. In recent studies also Pharmacogenomics have provided examples of causal relations between genotypes and drug responses to account for phenotypic variations of clinical importance in drug therapy. In recent progress the understanding of genetic contributions to major individual variability in drug therapy with focus on genetic variations of drug target, drug metabolism, drug transport, disease susceptibility and drug safety. Pharmacogenomics is the technology that analyses how genetic makeup affects an individual's response to drugs. It deals with the influence of genetic variation on drug responses in patients by correlation gene expression or single nucleotide polymorphisms with a drug's efficacy or toxicity. The main aim of Pharmacogenomics is to develop rational means to optimize drug therapy, with respect to patient's genotype, to ensure maximum efficacy with minimal adverse effects.

In a large patient population, a medication that is proven efficacious in many patients often fails to work in some other patients. Furthermore, when it does work, it may cause serious side effects, even death, in a small number of patients. Although large individual variability in drug efficacy and safety has been known to exist since the beginning of human medicine, understanding the origin of individual variation in drug responses has proven difficulty. On the other hand, the demand to overcome such variation has received more attention now than ever before. It is well documented that large variability of drug

efficacy and adverse drug reaction in patients is a major determinant of the clinical use, regulation, and withdrawal from market of lineal drugs and a bottleneck in the development of new therapeutic agents.

Pharmacogenomics and individualized drug therapy are increasingly influencing medicine and biomedical research in many areas, including clinical medicine, drug development, drug regulation, pharmacology and toxicology, a thematic reflexation of the post genomic era of today's medicine. This article is intended to provide a comprehensive review of recent progress in the understanding of the basis of individual variability of drug efficacy and adverse drug reaction with focus on genetic polymorphisms of drug targets, drug-metabolizing enzymes, drug transporters and targets of drug toxicity.

(i) Some individual drug responses

- *Individual variability in drug therapy:* Drug efficacy and adverse drug reactions dose dependently determine the clinical outcome of a medication. A higher dose boosts drug therapeutic effect but simultaneously increases the propensity for new or greater undesirable side effects. The drug dosage between its therapeutic effect and apparent adverse reaction, define therapeutic window. For many drugs the optimum dose required for effective and safe therapy varies significantly from patient to patient. A drug dose within the therapeutic window for the majority of a patient population can be too low or too high for a small number of patients who have an atypical dose response curve for a drug therapeutic effect, toxicity or both resulting in unexpected undesirable outcomes in the patients. Individual variability generally has a larger impact on drugs that have a narrow therapeutic widow than those with a wide one.

(ii) Factors affecting individual drug response

- Genetic and nongenetic factors affect individual variability of a drug response by modulating the dose response curves of drug efficacy and drug toxicity of patients. Genetic factors generally cause permanent changes in protein functions, whereas environmental and physiological factors and their impact on drug response are transient in most cases.
- Genetic polymorphisms of proteins involved in drug targeting and drug metabolism and transport are likely to be the most important sources of individual variability in drug efficacy. Drug target responsible for an adverse drug reaction can be the same as or different from the therapeutic target of the drug resulting in on target or off target side effect.

- At the molecular level genetic variations can change the structure of a target protein via mutation in the coding region of the gene or the amount of the protein expressed by modulating gene regulation both of which ultimately alter the function of the protein or the rate and kinetic constants in the cases of an enzyme. Mutations can also modulate gene expression by way of epigenetic regulation.
- Genetic polymorphisms of drug metabolizing enzymes and transporters can affect the absorption, distribution, metabolism and elimination of drugs and thereby modulate their plasma and target tissue concentrations. Defective DNA repair enzymes reduce the ability of cells to repair mutations induced by alkylating chemotherapeutic agents. Mutations that alter the structure or reduce the amount of the enzymes involved in the biosynthesis of glutathione are likely to reduce the intracellular content of glutathione, which is critical in protecting cells from oxidative stress and reactive intermediates commonly encountered adverse drug reaction.
- Environmental chemicals co-administered drugs, dietary constituents, tobacco smoking and alcohol use are all known to induce or inhibit Cytochrome P450 other drug metabolizing enzymes and drug transporters to alter drug efficacy and to induce drug-drug and drug chemical interactions and drug side effects.
- Physiological factors including age, sex, disease states, pregnancy, exercise, starvation and circadian rhythm can also contribute significantly to individual variations of the pharmacokinetic and pharmacodynamic properties of administered drugs. Some physiological traits are also genetic generally polygenic in nature such as sex, body weight and chronic diseases.
- *Human genetics in drug response:* Genetic influence on drug response involved variations in single genes (monogenic inheritance) in which polymorphisms of a single gene encoding a drug metabolizing enzyme responsible for the metabolism and disposition of a substrate drug caused aberrant response to the drug.
- Traditional approach was used to establish a genotype-phenotype connection in three steps: Identify individual phenotypes (normal or extensive metabolizers versus poor or slow metabolizers) by measuring drug levels in the urine or plasma before the genetic mechanism was known, establishing a correlation between drug pharmacokinetics and drug response (efficacy or toxicity) and

finally identifying the genetic defects that account for the low or lack of the enzyme activity later.

- Genetic variations can result from single nucleotide polymorphism (SNP) insertion, deletion or duplication of DNA sequences. SNP is probably the most common variation. More than 90% of human genes contain at least one SNP and nearly every human gene is marked by a sequence variation. More than 14 million SNPs have been identified in the human genomes.

(iii) Genetic polymorphisms of drug targets

- Polymorphism in genes encoding drug targets directly affect target protein function, drug target interaction or both to produce profound effects on drug response.
- ***Example:*** Warfarin is most commonly used oral anticoagulant. The main target of Warfarin is vitamin K epoxide reductase (VKOR), (especially VKOR complex subunit 1- VKORC1). VKOR catalyzes the conversion of vitamin K epoxide to reduced vitamin K, which is required for post translational γ carboxylation of the glutamic acid residues of coagulation factors II,VII,IX and X and the anticoagulant proteins C, S and Z by γ-glutamyl carboxylase (GGCX). Warfarin acts by inhibiting VKORC1, it leads to depletion of reduced vitamin K and consequently it leads to production of hypofunctional coagulation factors resulting in anticoagulation. In case of Warfarin dose determination depends on inhibition capacity of Warfarin on VKOR. But VKOR is exposes in so many types of polymorphic forms (like A41S, V45A, R58G, V66M and L128R etc.,) but these mutations are rare in human population (<0.1%) but influences the dose determination. Overall, polymorphisms of VKORC1 were estimated to account for 25% of the variation in Warfarin dose.

(iv) Genetic polymorphism of drug metabolizing enzyme

- Most of all clinical drugs are metabolized by one or more microsomal cytochrome P450 enzymes. P450 catalyzes the mono oxygenation of lipophilic drugs to give rise to metabolites with altered water solubility or metabolites more suitable to further metabolism by other enzymes. In many cases P450 polymorphism is a major variable affecting drug plasma concentration, drug detoxification and drug activation.

  Example: CYPED6 polymorphism is one of the important example for understanding of genetic polymorphism role in new drug discovery, by mutation of metabolizing enzymes may lose their

metabolizing capacity. Sometimes it leads to toxicity of those particular drugs. CYP2D6 is responsible for the metabolism of approximately 20 to 25% of all marked drugs like β adrenergic receptor blockers, antidepressants, antiarrhythmics and antipsychotics. CYP2D6 is highly polymorphic, variant alleles of CYP2D6 are classified on the basis of enzymatic activities. If CYP2D6 is mainly responsible for the metabolism of any drug it leads to high blood levels of that drug by polymorphic form. So by knowing of phenotype of an individual patient would allow physician to prescribe a safe and effective dose of the drug.

- Many non P450 drug metabolizing enzymes also play critical roles in the metabolism of a variety of drugs. Polymorphisms of these enzymes influence the metabolism and therapeutic effect of the drugs, some of them are clinically significant.

  Example: Thiopurine methyltransferase (TPMT) catalyzes the S-methylation of 6-mercaptopurine, azathiopurine and thioguanine, which are used for treatment of leukemia and autoimmune diseases. More than 20 variant alleles of the TPMT gene have been identified (like TPMT*2, TPMT*3A, TPMT*3C etc.,) they produce poor enzymatic activities. Approximately 90% of white persons inherit high enzyme activity, 10% inherit intermediate activity (heterozygous) and 0.3% inherit low or no activity. The persons carrying defective TPMT alleles accumulate higher levels of cytotoxic thiopurine nucleotides than those with the wild type alleles after receiving a standard dose of the drug, leading to severe hematological toxicity by the parent drugs. In these scenarios, a reduced drug dose should be prescribed.

(v) Genetic polymorphism of drug transporters

- Drug transporters modulate the absorption, distribution and elimination of drugs by controlling of influx and efflux in cells. With genetic polymorphism of transporters can have impact on drug disposition, drug efficacy and drug safety.

  Example: TheABCB1 gene encodes the P-glycoprotein (Pgp, ABCB1, multidrug resistance that transports many important drugs out of cells, ABCB1 is highly polymorphic, and some allelic variants exhibits ethnic-dependent distribution. The SNP C3435T of ABCB1 occurs with high frequencies in many populations (20-60%), it leads to disposition of digoxin (a substrate of Pgp). In some individuals it raises the serum digoxin concentration and in some others it may decreases serum dioxin levels, all these differences because of different polymorphic forms of ABCB1.

(vi) Genetic variables indirectly affecting drug response

- Many genetic variables affect drug response by modulating the functions of proteins that are not direct drug targets, drug metabolizing enzymes or drug transporters but influence the biological context of a drug response.

(vii) Genetic variables affecting adverse drug reaction

- Adverse drug reactions are highly variable in many cases and thus represent a major limiting factor in drug therapy and drug development. Idiosyncratic adverse drug reactions characterized by rare occurrence of multiple exposures are the most extreme cases of individual variability in drug safety. Drug safety issues are to understand the mechanism of adverse drug response, determine the gene or genes responsible for the adverse events and develop reliable biomarkers for screening sensitive individuals. Illustrate the utility of this Pharmacogenomics approach and bring the hope that drug dose or alternative drugs can be chosen according to individual genotypes and phenotypes to minimize adverse drug reactions in patients.
- Drug toxicity a result from the inhibition or activity of a therapeutic target by a drug or from an interaction between a drug and a target protein different from the therapeutic target of the drug. In some cases “on target” (such as excessive bleeding from high doses of Warfarin) is observed, in other cases “off target” toxicity (such as statin induced myopathy) may observed. All genetic factors that influence drug response-drug targets, drug-metabolizing enzymes, drug transporters and genes indirectly affecting drug action can modulate drug toxicity and contribute to its individual variability.
- The statins–simvastatin, pravastatin and rosuvastatin inhibit HMG-CoA reductase to reduce LDL cholesterol levels, which reduces the incidence of heart attacks, strokes and revascularization procedure by approximately one fifth for each reduction of 40 mg/dl in the LDL cholesterol level. On the other hand statins cause myopathy (muscle pain and weakness associated with elevated creatine kinase levels) in a small number of patients receiving statin therapy. Statin induced myopathy occasionally develops into rhabdomyolysis (muscle breakdown and myoglobin release) that may cause renal failure and death. The mechanism of statin is unclear. In study of healthy volunteers, a common polymorphism of SLCO1B1 (C.5211>c, V174A, or rs4149056) was shown to

markedly affect individual variations of statin pharmacokinetics. The plasma $AUC_{0-\infty}$ of simvastatin acid (but not simvastatin) was increased more than 2 or 3 folds in persons with the homozygous C. 521CC genotype compared with the TC heterozygous or the TT homozygous genotypes respectively as a result of reduced uptake of simvastatin acid into hepatocytes via OATP1B1 in the later genotypes. Increased plasma concentrations of simvastatin aid in patients carrying the C.521C variant allele may have increased risk of systemic adverse effects and reduced cholesterol-lowering efficacy as a result of reduced intracellular simvastatin acid for inhibition f HMG-CoA reductase in hepatocytes.

- Drug Hypersensitivity Reactions (DHRs) are the effective of drugs that occurs at a dose tolerated by typical subjects and clinically resemble allergy. DHRs may represent up to one third of adverse reactions and concern more than 7% of the general population. DHRs can be life threatening, require or prolong hospitalization or entail change in drug prescription. The pathogenic mechanisms of many DHRs remain unclear. Although DHRs are unpredictable for most part genetic polymorphisms of certain genes can predispose patients to drug allergy.

  Example: The use of abacavir a potent HIV-1 nucleoside analogue reverse-transcriptase inhibitor is complicated by a potentially life threatening hypersensitivity syndrome, Abacavir hypersensitivity occurs in approximately 5 to 9% of the patients receiving abacavir treatment and is characterized by multisystem involvement. The hypersensitivity was strongly associated with the HLA polymorphism HLA-B*5701 and its combination with a haplotypie polymorphism of Hsp70-Hom (M493T).
- Carbamazepin (CBZ) a commonly prescribed first line anticonvulsant for the treatment of seizures, frequently cutaneous DHRs including maculopapular eruption, hypersensitivity syndrome, Stevens Johnson syndrome (SJS) and toxic epidermal necrosis (TEN). CBZ- induced SJS/TEN was strongly associated with a HLA polymorphism HLA-B*1502 in Han Chinese.
- The Warfarin story reveals that prospective clinical trials demonstrating that incorporation of genetic testing can induced benefit the selection of appropriate therapeutic agent and drug dose for individual patients to improve therapeutic response, reduce adverse drug effects and reduce overall healthcare cost are critical for wide clinical acceptance of Pharmacogenomic testing.

Pharmacogenomics in drug development

Pharmacogenomics can be used to improve drug discovery and drug development in at least two ways: development of new drugs to overcome drug resistance or target new drug targets, and optimization of drug metabolism and pharmacokinetics (DMPK) to minimize variation in drug levels.

- A major challenge in targeted cancer therapy is the rapid development of resistance to targeted anticancer agent as a result of frequent mutation of drug targets in cancer cells.

Applications of Pharmacogenomics

Pharmacogenomics has applications in illnesses like cancer, cardiovascular disorders, depression, bipolar disorders attention deficit disorders, HIV, Tuberculosis, asthma and diabetes etc.

Toxicogenomics

Toxicogenomics is a field of science that deals with the collection, interpretation and storage of information about gene and protein activity within particular cell or tissue of an organism, in response to toxic substances.

- In pharmaceutical research toxicogenomics is defined as the study of structure and function of the genome as it responds to adverse renobiotic exposure.
- Toxicogenomics combines toxicology with genomics or other high throughout molecular profiling technologies such as transcriptiomics, proteomics and metabolism.
- It is also defined as study of inter-individual variations in whole – genome or candidate gene single-nucleotide polymorphism maps, haplotype markers and alternations in gene expression that might correlate with drug responses.

Clinomics

- Clinomics is the study of omics data along with its associated clinical data. The term - omis generally refers to a study of biology.
- Clinomics will be a bridge between basic biological data and its effect on human health.
- Clinomics takes the next step by looking at not only the genetics of the patient and proteins associated with a patient and a disease.

Genetic Engineering

- Genetic engineering also called genetic modification is the direct manipulation of an organism’s genome using biotechnology.

- A new DNA may be inserted in the host genome by first isolating and copying in genetic material of interest using molecular cloning methods to generate a DNA sequence or by synthesizing the DNA and then inserting this construct into the host organism. Genes may be removed or "knocked out" using a nuclease. Gene targeting is a different technique that uses homologous recombination to change an endogenous gene and can be used to delete a gene, remove exons, add a gene or introduce point mutations.
- An organism that is generated through genetic engineering is considered to be a genetically modified organism (GMO).
- Genetic engineering techniques have been applied in numerous fields including research, agriculture industrial biotechnology and medicine.
- Enzymes used in some medicines such as insulin and human growth hormones are now manufactured in GM cells, experimental GM cell lines and GM animals such as mice or Zebra fish are being used for research purposes and genetically modified crops have been commercialized.
- **Genome editing:** Genome editing is a type of genetic engineering in which DNA is inserted, replaced or removed from a genome using artificially engineered nucleases or "molecular scissors". The nucleases created specific double standed break (DSBs) at desired locations in the genome and harness the cell's endogenous mechanisms to repair the induced break by natural process of homologous recombination (HR) and non homologous and joining (NHJ).

1.4.2 Proteomics

Proteomics has been said to be the next step from genomics. Proteomics is the study of the proteome. The proteome is the complete complement of proteins found in a complete genome or specific tissue.

Proteomics and genomics are inter dependent

The main aims of proteomics

- Detect the different proteins expressed by tissue, cell culture or organism using 2-dimensional gel electrophoresis.
- Store that information in a data base.
- Compare expression profiles between healthy cells versus a diseased cell.
- That data comparison can then be used for testing and rational drug design.
- Protein identification
- Protein expression studies

- Protein function
- Protein post-translational modification
- Protein localization and compartmentalization
- Protein-protein interactions.
- Proteomics is very important study because possibility of parallel analysis of multiple proteins including their post translational modifications. Discovery of disease specific proteins is possible in this technique, by targeting to specific candidate.

Mainly 3 types of proteomics are important

1. **Functional proteomics:** This type of proteomics used for identification of protein functions, activities or interactions at a global or organism wide scale.
2. **Expressional proteomics:** This type of proteomics used for analysis of global or organism wide changes in proteins.
3. **Structural proteomics:** This type of proteomics used for high through put or high volume expression and structure determination of proteins by X ray, NMR or computer based methods.

Roles of proteins

- Proteins are the instruments through which the genetic potential of an organism are expressed. And these are active biological agents in cells.
- Proteins are involved in almost all cellular processes and fulfil many functions.

Some functions of proteins:

- Enzyme catalysis, transporters, mechanical support organelle constituents, storage reservoirs, metabolic control, protection mechanisms toxins and osmotic pressure.
- Proteome is a protein compliment of genome. Proteomics is study of proteome.

Transcriptiomics is often insufficient to study functional aspects of genomics

DNA	– RNA	– Proteins
Genome	– Transcriptome	– Proteome
DNA Sequencing	– DNA arrays	– 2D-PAGE

- Some post translational modification of proteins are chemically modified or regulated after synthesis.

  Those are identified by proteomics.

Some covalent post translational modifications.

Table 1.3 Some covalent post translational modifications.

Modification	Residues	Role
Cleavage	Various	Activation of proenzymes and precursors
Glycosylations	Asn, ser, thr	Molecular targeting, cell-cell recognition etc.
Phosphorylation	Ser, thr, tyr	Control metabolic processes & signaling
Hydroxylation	Pro, Lys	Increase H-bonding & glycosylation sits.
Acetylation	Lys	Alter charge & weaken interactions with DNA
Methylation	Lys	Alter interactions with other molecules.
Carboxylation	Glu	More negative charge, e.g., to bind calcium
Transmidation	Gln, Lys	Formation of crosslinks in fibrin

Different approaches for proteome purification and protein separation for identifications by MS (Mass Spectrometer)

1. Separation of individual proteins by 2 - DE (2 dimensional proteins electrophoresis)
2. Separation of protein complexes by non-denaturing 2 - DE
3. Purification of protein complexes by affinity chromatography +SDS - PAGE
4. Fractionate by organic solvent
5. Separate complex protein mix
6. Hydrophobic membrane proteins.

2-dimensional protein electrophoresis: This method is useful to purify proteins from desired organelles, cell or tissue.

In this method first proteins are separated by 1 D, after that separated in 2 D by using stain gel and data analysis processes.

In first dimension separation, IPG (immobilized pH gradients) method allows the generation pH gradients of any desired range between pH 3 and 12. In this method sample loading capacity is much higher.

This method is useful for micro preparative separation or spot identification.

In second dimension separation (SDS - PAGE) method pour linear or gradient standards are present to separate proteins visualized gels are recorded by scanning or CD cameras.

Mass spectrometer is containing some basic components. Variations of instrument components typically used in protein sequencing and identifying experiments.

Instrument components

Sample inlet: Direct probe or stage, Capillary column liquid chromatography

Ion Source: Electrospray, Matrix assisted laser desorption

Mass analyzer: Quadrupole mass filter, Ion trap mass analyzer, Time of – flight mass analyzer

Detector

In Data System

Instrument contract system

Types of mass spectrometery

1. Maldi – TOF
2. ESI tandem mass spec instruments
   - Quodropole mass analyzers
   - Ion trap mass analyzers
   - TOF Mass analyzers

1. Maldi – TOF MS

- In these instruments samples are placed on slide, spectra generate masses of peptide ions.
- Protein identified by peptide mass fingerprinting
- But this instruments are expensive
- These are good for sequenced genomes.

2. Tandem MS (Mass Spectrometer)

- In these instruments samples are placed as solution form
- Proteins identified by cross correlation algorithms
- These also very expensive
- These are good for unsequenced genomes.

Proteomis applications: Differential display proteomics

- DIGE difference gel electrophoresis
- MP – Multiplexed proteomics
- ICAT – Isotope coded affinity tagging.
- 2 DE is a powerful technique to separate of coulees protein mixtures and analyze proteomes.

- Mass spectrometry microsequencing an identify proteins from 2DE gels and other samples.
- There are multiple databases and computer programmes available to analyze MS data for protein identification.
- Proteomics approach can be used to identify all proteins in particular sample, elucidate additional components of biochemical pathways or analyze post translational modifications at a small or a large scale.

1.4.3 Array Technology

Micro array technology is revolutionizing current drug development. Gene expression micro-array technology is benefiting all phases of the discovery, development and subsequent use of new cancer therapeutic.

Micro arrays measure the gene expression profiles of the cell. These profiles provide clues to the cells genetic makeup and response to the environment. They are unique signatures that biochemical pathways and broader cellular functions.

For example studding the signatures of tumors and normal cells may pinpoint differences that can be exploited in drug development. Genes associated with failure to treatment or poor outcome can be identified. 'metagenes' a combination of individual genes that describes a particular pathway or gene activity, can also be generated providing additional therapeutic targets.

This technology is used as a complement to other genetic methods. "The development of safe and effective drugs remains challenging". These new technologies will improve the rate at which novel molecular therapeutics are developed and evaluated and provided wealth of information.

This technology generally consists 4 phases.

1. Fabrication of the array
2. RNA isolation and labelling
3. Application of the labelled sample to the array and measurement of hybridization.
4. Data analysis and interpretation.