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In Silico Design for Drug Discovery and Development: From Bench to Market

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ABSTRACT

The discovery and development of new medicines is a long, complicated and an iterative process. The portion of drug development process especially phase III clinical trials is usually funded by industry and other private organizations. This process is so meticulous that only four to seven percent of candidate drugs receive approval from Food and Drug Administration (FDA). It takes approximately 10-12 years to introduce a new drug to the market. Knowledge of the rigorous process for drug development and of the occasional dramatic failures opens up possibilities in the determination of new drug molecule with increased efficacy. The drug development process is meant to ensure that patients receive safe and effective medicine. The rapid growth in the *in silico* drug design has gained significant momentum in drug discovery and development to predict the biochemical, chemical and pharmacological activity of new drug molecule. However, by continuing to draw on the knowledge, scientific findings and expertise of pharmaceutical scientists, as well as applying both new and established methods to drug development into therapeutic interventions will undoubtedly continue to improve chances of developing new drugs for the future. This chapter aims to summarize the main areas of drug discovery and draw attention to the huge amount of scientific effort that goes into the production and development of modern medicines before they reach the market place.

Keywords: Drug design, Drug discovery and development, *In silico*, *In vitro*, *In vivo*, Investigation new drug, New drug molecule

INTRODUCTION

Drugs substances are chemical ingredients intended for treating different diseases. Drug substances are classified as inorganic substances, organic substances derived from animal or human origin and organic substance from synthetic or semi-synthetic or from herbal medicines [1]. Inorganic substances are chemicals which do not have any carbon in their molecular formula; mostly they are in salt form. Examples for inorganic substances are sodium chloride, ammonia, all metals, etc. Organic substances derived from animal or human origin are substances which are isolated/extracted from animal or human origin. Examples for organic substances derived from animal or human origin are antigens, insulin, enzymes, carbohydrates, hormones, nucleic acids, amino acids, vitamins, fats and oil, etc. Organic substance from synthetic or semi-synthetic or from herbals are prepared by chemical reaction or isolated/extracted from herbal plants. Most of the polymers, active pharmaceuticals ingredients like paracetamol, aceclofenac, metformin hydrochloride, glibenclamide etc are organic synthetic compounds whereas compounds like vincristine, vinplastine, digitoxin, resperine, alkaloids, flavonoids, etc., are examples for the compounds isolated from herbal medicines/plants.

Drug discovery and development are the two backbones of medicinal chemistry. The fundamentals of drug discovery and development of a new drug begin from a "scientific idea". Development of new drug substance is centered on the market potential of the drug substance or medical emergency need of the existing therapeutic effect. Lot of discussions will take place from the scientific idea as a starting point in discovery research with the ultimate goal to introduce a new drug molecule into the market.

PRODUCT DEVELOPMENT TEAM

Once an organization have decided to develop a new drug substance, different groups collectively as product development team involve in the development of new drug

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molecule. The team of product development includes members from different disciplines such as medicinal chemist, toxicologist, pharmacokinetics and pharmacodynamics, clinician and regulatory experts [2]. The role and responsibility of the product development team's member are:

Medicinal chemist

The prime responsibility of the medicinal chemist is to identify a basic scaffold with his chemistry knowledge to meet the project objective, frame a synthesis process and synthesis the molecule for further studies.

Toxicologist

Toxicologist will assess the no toxic effect dose, LD_{50}, ED_{50}, therapeutic index and toxicology of the optimized lead molecule. Pharmacokinetics and pharmacodynamics – Concern of these team members are to assess and characterize the new drug molecule absorption, distribution, disposition, metabolism and excretion. Also to assess the distribution of the drug in different organs like muscle, skin, heart, liver and kidney, etc.

Clinician

Role of the clinicians are to assess the toxicity, pharmacokinetic and pharmacodynamics properties in human volunteers and establish the *in vitro* and *in vivo* correction of the new drug molecule.

Regulatory experts

Accountability of these experts is to prepare the documents towards Investigation New Drug Application (INDA), New Drug Application (NDA), Drug Master File (DMF) and commercializing the new drug molecule into the market.

CURRENT SCENARIO

Recently, the drug discovery and development have stepped in new dimensional changes of drug substances from botanicals to synthetic. For the past few decades, majority of new molecules are discovered through synthetic chemistry process [3].

The key process in the drug discovery and development of new drug substances are:

- Understanding the disease
- Target identification
- Target validation

Different stages in the discovery and development of new drug substances are [4]:

- Preclinical investigation
- Investigational new drug application
- Phase I
- Phase II
- Phase III
- New drug application
- New drug approval

DRUG DISCOVERY PROCESS

Synthesizing a new drug molecule is an art. Synthesis of new drug molecules is performed by chemical transformations either creating a new process or with the existing process. In earlier stage, thousands of compounds were synthesized and assessed for its activity. Nowadays, receptor targeted research have been used to assess its biological activity.

Several key go/no-go 'decision gates' are followed in the drug discovery and development process. Decision gate tool is utilized to assess the properties of its predetermined specification/criteria to proceed further or to the next stage of new molecule [5,6].

Steps involved in the drug discovery and developments are [7,8]:

- Synthesis
- Non clinical testing
- Non clinical pharmacology evaluation
- Non clinical pharmacology evaluation through *in vitro* techniques
- Preliminary animal pharmacokinetics
- Preliminary drug metabolism
- Toxicity studies
- Non clinical pharmacology evaluation through in vivo studies
- *In vitro* and *in vivo* correlation

RESEARCH AND DEVELOPMENT

Research and development (R&D) have been considered as the backbone of any pharmaceutical organization. The R&D activities of the organization have a large impact and the ability to execute the company's targets in time. Around 10-14 years are needed to develop a new drug molecule with an average cost of more than $800 million. Out of 10,000 compounds, only one compound will get approval by the FDA as new molecular entity due to unacceptable toxicity observed during the drug discovery and development stage.

The time duration for the drug discovery, development and approval are [9]:

- Drug discovery research: 4.5 years
- Preclinical testing: 1 year

- Clinical Trial - Phase I: 1.5 years
- Phase II: 2.5 years
- Phase III: 2.5 years
- Submission, FDA approval and Product launch: 1.5 years

COMPUTER AIDED DRUG DESIGN

Considering several issues in pharmaceutical R&D sectors, different strategies are adopted in the drug discovery and development process such as combinatorial chemistry, DNA sequencing, High-Throughput-Screening (HTS) and computational drug design. Nowadays, computer modeling and bioinformatics tools are utilized to improve the drug discovery and development process, which significantly reduce the time and resources in synthesis of drug molecules.

These computational methods consist of computer aided biological system, biological system and chemical system. Computer aided biological system includes – hit identification, hit to lead selection, absorption, distribution, metabolism, excretion, optimization and toxicity profile. Biological system includes functional proteins, monocellular organisms, multicellular organisms, cells isolated from tissues and organs. Chemical system includes ligand-based drug design, structure-based drug design, Quantitative Structure-Activity Relationships (QSAR) and Quantitative Structure-Property Relationships (QSPR) [10-12].

Determination of protein structure

A modern approach to structural biology utilizes as many methods as possible to decipher a convergent molecular picture. 3D-structures are determined commonly by X-ray and neutron-diffraction methods or NMR spectroscopy. In all cases, computer assisted data manipulation is required but, in addition, computer modeling and bioinformatics methods help research. In order to get a reliable, broad and good enough picture on structure and dynamics of proteins, a careful evaluation of the experimental data is needed by typically using a variety of techniques. The first protein structures were determined by X-ray diffraction in the early 1950s and until now over 80,000 structures were deposited in the Protein Data Bank [13]. Structure determination by NMR spectroscopy is also possible, but the number of deposited structures to date does not exceed 10,000 entries.

X-ray crystallography

One of the major drawbacks of protein crystallography is the requirement of relatively large single crystals of the target protein. Hence, it is often a tedious task and hard to fulfill. Once a suitable crystal appropriate in size and quality is found, it is irradiated by X-rays and the obtained diffraction pattern is detected and subsequently analyzed. Using the information of primary sequence of proteins, it is further evaluated and refined. Finally, a 3D representation of the protein molecule is obtained and visualized by various techniques:

I. Sample purification and crystallization

II. Data collection

III. Structure solution

IV. Model building

V. Refinement and validation

Final structures are validated by various bioinformatics methods. Neutron diffraction is a method that requires high thermal-neutron fluxes obtained from nuclear reactors and provides special information on proteins. Hydrogen atoms can be precisely located, which is almost impossible by X-ray diffraction. A diffraction experiment can be performed on a crystal; the results can be evaluated similarly, as done for the X-ray technique.

Nuclear magnetic resonance (NMR) spectroscopy

NMR spectroscopy is an important method of modern structural biology, allowing determining three-dimensional protein structures in solution, and even in case of some proteins for which X-ray diffraction does not provide enough result. It became an almost routine method for the structure determination of proteins up to about 30 kDa molecular weight. For an NMR measurement, a protein solution of at least 95 % purity is needed, which is stable over a week and has an appropriate concentration (0.1-1 mM).

Stages involved in the *in silico* drug design are:

1. Determination of protein structure
2. Target structure determination along with a promising lead
3. Optimized lead molecule synthesis, new target structure determination and its complex
4. Lead compound optimization
5. Optimized compounds and its binding with target specificity

The advantage of *in silico* studies is the speed of execution, the low cost and the ability to reduce the use of animals. The use of *in silico* methods has been an interesting strategy to accelerate the discovery of potential new drugs. In drug discovery and development*, in silico* drug design procedure is performed in two ways [14,15]. They are Structure/Receptor Based Drug Design (SBDD) and Ligand Based Drug Design (LBDD).

Structure/receptor based drug design

In this approach, cell signaling or metabolic pathway is the key process for a particular disease.

Determination of protein structure: X-ray crystallography and NMR spectroscopy are used to determine the 3D structure of target protein [15].

Homology modeling: In homology modeling, 3D structure of target protein is predicted based on the sequence [11,16,17].

Protein folding: When the similar protein sequence are not available, protein folding technique is adopted for the determination of protein. In this technique, target proteins are identified based on threading methods or fold recognition [15,17-20].

***Ab initio* (*de novo*) modeling: W**hen the homologous structures of protein are not available for comparison, *ab initio* (*de novo*) modeling technique is adopted. The target proteins are identified based on the sequence [15,17,21-25].

Ligand-based drug design (LBDD)

This approach is grounded on the previous study results of the interaction between the small molecules and the target proteins [15,26].

Virtual screening: New chemotype molecules are identified based on the process of scoring, ranking and affinity from the chemical compound databases/chemical libraries [8,17,27-31].

Two categories of virtual screening are:

1. Structure based virtual screening
2. Ligand based virtual screening

Structure based virtual screening (SBVS)

In this screening technique, the chosen molecule is docked with the selected target protein. Molecules will be selected founded docked scoring and ranking, then it will be experimentally tested for its target protein binding site [32-34].

Steps involved in the SBVS are [35]:

i. Preparation of molecular target
ii. Selection of compound from the database
iii. Molecular docking
iv. Post-docking analysis

Ligand based virtual screening (LBVS)

This screening technique is adopted when the receptor structural information is unable to predict. Different approaches in this screening technique are:

- Similarity search
- Pharmacophore-based virtual screening
- Quantitative structure-activity relationship

Similarity search

Screening of the physical and chemical similarity of the compounds in the databases.

Lead molecule is selected from the 2D score value and 3D similar structure of compounds from the database [11,15,17,25,36-39].

Pharmacophore-based virtual screening

In this screening technique, pharmacophore model is generated and assessed for its binding efficiency to a target protein and its structural features such as H-bond donors, H-bond acceptors, hydrophobic, aromatic, positive, ionizable groups and negative ionizable groups [11].

Steps involved in the 3D pharmacophore model generation are [15]:

- Binding of the active compounds to the desired target.
    a. Defining the atom types and connectivity in the 2D pharmacophore model.
    b. Defining the nomenclature in the 3D pharmacophore model.
- Identification of common feature property for binding.
- Pharmacophore model generation.
- Selection of best models based on the ranking of the pharmacophore models.
- Validation of pharmacophore models.

QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP

This method correlates the relationship between molecular descriptors of the molecules of ligand and binding target relates to its biological activity [14,40-44].

MOLECULAR DOCKING

Molecular docking is a computer algorithm, used to predict the binding between the ligand and the target binding sites [11,17,45,46].

Three types of docking algorithms are used to predict the binding between the ligand and the target binding sites [47]. They are:

a. Rigid docking
b. Semi-flexible docking
c. Flexible docking

Confirmation of the binding between the ligand and protein active site is identified by Young [17]:

i. Accurate pose prediction.
ii. Accurate binding free energy prediction.

Confirmation of the ligand in molecular docking is confirmed by specific scoring function. Specific scoring functions are:

1. Conformational search: Conformational search in molecular docking is determined by either systematic search or stochastic search [48,49]
2. Evaluation of binding energetics: Evaluation of binding energetics in molecular docking is determined by Force-field based scoring function, empirical scoring function and knowledge based scoring function [50].
3. Covalent bonds in molecular docking
4. Molecular dynamics
5. Structural water
6. Protein-protein interaction inhibitors and molecular docking [51].

MOLECULAR LIPOPHILICITY

Molecular lipophilicity is defined as log P, it has an important role in molecule permeability, bioavailability, toxicity and also the interaction between the drug molecule and target site [52].

Lipinski "rule of five" [53] is used to distinguish drug like and non-drug like molecules. Lipinski rule predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules:

1. Hydrogen bond donors (sum of hydroxyl and amine groups) less than 5.
2. Hydrogen bond acceptors (sum of nitrogen and oxygen atoms) less than 10.
3. A molecular weight under 500 Da.
4. A log P coefficient of less than 5.

PHARMACOKINETIC PROPERTIES (ADME)

Pharmacokinetic parameters like absorption, distribution, metabolism and excretions are considered during the development stage itself to avoid any future failure. Nowadays, High Throughput Screening techniques are utilized to assess quantitative structure-activity relationship (QSAR) and quantitative structure-property relationship (QSPR) properties through mathematical model to predict the pharmacokinetic parameters of the drug molecules [17,54,55].

Development of luminescence due to the interaction between the ligand and biological compound, its measurement is the basic principle of HTS.

Several methods are used to measure the luminescence. They are given below:

- Fluorescence Anisotropy (FA)
- Fluorescence Correlation Spectroscopy (FCS)
- Fluorescence Intensity (FI)
- Fluorescence Lifetime Imaging Microscopy (FLIM)
- Fluorescence Resonance Energy Transfer (FRET)
- Total Internal Reflection Fluorescence (TIRF)
- Time Resolved Resonance Anisotropy (TRRA).

Other nano-bead based techniques for measuring the luminescence are [55]:

1. Scintillation Proximity Assay (SPA)
2. Amplified Luminescence Proximity Homogeneous Assay (ALPHA)

PHYSICOCHEMICAL PROPERTIES

Physicochemical properties of the new molecules has important role in the pharmacokinetic parameter. Different pharmacokinetic parameters include [56]:

- pKa
- LogP and LogD
- Polar surface area
- Lipophilicity
- Solubility
- Permeability

PREDICTION OF PHARMACOKINETIC PARAMETERS INCLUDES

1. Absorption
2. Distribution - Blood-brain barrier penetration and Plasma Protein Binding
3. Metabolism - Site of metabolism (SOM) prediction and Metabolite prediction
4. Excretion [56-63].

Prediction of toxicity

Damage on organism or substructure of organisms includes cells and organs are defined as toxicity. A good drug molecule should not produce any toxicity and to produce good therapeutic effect. Earlier stage prediction of toxicity can help to develop a desirable drug molecule. Other toxicity study includes acute toxicity, genotoxicity and hERG toxicity.

Five steps are generally followed to predict the toxicity [64-66]. They are:

1. Data collection based on the relationship among the drug molecule and the toxicity.
2. Calculation of the new molecule molecular descriptors.

3. Prediction model development.
4. Assessing the developed model performance.
5. Evaluation.

Lead molecule is optimized from the toxicity prediction. Once the lead molecule is optimized, the real process of drug development will start.

DRUG DISCOVERY AND DEVELOPMENT PROCESS

Synthesis

Medicinal chemist will prepare the final synthesis step and perform the reaction in batch to synthesis the optimized new drug molecule for further studies. Synthesis of initial quantity in batch scale is depends on its complexity, lengthy synthesis process and yield.

Crystallization

Crystallization is a process of separation and purification of new drug molecule, which involves formation of nuclei and molecular aggregation in a solution through diffusion process. Sequences involved in the crystallization are super saturation, nucleation and crystal growth. Approximately, 1,020 molecules are arranging in an order by themselves to form a definite as crystal lattice. These crystal forms differs in its physicochemical properties also its bioavailability and stability [67-68].

Evaluation of physicochemical properties

Once synthesis and crystallization (purification) process are completed, the synthesized compound will be evaluated for its physicochemical properties. The physicochemical properties include [69]:

1. Physical form – Assessing its crystalline polymorph
2. Organoleptic properties.
3. Melting point
4. Solubility profile
5. pH, pK_a or pK_b
6. Specific gravity or bulk density
7. Spectroscopic character
8. Isomeric composition

Non-clinical evaluation

Non clinical pharmacological evaluation [2] is performed in animal models to assess its:

1. Safety and efficacy
2. Mechanism of action
3. Pharmacodynamics properties
4. Preliminary protein binding
5. Dose fixation
6. Cellular uptake and membrane transport

IN VITRO STUDIES

Recently, gene based diagnostic test kits are used during in vitro studies. For an example, to measure the HIV resistance, HIV-1 TruGene Assay is used which can provide options for AIDS patients during treatment.

Metabolism is the key determinant to produce the therapeutic efficacy. Too rapid metabolism of drug leads to lose its therapeutic efficacy. Cytochrome enzymes like CYP2D6, CYP2C9, CYP3A4/5, CYP2C19 and CYP1A2 are the key enzymes in human liver. Around 50% of small molecule drugs are metabolized by CYP3A4 enzyme. For in vitro metabolism studies, the main metabolism enzyme cytochrome P450 from liver is used due to its ready availability. For metabolic profiling, liver slices from human and animal species can be used. Gastrointestinal mucosa, brain, placenta, skin and kidney are used as non-hepatic tissues. Non- cytochrome P-450 enzymes are responsible for the reaction such as glutathione conjugation, acetylation, sulfation and glucuronidation [70].

ANALYTICAL METHOD

To quantify the concentration of new molecule in the biological sample, a suitable bioanalytical method needs to be developed with good selectivity, sensitivity and reproducibility. When response components are unavailable to quantify the new molecule, different physiological and pharmacological biomarkers are used during validation studies [71-73].

PRELIMINARY PHARMACOKINETIC STUDIES

Developed bioanalytical method is used to assess the preliminary pharmacokinetic properties of the new molecule. Preliminary pharmacokinetic property includes absorption, distribution, metabolism and excretion. Organ distribution and toxicity studies are also performed in liver, fat, kidney, muscle, skin, heart and urine [74].

IN VIVO STUDIES

A suitable animal model needs to be chosen for determining the therapeutic efficacy of the new drug molecule with the availability, selectivity, predicivity and reproducivity of the animals. Before performing *in vivo* studies, complete knowledge about the medicinal chemistry, biological property of the disease, pharmacological and ADME parameters are to be known by the drug discovery team. Further, regulatory requirements and competitive therapies both in market and competitors have to be known by the project team. In vivo pharmacokinetic parameters can help to generate dosing and schedule selection [2]. Issues in the

drug discovery and development process are shown in **Figure 1** [75].

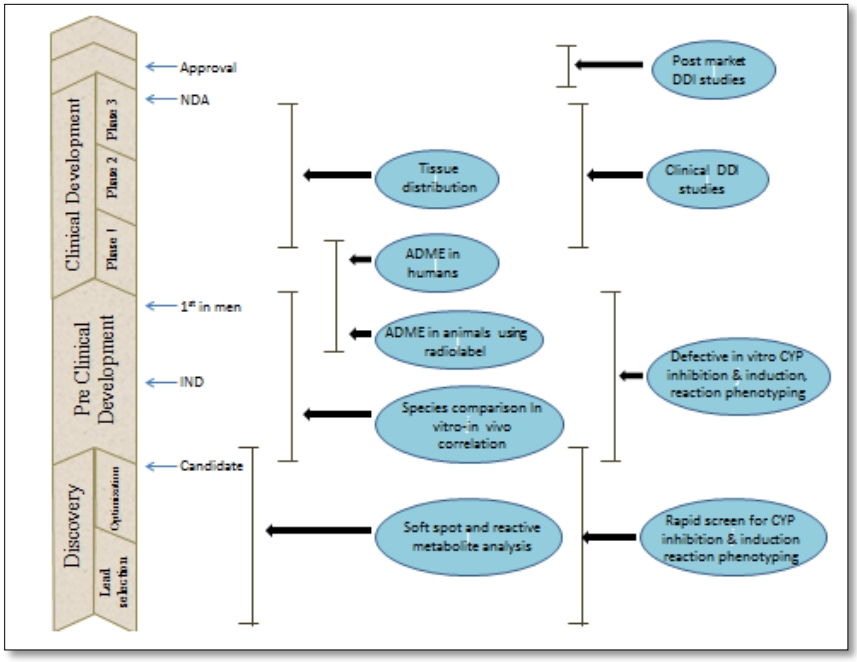

Figure 1. Issues in drug discovery and development process.

INVESTIGATIONAL NEW DRUG (IND)

When an initial clinical study results proves that the new drug molecule is safe, investigator can submit the investigational new drug application. Investigational new drug application comprises of drug chemical composition, preparation procedure, qualitative and quantitative analytical method, preclinical and clinical study.

REVIEW OF THE IND

Once investigational new drug application is received by FDA, FDA reviews the IND application. Different review committee members review the chemistry, pharmacological, toxicological and clinical efficacy of the new drug molecule. FDA gives an approval or clinical hold for clinical trials from the constructed review report [76,77].

CLINICAL TRIALS

In drug discovery and development process, clinical trial is the most critical and demanding phase. Clinical trial programme will be carefully executed in larger population to assess the drug efficacy.

Clinical trial is performed in three stages.

Phase 1: Human volunteers are exposed to a single rising dose study, a short multiple dose study and food interaction study.

Phase 2: Human volunteers are exposed to a dose ranging study.

Phase 3: Human volunteers are exposed to double blind comparative studies with registered compounds [78-81].

A survey of the drug development database suggest that the success rate for Phase III is decreased from 40% during 1999-2003 to 18% during 2011-2014 (failure in Phase III is increased to 50%). Moreover, the investment in Phase III is huge and more expensive when compared to other phases due to longer study duration, unpredicted pharmacokinetic and therapeutic effect among the human volunteers. To overcome the unpredicted pharmacokinetic and therapeutic effect, pharmaceutical organizations adopt different strategies to assess the pharmacokinetic parameters during *in vitro* and *in vivo* studies [82,83].

NEW DRUG APPLICATION (NDA)

After observing the results of clinical trials, pharmaceutical organization can submit new drug application. New drug application comprises of the chemistry, composition, strength, purity, non-clinical and clinical test results and its interpretation data, preparation procedure, qualitative, quantitative analytical methods and draft label of the new drug molecule.

The draft label consists of:

- Product description
- Clinical pharmacology
- Indications and usage
- Contraindication and Warnings

- Adverse reactions and Precautions
- Drug abuse and dependence
- Dosage

NDA review

Once new drug application is received by FDA, the FDA starts to review the NDA application. Different review committee members assess the chemistry, pharmacological, toxicological, clinical efficacy, statistical, biopharmaceutical and microbiology (if applicable) of the new drug molecule. After review by the expert committee members, a review report will be prepared and submitted. The review report includes written evaluation, observation, conclusions and recommendation/non recommendation regarding the new drug molecule [84-86].

Pre-approval inspection

The investigators from FDA will visit the applicant manufacturing premises with respect to their statements. After inspection by the FDA investigators, recommendations of the investigators, the Center for Drug Evaluation and Research (CDER) will issue an action letter stating an approval/approvable/not approvable. Any issues or deficiencies in the premises are addressed to the applicant before the approval and a time frame is provided for resubmission of the application by the applicant [4].

MANUFACTURING PROCESS AND PROCEDURE OF NEW DRUG SUBSTANCE

Starting materials and reagents

The key starting materials and reagents which are used for the synthesis should be commercially available with desired physical and chemical properties and must meet the desired specifications like USP, NF and ACS specifications.

Preparation process

Synthesis: A standard procedure with a complete diagrammatic flow chart for the preparation of drug substance with the desired purity must be given. Diagrammatic flow chart must provide quantity of raw materials, reagents, catalyst, equipment, process condition with isolation and purification of the new drug molecule if any.

Process control: A detailed process control is needed to be provided to ensure the quality (intermediate specification), quantity and purity of the material synthesis.

Quality control: Quality of the products is verified through a quality control process. Quality control tests are performed to ensure the physicochemical property of the drug substances with the desired quality control specification. A detailed specification with the acceptance criteria for impurity/degradation products and residual solvents is needed to be provided. If the product is manufactured in aseptic condition, microbiological quality control process and procedure needed to be provided. Physicochemical property of the drug substance includes description, physical properties, identification, impurity profile and assay.

ANALYTICAL METHODS

Analytical methods and its procedure are the most key parameters to ensure the quality of the product. When there is unavailability of pharmacopoeia monograph, it is sole responsibility of the manufacturers to develop of a suitable analytical method and its validation [87,88].

Manufacturing process controls

Manufacturing process controls include process validation, air handling unit and cleaning validation.

Process validation

Process validation is a systematic evaluation of the critical process in the manufacturing to ensure the drug safety and quality in a reproducible one [89].

Air handling unit

Microorganisms are present everywhere in the earth; it can damage any type of organic or inorganic compounds. A specified environmental monitoring system includes air filtration, heating, ventilation and air conditioning can provide a clean room and aseptic facility free from microorganisms for the manufacturing of drug substance. Environmental monitoring system also includes desired airflow, temperature, humidity, water and compressed gas to ensure the environment free from microorganisms [90,91].

Cleaning validation

Cleaning validation of an Active Pharmaceutical Ingredient (API) equipment/facility is important to prevent the cross contamination. A detailed cleaning validation programme includes selection of suitable cleaning agent, cleaning method, sampling techniques, recovery of the residue, analytical method, acceptance criteria, detection of residues and a cleaning report. Cleaning validation ensures that prepared products are free from the previous batch materials, unintended contamination materials, microbiological contamination as well.

Container and closure system

Suitable container and closures are selected grounded on the results of stability study in order to ensure the suitability and stability of the drug substance.

Product Label - Label of the product has an important role to guide the physician. Product label provides information about the appropriate dose, dosage regimen and adverse effects which are observed during the clinical trials.

Drug master file (DMF)

DMF is a document which provides confidential information regarding the manufacturing facilities, process, packaging and storage of the drugs.

OVERVIEW OF DRUG DISCOVERY AND DEVELOPMENT

Recently, drug discovery and development process have undergone enormous changes. After lead molecule identification and optimization, it is evaluated for its physicochemical properties followed by the determination of its therapeutic activity by *in vitro* and *in vivo* models.

There are four stages involved in the drug discovery and development.

1. Assessment of toxicity, preliminary pharmacokinetics and its therapeutic effect of the optimized lead compound.
2. Optimizing the dosage form founded on the pharmacokinetic properties.
3. Assessment of its therapeutic activity in human volunteers and patients.
4. Assessment of other toxicities like carcinogenicity, mutagenicity and reproductive toxicology study, etc., is performed.

Interaction between Industry and FDA using drug discovery and development process is shown in **Figure 2**.

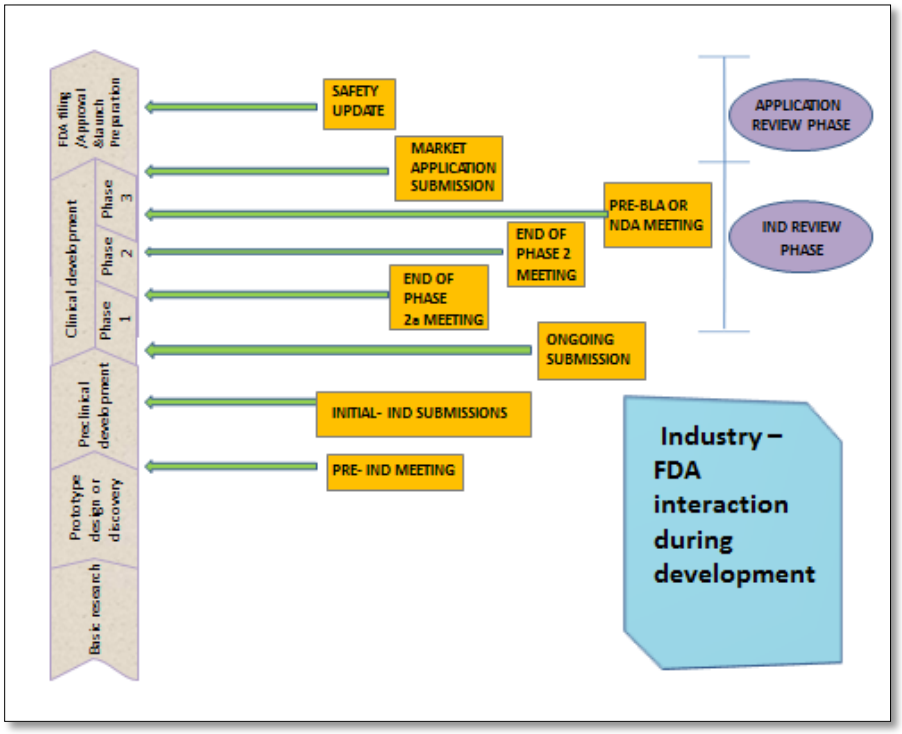

Figure 2. Interaction between industry and FDA using drug discovery and development process.

CONCLUSION

Development of new drug substances from botanicals to chemical synthetic process is a prime scientific development in the modern health care. Early drug discovery and development estimated that only 1 out of 10,000 screened compounds is approved by the FDA and it takes around 20 years to introduce a drug molecule into the market with an average cost of more than $800 million. Considering this huge cost, pharmaceutical organization have adopted new techniques in the drug discovery and development. In this review, early studies for the identification and validation of therapeutic targets and the *in silico* and high-throughput screening approaches that contribute to lead drug candidate selection which will advance the development process were briefly discussed. Also reviewed were the steps involved in the process of new drug development. As an outcome, these would result in the expansion and validation of several alternative methods that can be adopted and recommended by the main international regulatory agencies. Further, lead molecules can be identified and optimized in short duration to avoid further failures of the drug from the bench to the market.

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