Review article

Review of approaches from drug discovery process to drug targeting mechanisms

Faheem Maqbool[1]*, Amman Abid[2,] Ishtiaq Ahmed[3,4], Hafiz M. N. Iqbal[5]*

[1]School of Pharmacy, Pharmacy Australia Centre of Excellence, University of Queensland, Brisbane, QLD 4102, Australia

[2]Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan

[3]School of Medical Science, Gold coast campus, Griffith University, Southport QLD 4222, Australia;

[4] Department of Biochemistry, University of Agriculture Faisalabad, Pakistan;

[5]ENCIT - Science, Engineering and Technology School, Tecnologico de Monterrey, Ave. Eugenio Garza Sada 2501, Monterrey, N.L., CP 64849, Mexico;

***Corresponding Author:** E-mails: f.maqbool@uq.edu.au, hafiz.iqbal@my.westminster.ac.uk & hafiz.iqbal@itesm.mx.

Abstract:

Drug discovery in different time frames provides reflection in the struggle of scientists to generate synthetic products from an atural source. The complex processes exist in the development of new drug from novel ideas. The duration may vary 10-15 year with estimation cost of $1.5 billion. The stratification of different assays involved in development phase leads to the acceptability of drug molecule. In the discovery phase, implementation of powerful techniques such as combinatorial chemistry and molecular modeling has also participated in the identification of drug target molecules. The advancement in molecular biology especially genomic science has also imparted a deep role in the discovery phase. But, with bioinformatics branch, it allows us to derive more suitable points for attacking the drug molecule in different disease conditions. However, the review will describe the preclinical stages, identification, and validation of target through HTS (high throughput screening assay) and finally approval of drug molecule for further clinical development.

Key words: Targeted-drug discovery, monoclonal antibodies, spectroscopy, clinical trials, toxicological studies, Drug design.

INTRODUCTION

A process involving scientific disciplines that enable scientists to identify the new molecules (drug) having a potential to do some changes. The definition of drug discovery focuses on the development as well as the availability of some suitable products to combat against different clinical conditions. The different changes in the environment of the body motivate the initiation of the research that helps in elaborating a hypothesis e.g. the activation or inhibition of protein and some other pathways can help in the evaluation of therapeutic response in a disease condition. These types of hypothesis lead to the development of the strategies for measuring the tendency of response in the target population[1]. Sometimes, the validation of outcomes of such strategies is also necessary before next progression phase. Once a molecule has shown the valuable test result, then it is allowed to pass through drug development phase before trial phase. These steps are critically observed before the final step. All these efforts in development ultimately lead to the discovery of the drug. After achievement of successful results and FDA recommendation, now it is considered as marketed drug[2].

Historical Background:

A large number of pharmaceutical ingredients are produced by different species (plants, insects as well as fungi) in nature. These compounds are considered as drugs due to their use in the prevention of 80% of recognized human diseases. Many important therapeutic agents e.g. anticancer and cholesterol reducing agents have been discovered[3,4].

All natural products are providing a great diversity in their internal structures comparing with a standard. For this reason, it is a better opportunity for scientists to discover novel drug molecules with desired characteristics. The evaluation reports of biodiversity show that some natural compounds are in the pipeline for facing global challenges to gain in final forms[5].

After discovery, the main requirement for identified molecules is to show the desired response in target species e.g. the proteins or any substances involved in biological pathways displace position in the body to produce efficiency for drug target. Hence, different developing methods during a study of proteins expressed by genomes can lead to some major impacts in the discovery phase[6,7].

In early stages, the biological activity of different substances was screened and not given importance to the drug target. But after the identification of active molecule, a target had also estimated to describe its Pharmacology. Targeted-drug discovery utilizes the knowledge of biochemistry, structural biological principles and pharmaceutical chemistry as well as technology related approaches to explore high efficient testing of compounds and their targets to be discovered. It is seen that phenotypic drug discovery (PDD) also has a great impact on bringing innovation in drug discovery as strategies of gene-specific-targeted may reduce the risks associated with poor validation of target[8].

The working of Gertrude Elion on a group of < 60 people contributed to the discovery of Azathioprine molecule that is given during organ transplantation as it has immunosuppressant properties. Today, the most frequent approach to the discovery of drug is developing a mechanism of cloning of proteins. Thus, large libraries will be created that can be more beneficial in studying the linkage with specific diseases[9]. The advent of new technologies has made a tremendous change in efficacy of discovered molecules highly. Every novel method is promising to develop a large number of molecules with desired efficacy. Now, many companies have established a large number of unique libraries. So they are generating combinatorial libraries e.g. Pharmacopeia. The prediction from CEO of Joseph Mollica can be seen as right as there is increasing a trend of productivity from a large number of novel molecules to 100,000 per year with little change in original cost. Combinatorial libraries >1250 have also been described from industrial laboratories since 1992[10]. The concept of use of natural products had established in ancient times when people used it as traditional medicine. This perception has led to the discovery of many crude drugs. It was a time when the medicinal chemistry has been recognized as many scientists had started a research on the discovery of potent molecules e.g. the isolation of morphine from opium plants by Sertuner in 1815. Meanwhile, analytical chemistry also emerged its role in the development of many bioactive molecules from plant sources in 19th Century. No doubt, the purity of extracted molecules was not uptomark. But these approaches enabled scientists to explore their knowledge in the field of medicine. Ultimately, inthe20th century, the generation of different concepts had established in chemistry that in turn lead to theories. For example, in 1865, aromaticity in Benzene given by August Kekule influenced "Chemoreceptor Theory" that is originally proposed by Paul Ehrlich (1872-74). The advancement in concept to be more functional had been started since 1905. For example, the response of receptor toward the signals whether they were accepted or generated a response in the body had changed the attention of researcher toward the field "Pharmacology". Novel concepts were more polished to develop an idea of pharmacophore. Both the instrument and institutional support were also a key role in drug discovery as they were used for bring innovation in identifying the molecules[11-13].

In 1942, the antibiotics were recognized as a wonderful drug for combating the different life-threatening infections. The determination of the structure of penicillin imparted a new shape to a scientist for the discovery of antibiotics of a different spectrum. In 1948, an alternative to Penicillin, Cephalosporin came into existence. Some semisynthetic molecules e.g. surfactants had introduced by Pfizer pharmaceutical. The compound showed a tendency of oxidation of thiazoles sulfur to sulfone[13]. Such type of mechanism made a new pathway for development of many new drugs e.g. diuretics, antihypertensive and hypoglycemic[11].When compound had shown *in vivo* activity in different animal models, furthermore parameters evaluated to ensure the efficacy of molecule. There might be a chance of occurring of errors in Pharmacokinetics. After successful results, the drug behavior was determined in the human body.

The discrimination of Medicinal Chemistry had been established in 1950. The in vivo tests were extensively used in the understanding chemistry of molecules till 1980. After, it was captured by the emergence of new technology design in the study of drug entity[14].With the advancement of technology, another breakthrough appeared in 1975 when there was the introduction of hybridoma technology. A large number of monoclonal antibodies were produced and used as therapeutics. In 2000, 25% of drugs that are developed were mostly monoclonal antibodies[15].

Another boom had seen in the discovery of human insulin (Humulin) that was developed by DNA recombinant technology in 1982[16]. The survey was conducted in 2006, and almost two hundred prescriptions contained a drug that was recombinant proteins to treat autoimmune diseases e.g. multiple sclerosis and rheumatoid arthritis. The role of biopharmaceuticshas also emerged in the introduction of Biogenerics. They have been got approval in Europe since 2006. Baar, Switzerland offer the products Valtropin and Omnitrope. The USA has also approved Sandoz's Omnitrope that is biogeneric with Genotropin. But today, the great diversity of generic industry and different companies has agreed to bring innovation in molecular level. Some drugs (Binocrit, Hexal, and Abseamed) similar to erythropoietin had been adapted as marketed drugs since 2007. These all are still used in the treatment of anemia[17,18]. The role of Pegylated (Peg) interferon α-2a in prevention of hepatitis B and C has also been studied. The data showed the achievement in maintaining the low viral load in HIV treated patients. However, the relevant study is needed for appropriate preventative strategies for patients having early stage of infection with HIV[76].

Finally, we can say that whole process of drug discovery involves intercorrelation with all displaces because each discipline provides expertise and knowledge, both. Ultimately, all disciplines brought together will give successful results. However, if we want to achieve a drug molecule that is highly efficacious, then three key points (the molecule, its target and its access to target sites), should be focused. Some other considerations e.g. interaction of the drug with its target, biological pathways, and pharmacokinetic parameters describe the drug specification as well as drug behavior[19]. It is necessary to overview all disciplines that can provide important information related to drug therapy. Sometimes, to capture mechanism of action after identification of disease, can be helpful for the scientists to derive their knowledge toward the successful development of the drug entity.

In early era, most of the drugs obtained from natural sources did not provide any useful therapeutic response. Later on, a more focus is given to the development of clinical trials to determine clinical response along with drug toxicity. The advancement in genomics has also contributed to involvement in the better identification of the target of the molecule to disease. Now in the modern era, a close relationship between pharmaceutical companies with innovation in scientific disciplines has encouraged the researcher to adapt "multifaceted approach" to bring improvement in the discovery of new molecules[20].

Identification of target

Target identification is considered as the first step in the discovery of the drug. It is seen that the tendency of the drug to show apoor response in the clinic is due to non-working and poor safety of the molecules. To introduce the drug in the market, the most important step for the drug is to have a full affinity toward the target to elicit the response e.g. binding with the receptor to activate gene or RNA. It is necessary that target should be safe, efficacious and meet clinical needs. A drug is a biological target in which molecules (small or large) have high affinity to thetarget site, upon binding, different biological response can be measured via both *in* vitro and in vivo.

The relationship of the target and disease can be judged by identification of the target which enables us to understand mechanism-based phenomena whether an attachment of the molecule to the target sites will lead to side effects or efficacious response. Both if the target identification, and validation will meet the needs, the confidence level will also increase. It is also seen that data mining of biologically available data is also helpful for the reorganization of the target. Data mining is a tool in which we use biological information approach to identify, select and then prioritize some disease-targets[21]. Such type of data is obtained from different sources e.g. publications, proteomics data, transgenic phenotyping data, gene expression data and compound profile data. The other approach e.g. mRNA level is examined to check the co-relation with disease progression or exacerbation, and it may express in the disease state. The genetic association is also a powerful approach to explaining the linkage between dissemination of disease and genetic polymorphism e.g. mutation in presenilin gene or amyloid precursor protein can be seen inpatient of Alzheimer's disease. The result of the mutations is production and deposition of Abeta (peptides) in the brain that is a feature of Alzheimer's disease[22]. In human, mutation has also important role because of over-activation of the receptor or sometime nullify the response. For example, a mutation in NaV1.7 (voltage-gated sodium channel) causes a decline in expression of nociceptive neurons that show the impact on the sensitivity respectively[23,24].

Another approach is phenotypicscreening that is used to identify targets related to the disease. The data from an experiment shows the human monoclonal antibodies that are derived from phage display antibody library. Such type of antibodies has an ability to bind with target surface of the tumor cell. Immunostaining method was used to screening clones. Mostly chosen were those that had great potential to stain the malignant cells. Immunoprecipitation was a technique used to isolate the antigens (that are recognized by clone cells), and identification was done by using a mass spectroscopy. Out of 2114 MAbs (having unique sequences), only 21 antigens of high expression on different carcinomas, were identified. In this way, different Monoclonal Abs against several determined-targets may become useful for the therapeutic purpose[25].

Rationalization of target

After recognition of the target, the next step is a validation of the target. Different tools have been adaptedto it for optimization of desired target sites. The overall aim of validation is to provide confidence of accuracy of target and targetedmolecules. Both in-vitro and in-vivo techniques operate side by side, and the evaluation of the results gives the precise decision of optimization of the target. For example, antisense technique which is considered as a more powerful technology that utilizes oligonucleotides (chemically modified like RNA) that are designed to be complimentary to a region of target-mRNA[26]. in this way, encoded proteinsynthesis is blocked by an attachment of antisense oligonucleotide with target mRNA. Therefore, a translation mechanism is also hindered.

We can observe researcher demonstrated the power of this technique as the development of antisense probe to the P2X3 receptor[27]. The study shows that function or analgesic response can be returned after the discontinuation of administration of antisense oligonucleo--nucleotides. We can find more reversibility of the effects with antisense oligonucleotide as compared to gene knockoutapproach. Continuously presence of antisense achieves the target to protein inhibition[28]. Sometimes, toxicity and less bioavailability of developed chemically oligonucleotides create a problem e.g. in-vivo, use of such oligonucleotide can express undesirable action. This type of situation has been occurred due to lack of variety and diversity in choosing appropriate nucleotide probes[26]. For the validation, transgenic animals can also be used as they are more attractive tools that provide the assay of the whole animal as well as the full judgment of phenotypic-endpoints to explain the consequence of gene manipulation.

The idea of producing of gene-targeting animals having a lack of function of agene from beginning to throughout life enabled the scientist to use in-vivo tool to determine the function of different genes. For example, the confirmation the role of anion channel in the maintenance of inflammatory pain was possible after the use of P2X7 in mice[28].

Whereas the mice are having a lack of P2X7 receptor had shown the complete absent of both neuropathic and inflammatory hypersensitivity in response to mechanical stimuli.While these type of animals were also used to predict the mechanism of action of the drug. The study showed that some transgenic animals were not releasing the mature pro-inflammatory cytokines (IL-1beta) from the cell. Even though, no deficit was found in IL-1beta messenger RNA expression.

Gene knocks (in) is also an another alternative to gene knock out. The replacement of non-functioning enzymatically protein to endogenous protein is clearly seen in this approach. These animals had shown different phenotypes to knockout e.g. enzymatic and structural functions exhibited by protein[29,30]. The appearance of true for mice mimic the more closely what were happened during the treatment with drugs. The actual protein was there but not showing functioning. These types of approaches are mote challengeable. There may be a reason involving an avoidance of compensatory mechanism or developmental phenotypes. It is also due to the requirement of overcoming the embryonic lethality of homozygous nullanimals. But in another

sense, the use of transgenic animals is not fit due to expensive as well as time-consuming. The solution of all is that we will have to use si-RNA (small interfering RNA) because it is now considered as a popular approach for target validation. In this, the first step is to the introduction of dsRNA (double stranded-RNA) having specificity to gene into a cell. Then, it is identified as exogenous material. In the end, the activation of the RNA pathway starts. The activated ribonuclease protein Dicer is then attached and cut dsRNAs to generate fragments of double stranded (21-25 base pairs with some unpaired bases on each end). These generated fragments of short double stranded are known as si-RNA that is then quickly separated into single strands. Then, integration leads to the formation of a complex that is known as RISC (RNA-induced silencing complex)[31]. But the major problems have been reported e.g. the problem of delivery to the target. Anyhow, a delivery system with both viral and non-viral is still under investigation phase[32].

There is also an excellent tool for target validation that is monoclonal antibodies because the interaction of such MAbs with a larger area of target molecule's surface is greater that in turn lead to better understanding between closeness and affinity of molecules. On the other hand, the no crossing of the antibodies causes the restriction of the target to the surface of the cell. For example, the study confirms the efficacy of MAbs that has been determined in in-vivo, that is"function neutralizing anti-TrkA antibody MNAC13 reduces the both inflammatory and neuropathic pain hypersensitivity"[33].

Hence, we can consider best tool for target validation is the small bioactive entity as there is the appearance of interaction with and modulation of effector proteins. Recently, the field of chemical genomics in which we study or observe the responses (genomic) to chemical, has also been emerged in both target identification and validation. In the drug discovery phase, the identification of novel drugs and their targets goes to next phase of validation, designing and biological testing. The chemical genomics is overcoming the problem, occurring in drug discovery phase. The main objective is to find the chemical molecule of optimized efficacy in a disease condition. Genomics provides great diversity in structure based designing of the drug. This approach is also effective for those target classes that contain members of structural-related and are considered to be having therapeutic application[34].In the end, we can summarize the overall aim to use all of these tools is to evaluate cellular function before going to next phase.

Discovery process:

"Hit" molecule describes different meaning, but for the researcher, it is defined as a compound having the desired activity that can be reconfirmed upon retesting[35].There are different screening examples that are used to identify hit molecules. For example, the screening of molecule against drug target is donewith the help of HTS (high throughput screening). Sometimes, the complex system e.g. cell-based assay, although, its activity depends upon the target, but it requires secondary assay to provide confirmation the site of action of the molecule. However, the use of complex laboratory is needed in the screening where it is ensured that there is no prior

knowledge about the activity shown by molecule at the target site (See figure1 and 2). If we know the tendency of activity toward the target site, then we select from chemical libraries that in turn to produce their desired action at the target site[36]. The study shows that most drugs are developing and gaining acceptance using HTS method and pharmacophore based screening. The virtual screening methods, especially HTD (high-throughput docking) are mostly used and need to be refining it[37].

Fragment screening, considered as an important technique for drug discovery institutes, has an involvement of production of very small M.W compound libraries that are screened at high concentration. The evaluation of small molecule fragment represents an alternative approach as it permits control of compound properties.

Physiological screening should also be focused. It is a tissue based, and the observation of response in tissue is measured to identify molecules that show interaction with targets e.g. contractility of muscle. Different approaches such as chemistry are used for developing potency and selectivity highly to target. The evidence should be fully supported the efficacy of drug target in the disease state. Pharmaceutical industries have now established the objectives of identifying, assembling and infrastructure the molecule for screening. In this way, we can finally identify and optimize the hit molecules (from screening models) that turn into clinical development. News assays are developed by the academic scientists for the screening of drug that are passed on the different centers e.g. academic drug discovery center. The participation of academic sectors in drug discovery has also helped the pharmaceutical industry to introduce the molecule of optimized target validation. The success story behind this approach depicts the transfer of skills between academic and industrial sectors[38]. The different activities performed in the pathway of the discovery phase, starting from biological assay to identify molecule with the desired action at the target site. The hit molecule is termed as the output of screening compound showing a specific action at target protein. In the discovery phase, clinical safety is determined after screening of molecules with the help of cell-based assay in a predictive model of disease state and animal model of the disease[39].

Drug development phase:

There is a similarity of drug development with evolution. Many compounds are shifting their attitude to become more successful drugs, pass from a selection process with a high level of confidence. Many of the molecules are modification of their earlier generation e.g. first, second and third generation. These types of the happening of molecules do not appear from reproduction process. Variation (key to evaluation) is not in short supply. In the USA, the National Cancer Institute has screened many natural products for determining the activity e.g. 16000 compounds from marine, 180000 from microbes and 144000 from plants. There is also a role of the pharmaceutical company that can hold a list of 2 million molecules, available for measuring the biological activity. However, the variation in risk and benefit ratio of the profitable molecules can bring a successful selection of the molecules.

Drug development is seemed to be very slow. The data from regulatory board of US and EU depicts the number of the application that they have received from time to time. For example, the application was 131 in 1996 as compared to 72 that were in 2003 and the data of 2009 was 48 applications. The approval from FDA was recorded as in ratio of 56,27 and 25 in subsequent year[40]. The decline trend focuses our thinking to endangered species. Then, it becomes more necessary to determine deteriorating environmental factors for the purpose to decline extinction[41,42].

It has been seen that that the success rate in EU for processing applications have never been too high. The reported documents give the percentage regarding acceptance of the application that are 40% in 1996, 29% in 2003 and 60% in 2009[43]. The drugs approved in 2014 were reported as 41, as compared to 45 drugs that were in 2015; approved by The Center for Drug Evaluation and Research (CDER). Although, in1996, a strange year in which regulatory board approved 53 drugs. The rate of approval is noted as doubled the acceptance rate during 2005-2009[44].The main objective in development should include the enhancement of therapeutic efficacy. Now a day, a competitive environment in pharmaceutical industries allow them to invest a large amount of money even in an expensive phase 3 trials in order to innovate a molecule of optimized standard. FDA also ensures its responsibility in evaluation of all parameters regarding the study of drugs e.g. clinical trials, toxicological studies. All these protocols should be ensured before starting the experiment in human[45].

Most industries have a main focus on different biological approaches e.g. MAbs or gene therapy for targeting specific patients. There is also a need for expanding our knowledge of human genome e.g. 1/3rd part of approved drugs was antibodies, enzymes and peptides in 2015 as compared to about a quarter in 2004[46].

Evaluation of development (Assay development)

The majority of the assay, used by industries, rely upon the recombinant expression in which drug target is expressed to establish the biochemical assays e.g. HTS has been used in helping the screening of the molecules.[39]Cell-based assays are mostly adapted for target classes e.g. ion channels, membrane receptors etc. Such type of assay shows many advantages such as multiplexing, miniaturization and possibility of automation still this tool present more challenging. It exhibit more demand on the early stage of drug discovery. It shows result that can be read out as a consequence of chemical activity[47]. The biochemical analysis that has been applied to targets (enzyme or receptor) for simply observing the affinity of test molecules for target protein. Although benefits still exist, but there is need of debate regarding to merits of both biochemical and cell-based assay[48]. The success in identification of hit and candidate molecules can be easily achieved by both assay models (mentioned above). The different formats have been used that are helpful for compound screening. But the choice of format for assay depends upon the many things e.g. the experience of scientists, biology of drug-target protein, equipment used in laboratory and the need of an activator or inhibitor. In G-protein coupled receptors (GPCRs),

compound screening assay can be used to determine the binding ability of radio-labelled ligand to the receptor, to determine the activation of gene, to observe the exchange guanine nucleotide at point of G-protein and to observe the changes during in one of second messenger e.g. cAMP. When there is a selection of any format, following parameters should be considered: pharmacological assay, cost assay, assay quality and effects of compound in assay. In pharmacological assay, main focus is to identify the mechanism of action and desired therapeutic response. Assays are mostly performed in microtitre plates that are formatted in 96 well while in industry; assays are formatted in 384 or 1536 well. It is necessary to minimize the cost of the assay to select the reagents of minimum cost in each case. Z-factor should be considered in the case of assay quality[49]. This factor, ranging from 0 to 1, has become significant in industry for standard means of measuring quality of assay. Pharmacological assay has a great impact as if it lies within desired limits, then assays should be considered acceptable. Many factors influence the assay quality. Mostly, implementation of simple assay procedures should be used in creating a high quality assay e.g. to minimize the plate to plate transfer of reagents, to use of stable reagents and to ensure the working of all instruments of optimally. Definitely, it is achieved by adapting QC practices for all items used in laboratory. The libraries (chemically) are stored in solvents e.g. ethanol etc. The level of assay should be in such a way that it shows no sensitive to concentration of solvents. The biochemical assays are usually performed in solvent (concentration are upto 10% DMSO). In other side, cell-based assays show fully intolerance to solvent (concentration are greater than 1% DMSO). Different studies are also conducted in assays to establish false +ve or false –ve hit rates. If there is finding of unacceptable, that are high, then there is a need for reconfiguration of assay. Molecule screening assays are run at 1 to 10μM molecular concentration for hit discovery. The variation in test cons. can be helpful in the identification of compound with lower or higher activity. For example, use of HTS technology for purpose of identification of hit molecules (having activity at GPCRs) is aequorin assay[50]. Basically, aequorin is a bioluminescent protein that is Calcium sensitive, cloned from Aequoreavictorea (jellyfish). Now we create stable cell lines, then transfect to show expression in the GPCR-drug targets as well as aequorin biosensor. For the receptors having coupling ability to heterotrimeric G protein of Gαq/11 family, an increase in the concentration of intracellular calcium has been resulted after the ligands activation. An increase in concentration of intracellular calcium was detected when expression of aequorin was implicated in same cells as it was due to binding of calcium with aequorinphotoprotein. But the presence of coelenterazine as a cofactor resulted in the production of light flash. The detection of this phenomena noted in a microtitre plate-basedluminometer e.g. Lumilux platform. A simple protocol has been adapted to such type of assay. It has been developed for HTS in 1536 well plate in 6ml volume of assay. The activities of the molecules-profiling have been performed in 384-well plate format. Anyhow, when any HTS assay has been developed whether the requirement of screening of molecules are large in number, it is better to screen the training set of molecules to ensure that the performance of assay is upto the mark or acceptable. The study was conducted for screening of twelve thousand compound training set against the H1-receptor, expressed in CHO cell (Chinese

hamster ovary) in 1536 well format assay. However, the running of training set was done on three occasions to recognize the hit rate as well as the false +ve or false –ve hit rates. In all these cases, statistical approach selected for determination of above parameters. In aequorin assay, the hit rate was less than 0.5% as compared to statistical assay for screening of compounds. A tendency of agonist-signal observed as less while in standard agonist ligand, it was good. But false +ve or –ve hit rates were low in this assay format.

In the case of screening of the antagonist, the hit rate in aequorin assay was in 2 to 3%. It is seen that hit rates in antagonist appeared as higher as compared to hit rates in agonist assay. This type of activity is described by the appearance of a reduction in assay signal that also detects the molecules are having some interference in the generation of signals. It is said that assay plates (upto 200) are screened every day during HTS although using complex laboratory automation. The performance of the assay is also evaluated according to "Z" and variance in the pharmacology of standardized molecules during the screening of molecules. These two points are measured with assay plates that have failed or rescreened if the measurement of QC falls outside the predefined limits[49].

Advancement in the drug design and development process:

With the passage of time, there is need of advancement in drug development process. This challenge appears to be slow due to complexity in drug discovery process. Some uncertainties may show association with pharmaceutical industries. The more advancement in scientific discipline e.g. organic synthesis, genomic, cognitive science, some computational methods, and chemobioninformatics, are resulting in taking a boom in drug development process. The innovation can be achieved by adapting modern biological, chemical and pharmacological principles. The understanding regarding target validation comes in pharmacological approaches[51]. Epigenetics that have gained more interest in drug discovery because there is therole of epigenetics in combating different diseases[52]. Targets have also been validated for drug therapy[53]. For the development of new drug entity, a large number of resources are invested in different companies. The main aim is to provide the satisfaction of the properties (physicochemical or pharmacological) and developing methods regarding new drug entity[54]. As there is rapidly production of analogs, the structural modification helps in the development of new molecules. Sometimes, an alternative approach is adopted in which there is theuse of pharmacophoreto direct divergent C-H (functionalization) of lead molecules[55].

Mostly scientists are carrying out research related tasks after using routinely and easily accessible different approaches such as computational methods, cognitive science and an updated bank of information (e.g. Google). Some factors e.g. target-ligand match-pairs (TEMPS), biomarkers, matching molecular-pairs (MPPS) and the biological assay can provide information for identification and validation of target as well as optimization of the process[56].

However, the updated data of selected drugs, compound libraries and symmetry of molecules should also consider and implied in identification phase through in vitro and silico screening against targets[57]. Docking programs and molecule-modeling are also strengthened and applied to help in both identification and optimization of the process. The program for measuring "ADMET" should be determined. In this way, computational tools are allowed to daily practice as they are considered as complementary components of the design process for the drugs to explain innovation and creativity. Such type of practice is routinely adopted successfully by different companies. For example, for prediction of ADMET properties, the Schrçdinger Software Suite Quickprop is used, and for molecular docking, Glide is used[58].

There should be necessary to make precise rules or guidelines for determining the properties of compounds and for screening a suitable drug. Various important factors i.e. indication, method administration and structure of compound should be intensified in the case of molecular criteria. Industrial natural product chemistry has a great importance for product development because the source of approximately 80% of commercial medicine is from natural products. Some natural products e.g. taxol and vancomycin are also inspiring products although they lie outside the rule of five[59,60]. Both collecting and sharing knowledge among scientists should be adapted. The ultimately, it will result in better productive and cost effective both drug discovery and development. For scientists having specialization in medicinal chemistry, their work can bring improvement in discovery stage to yield the suitable drug candidate. The more important step is to ensure the quality of libraries of the compound for any biological screening to be improved.

However, the undesirable properties have been observed as in the case of excess of aromatic moieties[61,62]. That is why it is necessary to get rid of such type of moieties in the library of a compound or it should be limited. However, selected-ligands that have an optimized efficacy may consider as acceptable compound because early modifications are inserted to achieve the more favorable properties. The natural products due to their early success as drugs and the biological tool should be the most integral part of such a compound library. The study shows that the %age of NP, NB, and ND of Total drug approval globally are in the range of 30 to 50%[59,60,63].

The motivation for drug designers may be the presence of three-dimensional structures of the molecules as well as the chiral center in the molecule. The potency, diversity and biological properties can be explained from chirality or dimensionality of biological receptors[56,54].

Initially, high investment along with longer plan is needed in the understanding of the chemistry of natural product. The discovery of ADCS (antibody drug conjugates) has lightened the scientists to reinvigorate the chemistry of natural substances through new directions and heavy investment by industry. The use of modern biotechnology and high-techbiological screening of discovered molecules and trials in living things are some important facts that generate data for the investigator to describe their future role. Structural pattern of molecules

hasbeen discovered that impart some beneficial properties in molecules of the drug[54,55,65]. Some halogen or halogen-containing substances have also been found to improvement in both the pharmacokinetics and pharmacological properties. For example, higher metabolic stability or target affinity can be achieved easily with the placement of Fr or Cl within drug molecule. In another case, the addition of Br or I results in strong halogen bonding that ensure the improved selectivity. But their inclusion in the molecule is seen as less because of toxicity, depending upon the frequency of dosing regimen e.g. interference with TH-receptor. The method such as Organic synthesis is desirable in which halogen entity is added as it helps in choosing the best drug candidate. No doubt, initially involvement of more time and effort include, but outcome obtains as generation and development of the potential better drug[66,67].

Now the delivery of the drug is resulting in a study of a large number of reactions that is responsible for assembling the families of compounds that in turn lead to the selection of optimized drug candidate. The scientists in the field of discovery phase have shown the courage to accept a wide range of newer exotic reactions to shift their thinking beyond the traditional "flatland" compounds[62]. The organic synthesis has also excelled researcher's knowledge in the art of discovery as they are easily solving the restrictive model. The researchers should be utilized the full range of technologies along with the improved synthetic methods to develop the new molecules of high quality and efficacy[68,69]. There is also a need for assessment of different structural pattern with the help of chemical reactions. In this way, the replacement of traditional aromatic compounds e.g. benzenoid rings can provide an improvement in pharmacological properties of the drugs[55].The newly advanced techniques such as flow and microwave technique should also be implemented in drug discovery process. The main objective to improve a biological process or pharmacological inputs should be focused. The continuous advancement in sequencing is motivating the researchers to understand human genomes of normal or disease-associated to develop novel drugs that have aimed to reach their targets with anew mechanism. The use of such approach can be easily seen in cancer area because of identification of mutation genes, acting as disease drivers[70]. The conversion of undruggable to drugable is also a challengeable task. It can bring the innovation of some potent molecule having a desirable biological target. Although risky targets for academic work are the ideal choice, the outcomes after completion of the project are eminent in the discovery phase[71]. As we know that the main primary source of tumor growth is cancer stem cell, but measuring the drug resistance gives us ways to observe progression in cancer chemotherapy. Hence, the anticancer drugs have been discovered because of eradication of cancer from individuals after the destruction of cancer stem cells. To do this, there is a requirement to determination and characterization of cancer stem cell subtype from biomarkers. The target of the drug to destruct cancer cells is closely observed in the case of drug development process. The patient-derived xenografts (PDXs) model, a powerful clinical predictor, gives scientists to broad challenges in the field of cancer's biology as well as its complexity also encourages the pathologists, chemists, and biologists to find ways to success in the discovery of personalized medicines through collaboration. The collections of genetically annotate-tumor are being offered by a large number of vendorsfor PDX model testing. As a

result, investment in clinical trials has been reduced, and better drug molecules are going to be scrutinized, which in turn shift into personalized medicine in drug development process[72,73]. Both the improvement in drug development process and mutual sharing of knowledge are important key points that will ensure clinical efficacy and overcome the current status as academia. The aim of Academic Drug Discovery Consortium (ADDC) provides collaboration among the scientists in different university discovery center to design and develop the novel drug[74].However, the replacement of term "integrated-pharmacy Company" with "integrated pharmacy network" should be adapted to improve productivity as sharing and utilization of information from compound libraries and websites by companies or Government institutions lead to finding new ways to develop paradigms to sustain profitable pharmaceutical industries[75].

CONCLUSION

It is clear that the challengeable issues emerge in the field of discovery. However, it mobilizes the scientific community to interact and make more advancement in academics to investigate a molecule with new advanced technologies. Pharmaceutical industries are providing friendship environment for investigators to bring research activities in their fields. The biological target should be validated. The preclinical models should be competent to explain efficacy and, the safety of the drug. But, the occurrence of some failures in clinical trials is recognized as a cause of hindrance to drug development. It can be minimized by developing unique techniques to support the clinical efficacy of the drug. The development in genomic level also helps in improvement disease –target identification. The most important step in drug discovery for improvement in finding a new molecule is possible with the adoption of advanced principles of science as well as the close collaboration of scientific disciplines with the pharmaceutical industry.

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Figure 1: Steps in Drug Discovery process

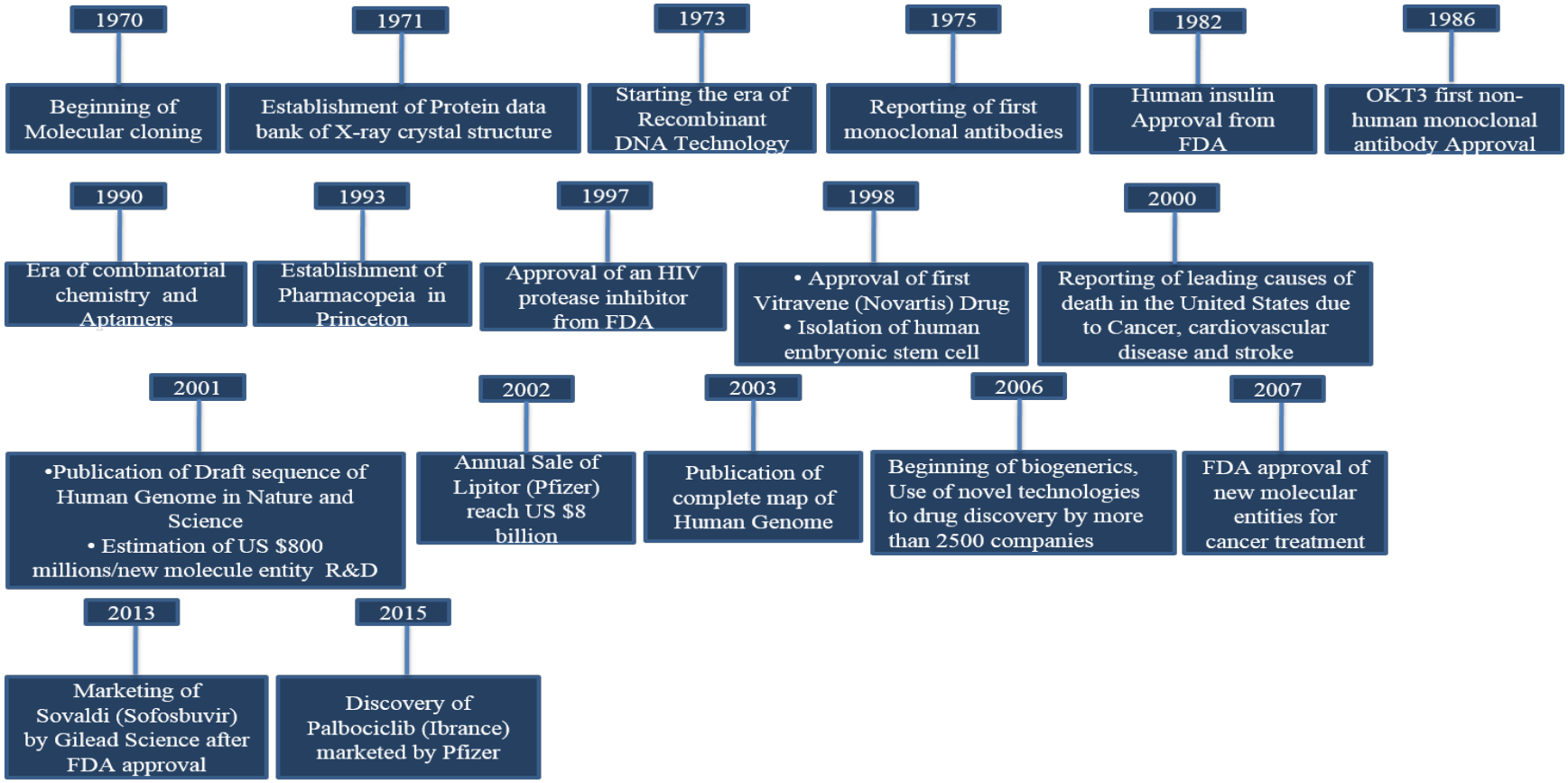

Figure 2. Important medical discoveries