

Target Based Approach A Way towards Protein Structure Determination in Drug Designing

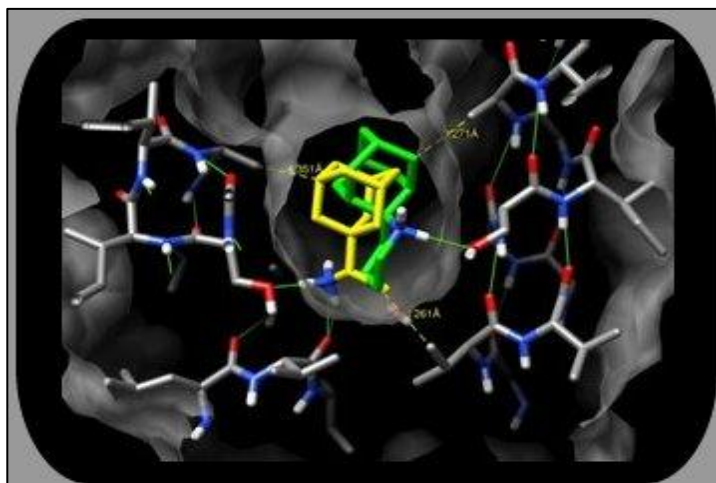
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ABSTRACT

Drug designing is a field of pharmaceutical chemistry, pharmacology & biotechnology by the process through which potential new molecules are identified based on target & being developed into novel compounds. Drug designing started from Serendipity to Rationality (i.e. identification of targets followed by screening of hits & optimization of hit molecules). Target is a special site for substrate or drug molecules which binds to it & leads to changes in physiological response. There are types of target for which a drug molecule can be designed. Thousands of drug molecules are been developed based on determination of targets for various diseases. Targets can be examined by use of different computational tools like X-Ray crystallography (XRC) & Nuclear Magnetic Resonance (NMR) technique. After the determination of target its validation is an important step to be carried out by particular bioassay. In the recent years cancer as an example has become a major cause of death for which various therapies are already available but still there is need for newer therapeutic approach in treating cancer. Identifying & determining the structure of targets in cancer cell would lead us a way towards designing drugs for cancer treatment helping in reducing the cost for therapy. The limitations in drug designing can be overcome by "Target based drug designing".

Graphical Abstract:



Abbreviation:

TBDD: Target Based Drug Designing, XRC: X-Ray Crystallography
NMR: Nuclear Magnetic Resonance, CADD: Computer Aided Drug Designing
VLS: Virtual Ligand Screening, CSP's: Chemical Shift Perturbation's
HSQC: Hetero-nuclear single quantum correlation, HER-2: Herceptin-2 Receptor
EGFR: Epidermal Growth Factor Receptor

Keywords: Drug designing, drug targets, Cancer, computational tools (XRC and NMR), rationality, and target based drug designing

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1. INTRODUCTION

Drug molecules helps in treatment & prevention of disease [1]. Pharmaceutical chemistry, pharmacology & biotechnology is a branch of science through which drugs are discovered or designed [2]. Previously drugs were discovered using substance occurring naturally in the environment. Drugs are also prepared by chemical alteration of natural substance. An increase understanding in nature of disease, how biomolecules work & how drugs influence the responses has led to deliberate design & synthesis of new molecules [2, 3].

Drug design [4] is an integrated development which involves era of tailoring of drug molecules. It involves the study of effect of biologically active compounds on the basis of molecular interactions in terms of molecular structures or its physico-chemical properties & based on these drug designing is built up [3].

In earlier day's randomized or virtual screening procedure were involved in new drug design which led to generation in number of chemical compounds but it's been no longer effective, time consuming, guarantee of little success & is expensive too. The discovery/designing of new drug molecules has diminished to 1 in 10,000 & will decrease further [3]. Now a days the development of molecule costs for more than \$2.6 billion per single drug [5, 6]. As per the need, drug designing can be revolutionized with the help of genomics, proteomics, bioinformatics & efficient technology which helps in target based drug designing of new lead molecules [7, 8]. During early 1980s, the rational drug designing using biomolecule structures was an unrealised idea for biologists. In early 1990s the first successful stories were published [5, 9]. Computational techniques like NMR & XRC are used in determination of various biomolecules (receptor). Basically it is useful in simplifying the receptor structure for determination of new drug molecules [3, 4]. Target based drug design (TBDD) can be powerful part of an entire drug discovery process. As per current definition we could rationally predicted as there are 5,000-10,000 potential drug targets are present in human body, hence

TBDD will be a promising method in drug designing [10].

Basically this review is to provide basic knowledge related to the process of TBDD which allow us in selection of the targets & evaluation of same for generating newer drug molecules with the help of different computational techniques with less in cost. Target based is a tailor-made approach & it also involves the study of biodynamics of a drug besides the interaction between drug molecules and biological targets.

2. Landscape of Modern Drug Designing:

Historically, drugs were discovered by identifying the actives from traditional remedies or by serendipitous discovery [2]. In modern period many research group & the scientist have dedicated their efforts towards Target Based Drug Design (TBDD) & Virtual Ligand Screening (VLS), as promising approaches to identify ligands (drug molecules) for biological targets [11]. TBDD provides insight in the interaction of a specific protein-ligand pair, allowing medicinal chemists to devise accurate chemical modifications around the ligand scaffold, whereas the strength and versatility of VLS are self-evident as it can be used to filter compounds to be assayed based on any rationale that can be expressed in terms of molecular properties (pharmacophores and/or physico-chemical descriptors). In VLS studies, docking programs can be used to filter libraries of compounds. However, it remains extremely complicated to rationally analyse docking results for libraries containing chemically diverse compounds. TBDD offers advantages compared with the classical ligand-based techniques such as the comparative molecular field analysis and pharmacophore-based techniques which are based on only ligand information has some limitations [1, 12].

Since in early 1990s, the TBDD paradigm has become an important approach for pharma industries [10]. TBDD reveal information on the structure activity relationships (SAR) of a chemical series of compounds, especially in the lead optimization phase, when accurate modifications are needed to adjust an ADME/toxicity profile while maintaining binding affinity [12]. An X-ray structure & NMR technique is available for the target determination, or that a 'reasonably' valid

homology model has been constructed by using CADD [3]. Knowledge of the target structure plays an important role as providing maximum possible sites where the drug molecule can bind, and the particular loci and orientations where hydrophobic and/or hydrophilic interactions can be engaged [12].

3. Drug Target Identification & Functioning:

3.1 Drug Target: A drug target can be defined as a single gene, gene product or molecular mechanism i.e. Genetic target [13] or Mechanistic target [14], which is identified on genetic analysis or biological observation [6]. Drug target is a specific binding site for a drug in vivo through which a drug exerts its action. Target identification is the first key step in drug designing pipeline. The drug target has following characteristics [2]:

Types of Targets:

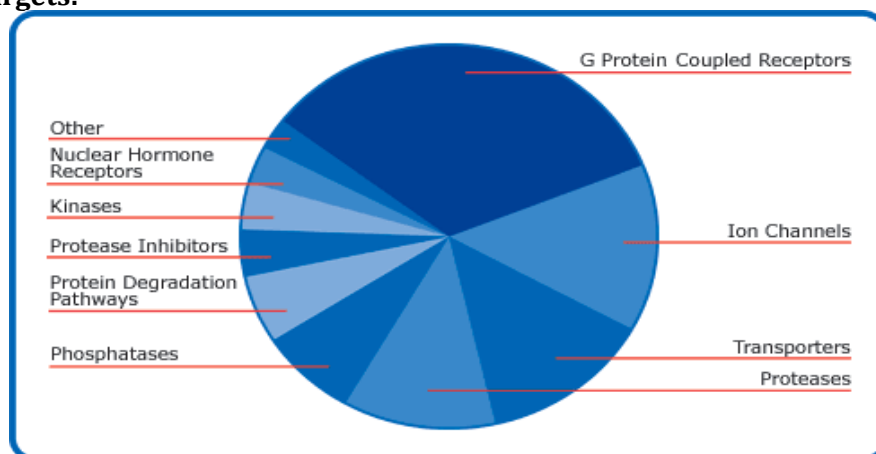


Figure 1: Different Types of Drug Targets [15]

3.2 Number of Drug Target:

Since the development of science and technology helped humans to identify & determine the number of targets in the body. As Drew's and Reiser was the first to propose and identified 483 drug targets [16, 17]. Later, Hopkins and Groom revised that figure to 120 molecular targets [18]. Subsequently, Golden proposed that drugs act through 273 proteins (biomolecules) [19, 20]. In contrast, Wishart reported 14,000 targets for experimental drugs [21], which then revised to 6,000 targets on the Drug Bank database. Imming catalogued 218 molecular targets for drug substances [22] & Zheng cited 268 'successful' targets in the current version of the Therapeutic Targets

1) The drug target is a biomolecule, i.e. a protein that could exist in isolated or complex form.

2) The biomolecules has special site to which a specific molecules binds, which could be endogenous or extraneous substances such as chemical molecules (drugs).

3) The bimolecular structure changes when the biomolecule binds to that specific molecule and the changes in structure normally are reversible.

4) Thereafter the change in the biomolecule's structure leads to various physiological responses and induce regulation of the cell, organ, tissue, or body status.

After the drug target is determined, it is necessary to obtain structural information of target, which could be determined by computational tools like NMR & X-Ray Crystallography (XRC) [3, 4].

Database [23, 24]. John proposed 324 drug targets for all classes of therapeutic drugs [25]. With the publication of an interim agreement that the human genome consists of approximately 21,000 genes. According to the current definition, one could rationally predict that there are 5,000 to 10,000 established and potential drug targets in humans [2, 26].

3.3 Techniques used in determination of target:

Once a target is selected, use of computational tools like NMR & X-Ray crystallography determines the precise three-dimensional molecular structure of the biomolecules. This structure serves as a

blueprint in drug designing of compound [27].

3.3.1. NMR Technique:

As per structural genomic programs the high-resolution structure determination utilize to determine the structures of the biomolecules (protein structure). The secondary structure of a protein are identified rapidly by NMR which is identified from a qualitative analysis of NH exchange rates, the $^{13}\text{C}\alpha$ and $^{13}\text{C}\beta$ secondary chemical shifts & ^3JHN coupling constants. The resulting secondary structure can then be used to generate a sequence alignment based on a correlation between the

secondary structure of the target and reference proteins [28]. To detect protein structure by NMR it uses chemical shift perturbations (CSPs), i.e. changes in chemical shift of certain resonances in the NMR spectrum. Typically, 2D $^1\text{H}^{15}\text{N}$ -heteronuclear single quantum correlation (HSQC) spectrum [29], is obtained in target-based screening of a labelled protein. Alternatively, $^1\text{H}^{13}\text{C}$ -HSQC spectrum can also be use but it is costly. Where ^{15}N -labelling allows for the identification of amides, which are often used for structure interpretation because of their strategic location in proteins [4].

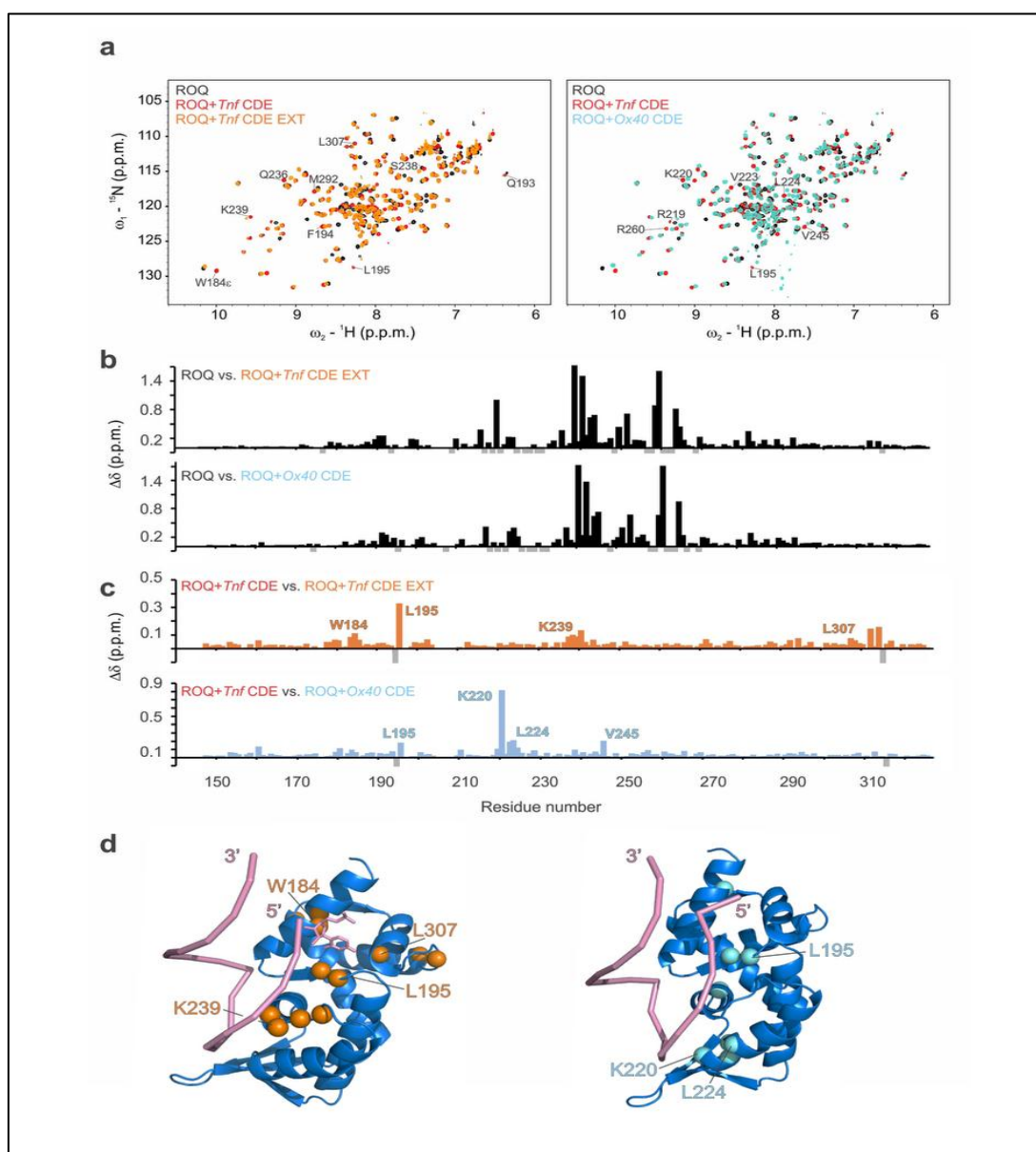


Figure 2: Use of NMR technique in Comparison of NMR chemical-shift perturbations (CSPs) for different CDE-like RNAs [30]

3.3.2. X-Ray Crystallography (XRC) Technique:

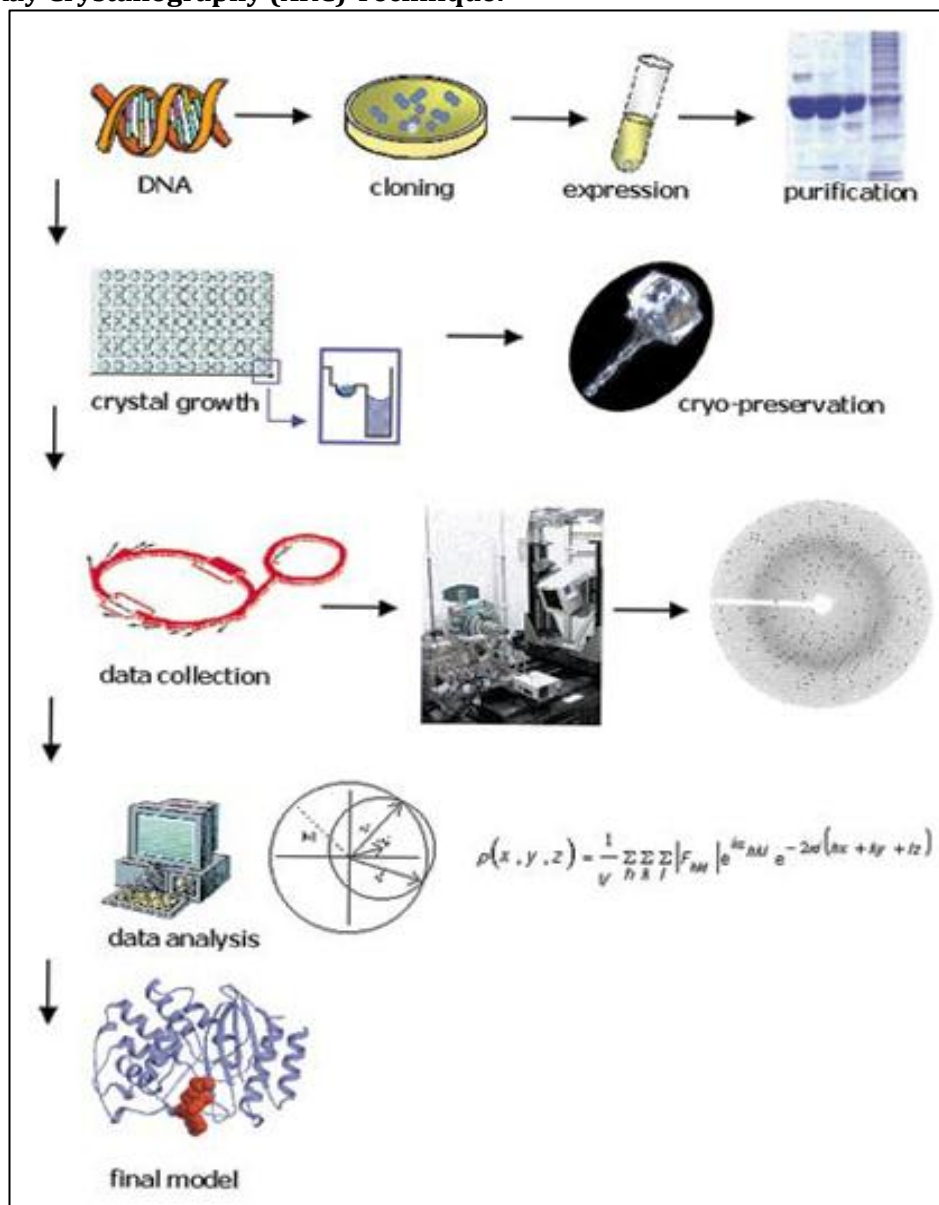


Figure 3: Flowchart for Protein Structure determination by X-Ray Crystallography [31].

Structures of biomolecules, determined by X-ray crystallography [32], reveal their shapes and sizes & also provide precise details of the intrinsic arrangement of atoms in the molecules [33]. X-ray crystallography provides atomic or near atomic resolution of matter which would reflect the repeating units of molecular structure. Crystallization of small molecule like protein or nucleic acid target followed by XRC determination of the structure is the basis and hallmark of TBDD [34]. Diffracted X-rays according to Bragg's law: $nh = 2d\sin\theta$, where 'n' is the order of diffraction, 'h' the wavelength of the radiation, 'd' the spacing or distance between a family of lattice planes in the

crystal, and 'θ' the angle of the diffraction [35]. X-ray radiation is used to analyse atomic structure, because the wavelengths used are in the order of 0.1-2.0 Å with 0.75 Å being about one-half the distance of an aliphatic carbon-carbon bond. The images of diffracted crystal lattices can be observed with specialized precession photographic equipment [5]. This knowledge is of immense help in designing drugs for specific diseases by targeting molecules. 3D structures of several macromolecules, especially those proteins, have been determined, enabling treatment of several diseases [1].

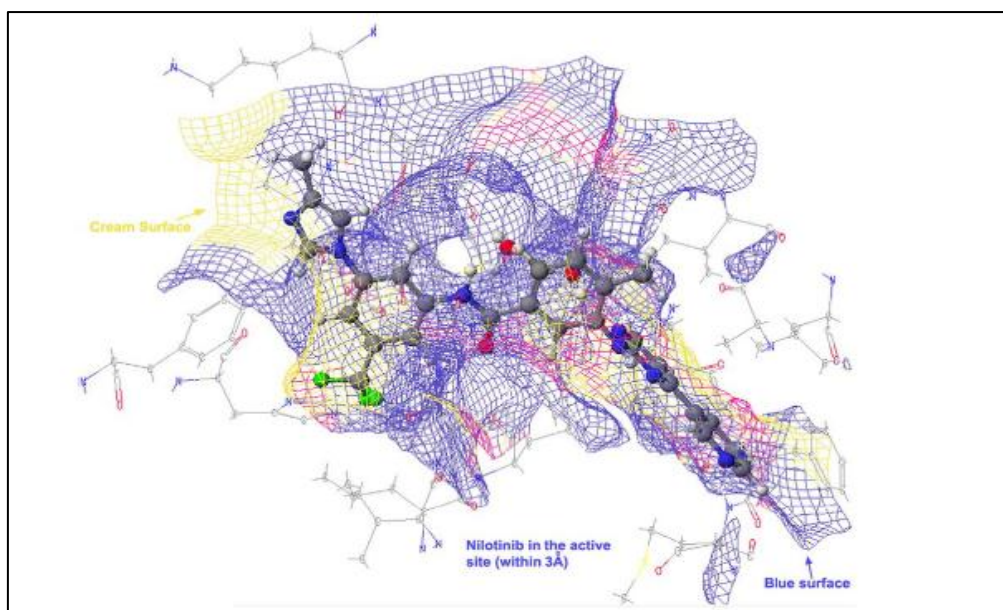


Figure 4: Use of X-Ray technique in showing the nilotinib drug to the active site of the target (BCR-ABL) [1].

3.4 Target Validation:

Drug target validation is useful not only for new drug research and development but also it provides idea for target related diseases. Basically, target validation process include following steps [2]:

1. Discovering a biomolecule (target) of interest.
2. Evaluating its potential as a target.
3. Designing a bioassay to measure biological activity [36].
4. Constructing a high-throughput screen [37].
5. Performing screening to find hits.
6. Evaluating the hits.

After the target is identified, it becomes necessary to confirm, that the correct

selection of target has been done. The use of suitable animal models and the techniques in gene targeting and expression are essential to validate the process. Validation also helps to identify any secondary target that the drug may bind to, which may lead to unwanted or adverse reaction. Ideally the drug candidate should be such that it binds to a single target only. Thus, binding to incorrect target leads to unwanted pharmacological actions which cannot be completely avoided but can be minimized to negligible extent [2]. Once an assay that measures the biological activity of the target, then compounds can be tested in the bioassay to see if they inhibit, enhance, or do nothing to this activity [5].

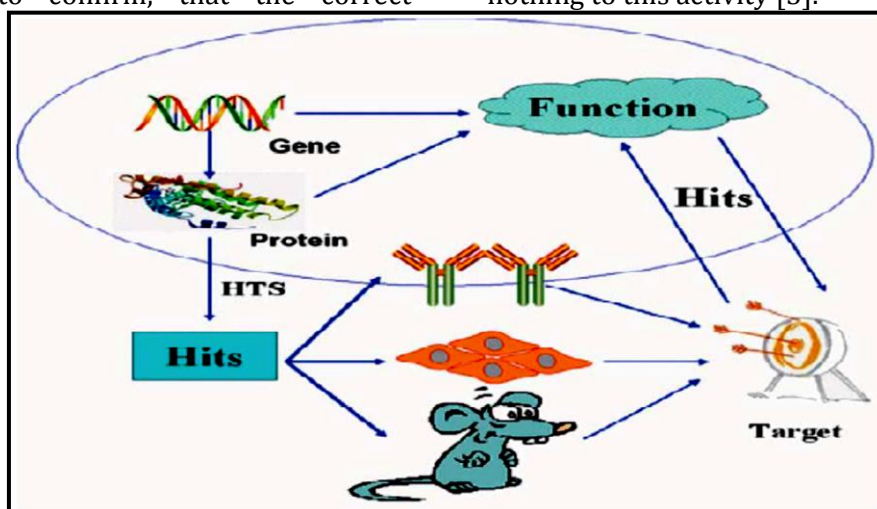


Figure 5: Drug target validation: hit discovery and target function research [2].

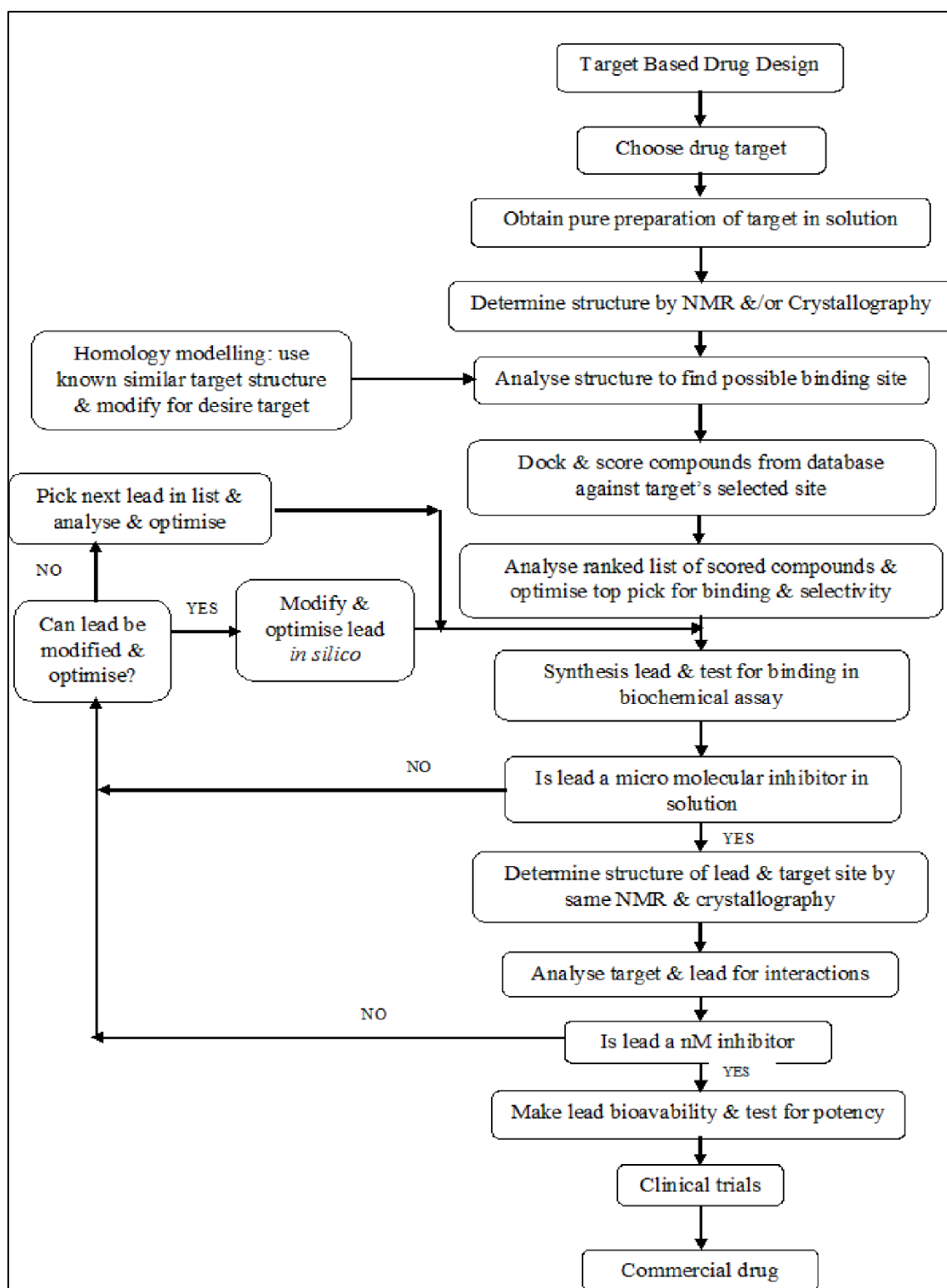


Figure 6: Flowchart for Target Based Drug Design (TBDD) [1, 5].

4. Example for Target identification & Drug design in Cancer Research:

Cancer [38, 39] in the human population has become a larger problem and the medicines available for treatments still have some limitations. There has been a tremendous increase in the knowledge of the molecular

mechanisms and pathophysiology of cancer. Many of these mechanisms has been utilised as new targets for drug designing & development to have greater antitumor activity with less toxicity that which is seen in currently used medicines [40].

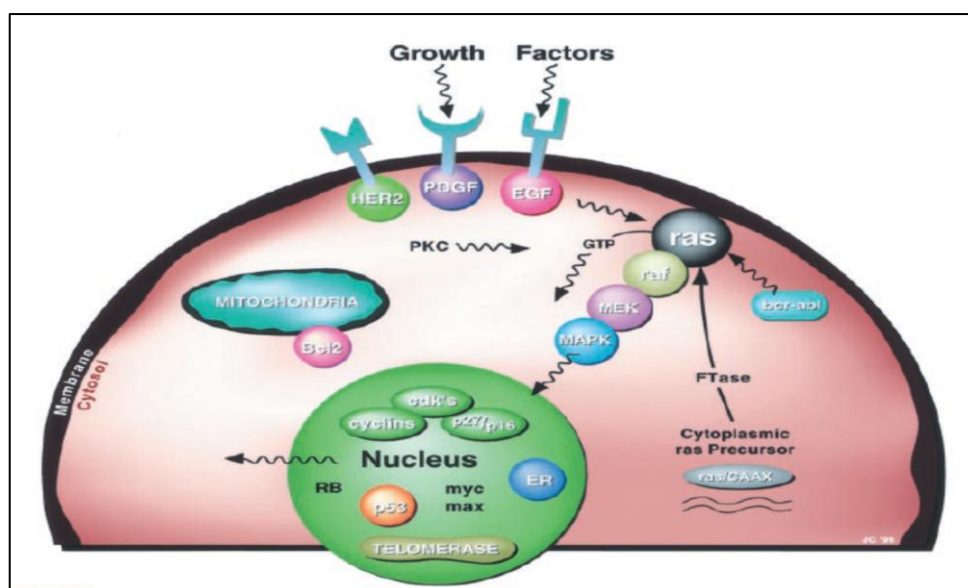


Figure 7: Example of molecular targets in tumour cells for cancer drug development [40]

Advances in molecular biology allows us to identify genes & molecular mechanisms of the disease. There are many targets known to affect tumor growth and it is to decide which will exploit the areas of signal transduction, apoptosis, telomere biology, cell-cycle regulation and angiogenesis. In cancer research for drug design, the choice of target is often selected as the mutated

gene is underlying the cancer such as *ras*, *raf* [41], *myc*, *myb*, *src* [42] and *bcr-abl* [43] shown in (Fig. 7). Overexpression of gene products, such as HER-2, epidermal growth factor (EGF) [44] and insulin-like growth factor receptors, and cyclins, is also been correlated as a causative factor in cancer for development of new drugs acting on the target site [40].

Some lists of targeted drugs in cancer are:

Table 1: Cancer drugs for specific target site [40]

Drug	Target
Raloxifene	Estrogen receptor
CGP 57148/STI 571	Bcr-abl receptor
R115777	Farnesyltransferase enzyme
Vitaxin	Integrin
ISIS-3521	Protein kinase C enzyme
Flavopiridol	Cyclin dependent kinases enzyme
Marimastat	Matrix metallo-proteinases enzyme

Further computational tools avail help in recognition of telomerase enzyme which regulate the immortalization of tumour cells. Telomere [45] consists of repeated units of the "Hexameric DNA sequence i.e. TTAGGG", ranging from 15 kb to 5 kb in chronic diseases. Telomeres have two important function, first to protect the chromosome ends from destructive nucleases & second it enable for complete replication of DNA. The expression of telomerase in normal and tumour tissues remarkable and largely superior [1]. It can be taken into

consideration for future aspects for designing of drugs in Cancer treatment.

4. Application:

Some application relates to development of drugs using target based drug designing method:

- Three-dimensional structures of dihydrofolate reductase have been the basis for the design of several improved inhibitors.
- Knowledge of the three-dimensional structure of HIV protease, a small but crucial protein from the AIDS virus, has led

- to structure-based design of nanomolar, bioavailable, non-peptide inhibitors.
- c. Selective inhibitor design is yet another opportunity offered by structural knowledge for trypanosomal glyceraldehyde-3-phosphate dehydrogenase in the treatment of sleeping sickness.
 - d. High-resolution NMR is useful in determining the structure of FK506-binding protein (FKBP) complexed with the immunosuppressant ascomycin.
 - e. Few proteins have been crystallographically studied which provides an extremely precious database for known structures of protein-ligand complexes with associated binding constants [46].
 - f. Development of antibiotics for Bacterial RNA [47].
 - g. Designing of drugs for Cholestatic liver disease were nuclear receptors as a drug target [48].
 - h. Therapeutic drug targeting approach in Atherosclerosis [49].

5. Limitations:

1. It's a questionable strategy, were over a period of time developed drugs may be ineffective when they are finally tested in the selected disease model.
2. A genetic target has a limited impact on disease treatment, because of the small size of patient populations.

6. CONCLUDING REMARKS:

Target Based Drug Design is a powerful & promising method for designing drug molecules at important targets. After the target & its structure is being determined, leads can be developed. Libraries of compounds can be generated for drug targets using computational tools & as per genomic investigation new targets have opened a door for new drug research. TBDD is a multidisciplinary approach in drugs designing & development which will help in combating disease to improve quality of life.

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