Review-I

Anticancer Drug Discovery: Role of Pharmacogenomics

ABHIJIT RAY

ABSTRACT

Anticancer drug discovery is an expensive and time-consuming exercise. It involves extensive profiling in experimental animals as well as in human subjects. Evidence suggest that many compounds that work in animal models and appear safe, fail in human trial on account of poor efficacy and / or safety. In recent years it has become clear that genetic makeup of human subjects regulate biological response of a new chemical entity. Anticancer drug discovery is moving towards target based drug discovery. Success in human trial can be maximized by stratification of patients based on expression profile of molecular targets as well as drug metabolizing enzymes. At the same time molecular finger printing of gene expression pattern between animals and humans may help in predicting potential toxicity. The use of pharmacogenomic based information is gradually getting acceptance in discovery research for new drugs and may improve success rate and bring down cost and time of drug discovery.

INTRODUCTION

Need exists to find novel anticancer agents that are high on efficacy and low on adverse event.[1,2] Effort to discover novel anticancer medicine faces several challenges. Firstly, drug discovery is a time consuming, expensive and risk intensive process, which is under commercial pressure to minimize cost, reduces time and improve overall success rate.[3] Secondly, anticancer drug discovery is undergoing a paradigm shift where unique cancer cell specific molecules are increasingly being targeted for drug discovery effort.[4] Several novel anticancer agents that inhibit activity of enzymes playing important role in cancer pathophysiology have reached market and many are in different stages of clinical development.[5] Pharmacogenomics is assuming an important role in anticancer drug discovery because lack of uniformity in patient response to targeted therapy can be attributed to individual's genetic makeup that determines both efficacy and side effect of anticancer drugs.[6,7] Main objective of this article is to familiarize readers with the complexity and pitfalls of anticancer drug discovery process. At the same time attempt will be made to bring to light that a sense of predictability can be brought into biological response using our knowledge and understanding of genome.

The review is divided into the following sections, - the drug discovery process, paradigm shift in anticancer drug discovery, pharmacogenomics and toxicogenomics in drug discovery.

DRUG DISCOVERY PROCESS

Drug discovery, is a cost, time and risk intensive exercise.[2,3,8] As shown in figure 1, the discovery process starts with identification and validation of a target and synthesizing molecules that recognize the target. Once a hit is identified from tens of thousands of molecules through the process of screening, it undergoes extensive optimisation to improve its affinity and potency for the target and selectivity against related family of proteins. Subsequent to demonstration of *in vivo* efficacy in preclinical models and establishment of appropriate metabolism and pharmacokinetic parameters a molecule is selected as candidate for safety evaluation. Following extensive testing for cardiotoxicity, genotoxicity, hepatotoxicity as well as repeat dose toxicity in more than one animal

Department of New Drug Discovery Research, Ranbaxy Laboratories Ltd., Udyog Vihar Industrial Area, Gurgaon, Haryana 122001.
Correspondence to: **ABHIJIT RAY**
E-mail: abhijit.ray @ranbaxy.com

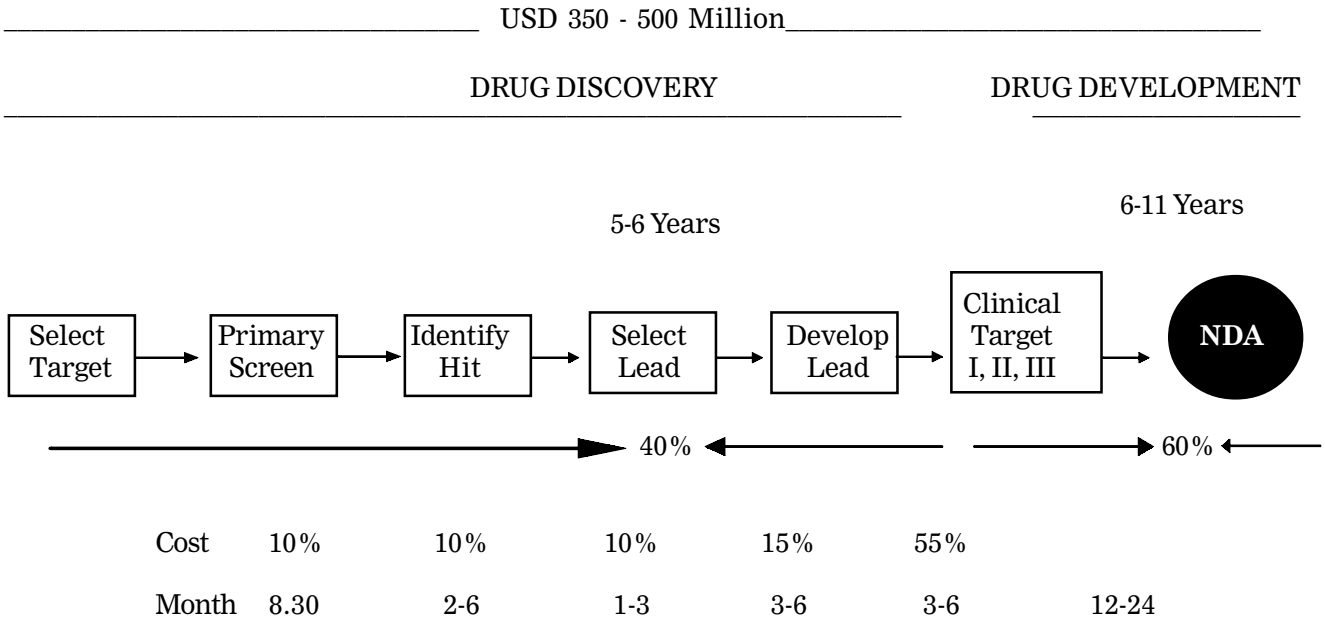


Fig. 1. Drug discovery a lengthy and expensive process

Drug discovery from concept to bedside involves extensive profiling in experimental animals and human subjects. The time taken for drug discovery is on an average 11 years. Cost involved from concept to clinic is upwards to $500 million (adapted from reference 19).

species a molecule becomes ready for human studies. Of three stages of human trial, phase I trial evaluates safety and tolerability of the molecule. Actual efficacy of the test compound is evaluated in phase II and phase III trials involving patient population.

It takes upwards of 500 million dollars and on an average about 10 years to bring a new drug from concept to clinic. Success rate in discovering a new drug is only 10%, that is if 10 molecules reach first stage of clinical trial (phase I trial), only one has statistical chance of reaching the market. As shown in figure 1, cost of drug discovery increases as we select a candidate molecule and undertake extensive efficacy and safety studies in experimental animals and humans. The cost of human studies takes up nearly 55% of budget for developing a molecule. Inspite of such extensive undertaking, a molecule may fail to meet the criteria for moving ahead at any stage of development as well as post launch. Keeping the expenditure of time and money involved in drug discovery research, it becomes very important to improve predictability of experimental models as well as human studies such that one can discard losers early and run with potential winners.

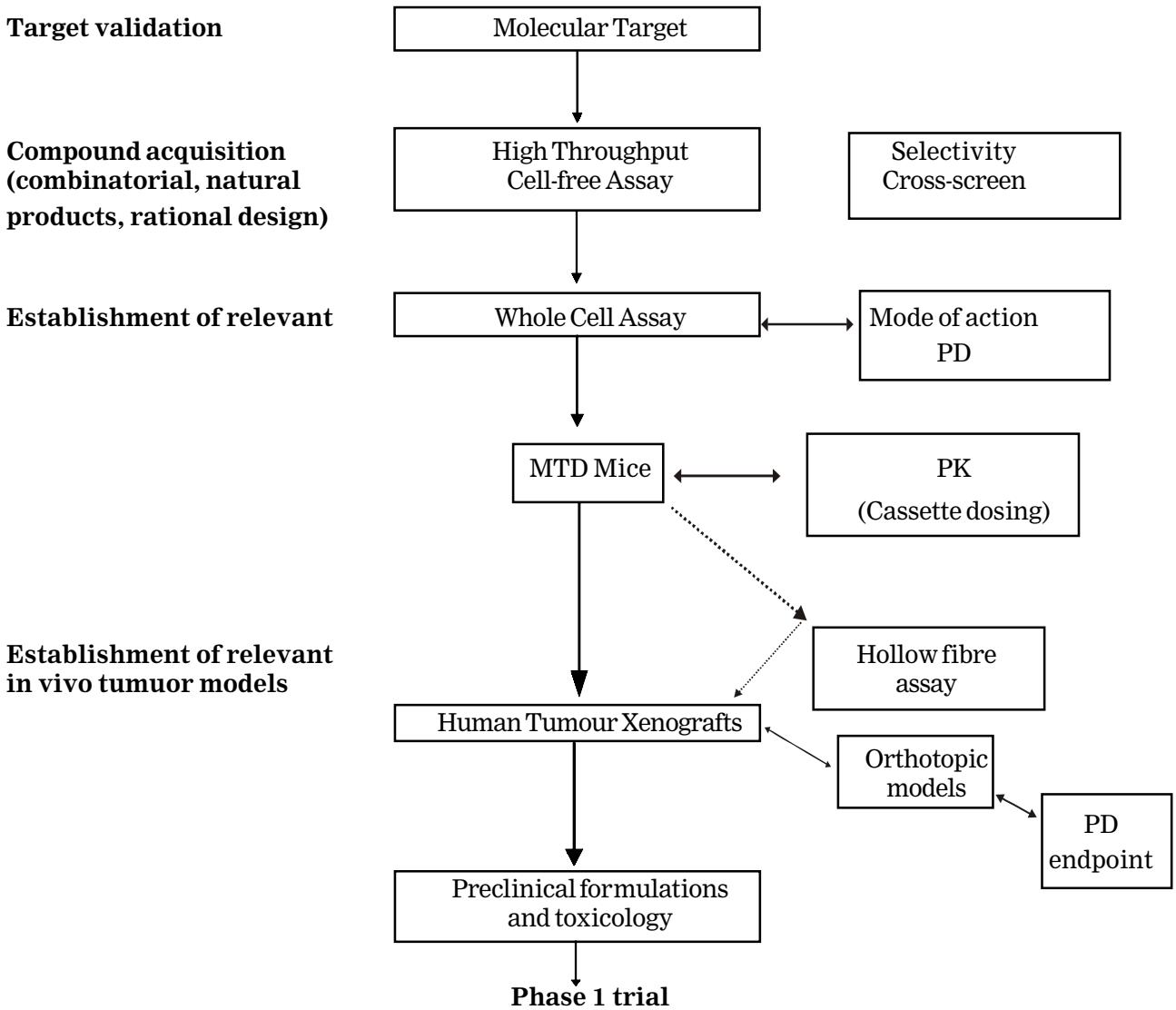
PARADIGM SHIFT IN ANTICANCER DRUG DISCOVERY

There is a need to discover novel anticancer drugs, because existing agents have severe limitations.[1] Being cytotoxic in nature, these agents do not discriminate between normal and cancerous cells. The assumption that tumour cells grow at a different rate relative to normal cells have not really translated into discovery and / or design of tumour selective molecules. In recent years effort has been made to identify and target pathway / protein unique to cancer cells.[8;4] Advances in recombinant DNA technology has made it possible to explore genes and gene products that are expressed in a differential manner in cancer cells relative to normal cells. For example, the understanding that epidermal growth factor receptor is over expressed in many human cancer has lead to discovery of anti EGFR monoclonal antibody - Tratsuzumab and small molecule inhibitors of EGFR tyrosine kinase – Erlotinib,

Geftinib etc.[5;9] Many such targets can be identified based on our understanding and use of recombinant DNA technology. It is believed that drugs developed around cancer cell specific targets may be free of adverse effect of cytotoxic agents.

Fig. 2. Progression path of a molecule through preclinical testing to human trial

A typical screening process involves testing compounds against a molecular target. Once affinity and selectivity are achieved, molecules are tested in a cell based assay. Potent molecules are tested for efficacy, pharmacokinetic and toxicity studies before it is taken up for human studies (adapted from reference 20).



The progression path of a molecule from first preclinical testing to human trial is shown in fig. 2.[20] A molecule has to pass through all the stations depending on its position on the ladder. However, the point worth noting is that the first stop is evaluating affinity of the compound for the molecular target, followed by inhibition of target mediated processes in a cell based system. Cell based assays can be designed to express target of interest or one can choose cell lines where target of interest is known to play a dominant role in cell

prolifereation. This is different from process where cell based assays used to be the first step and cytotoxicity used to be the measure of success to progress a molecule onwards. *In vivo* efficacy of the compound is assessed primarily using human xenograft injected into athymic nude mouse. Although a wide variety of tumour types can be tested in nude mouse, a first line screening could use cell lines where molecular target is driving cell proliferation. A case in point is Bcr-Abl-transformed hematopoietic cell line was used to evaluate efficacy of Bcr-Abl kinase inhibitor *in vivo* in mouse.[10] Similarly, a salivary tumour cell line over expressing IGF-1R is used to test efficacy IGF-1R receptor tyrosine kinase inhibitor.[11] The principal advantage of this approach is establishing a correlation between *in vitro* potency and *in vivo* efficacy, preclinical proof of concept and aid in decision making about progressing a compound from efficacy study to preclinical toxicity study with a reasonable degree of confidence.

Besides, establishment of target mediated biochemical and or molecular markers at preclinical level can be tracked in the phase I trial in clinic. A positive correlation will establish consistency in the behavior of the molecule from preclinical setting to the clinic. This approach improves the hit rate in discovery and sets up a clear path from discovery to phase I development.

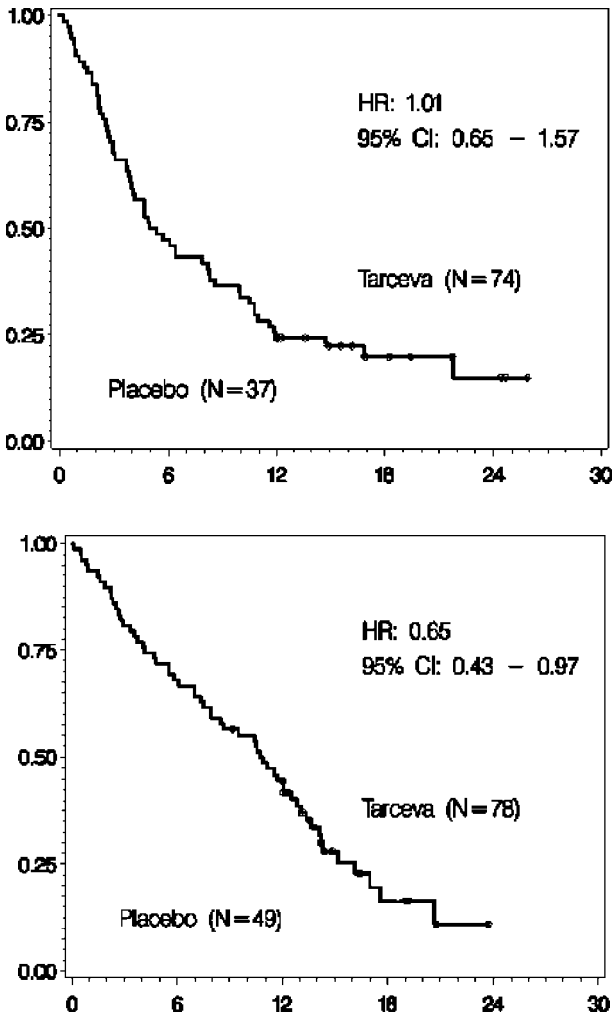
ROLE OF PHARMACOGENOMICS AND TOXICOGENOMICS IN DRUG DISCOVERY AND DEVELOPMENT

The greatest disadvantage of target based drug discovery is the uncertainty about the role target plays in the initiation and maintenance of human cancer. In recent years it has become clear that individuals respond differently to a drug depending on their genetic makeup – the science of pharmacogenomics. This difference may be manifest in the way a drug reacts to its target in a biological system – pharmacodynamic response, and how the drug substance is cleared from the system – pharmacokinetic response.

Molecular targets may vary in their expression level in human cancer and between individual exhibiting symptoms of cancer. As a result some patients recruited in a trial may respond to therapy whereas others may not .[6] Thus selecting correct patient population becomes very important for the successful outcome of clinical investigation.[12] This has been highlighted in a

Fig. 3. Beneficial effect of patient stratification based on EGFR expression (adapted from ref. 6)

Grouping NSCLC patients based on the expression pattern of EGFR improves clinical trial outcome.



study using with EGFR tyrosine kinase inhibitor – Tarceva (fig. 3). As shown, EGFR positive patients exhibited survival benefit in response to Tarceva.[6] Whereas patient population not expressing the target were not different from the group exposed to placebo.

It has also been shown using Trastuzumab and HER2 neu positive patients (Table 1) that with the help of appropriate stratification it is possible to improve success rate and bring down the number of patient needed to see a positive outcome. This has direct impact on savings as well as faster and longer access of the drug to patients with impact on profitability. More recently, the same principle has been used to establish clinical efficacy of two novel Bcr-Abl kinase inhibitors in patients with Imatinib resistant chronic myelocytic leukemia.[13,14]

Table 1: Herceptin: The business case for subdividing the target population

Trial Design	**With HER2 neu**	Without HER2 neu
Number of patients	**470**	2200
Response rate	**50%**	10%
Years of follow-up	**1.6**	10

- **Savings in clinical trial cost ~$35 million**
- **Income from 8 year acceleration of product ~$2.5 billion**
- **Access to drug from acceleration ~120,000 patients**
- **Recent success in adjuvant therapy**

Adapted from reference 18

Once a drug is inside the body, it is deactivated by drug metabolizing enzymes into inactive and water soluble metabolites to facilitate elimination. The expression pattern of drug metabolizing enzymes may vary between individuals leading to altered pharmacological effect of jngested drug. For example, Irinotecan, an anticancer drug, is known to cause neutropenia and diarrhea in patients that cannot metabolise it properly. The drug is metabolized by Uridine glucouronyl transferase (UGT1A1). It has been shown that promoter region of UGT1A1 gene has $(TA)_6TAA$ repeat. Homozygotes with $(TA)_7/(TA)_7$ and heterozygotes with $(TA)_7/(TA)_6$ in the promoter region of UGT1A1 metabolise Irinotecan slowly and exhibit adverse event.[7] Genotyping of patients receiving Irinotecan has become very important before therapy is initiated inorder to minimize side effect.

The genotype screening and molecular analysis of DNA polymorphism are increasingly being used in drug development. Understanding of polymorphic drug metabolizing pathways may help predict undue toxicity of drugs as well as

appropriate phenotyping of patients may reduce failure rate due to adverse effect and improve therapeutic index of anticancer agents.[15]

Over the years it has become clear that many compounds fail in the clinic because of adverse events. A potential drug candidate undergoes extensive toxicology evaluation for signs and symptoms of toxicity, experimental animals like rat and dogs do not always predict human toxicity.[16] It has been suggested that predictability of animal toxicity data can be improved by evaluating effect of drugs on expression pattern of different genes and gene products in body fluids, peripheral cell types as well as in biopsy samples. Tracking these changes following drug treatment preclinical to clinical settings may help predict toxicity early as well as give confidence about efficacy of a molecule. In this context, it is worth mentioning that regulatory agencies are encouraging use of biomarkers that can predict efficacy and toxicity of compounds based on molecular events.[17; 18] To the extent regulatory agencies are willing to approve a molecule for human use based on compelling evidence from biomarker data.

It should be pointed out that despite drawbacks of traditional safety evaluation studies, a certain degree of comfort has been established because of their use over a long period of time. It is still early days for using genomic information to decide fate of a molecule. A lot of validation needs to be undertaken about translatability of genomic information into proteome level and contributes to overall toxicity patterns. Besides, many decisive biomarker based studies need costly investment, which, except for a select few, may be out of reach of most companies.[17]

SUMMARY

In summary, need exists to discover novel anticancer agents that exhibit their biological effect by binding to molecular targets unique to cancer cells. The cost, time and predictability of anticancer drug discovery effort can be improved by taking into consideration interindividual variability in responsiveness to drugs and designing studies accordingly. In addition to drug response, adverse effect of drugs also depends on individual's genetic makeup. Using appropriate screening tool, it is possible to track drug induced changes in molecular events from laboratory to clinic and predict potential winners. In short, utility of molecular markers based on pharmacogenomic and toxicogenomic information is gradually being appreciated in drug discovery research. Used prudently, this may bring down cost and time of drug discovery.

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COMMENTS

It is a pleasure to review the article "Anticancer Drug Discovery: Role of Pharmacogenomics" by Dr. Abhijit Roy. Pharmacogenomics aims to predict the safety, toxicity and/or efficacy of drugs in individuals. [1] Anticancer drug discovery is truly moving towards a target based drug discovery. There are many challenges in the path of drug discovery as well. Due to huge costs and time incurred in churning out a new drug molecule, the pitfall of science remains that is not necessary that a drug in experiment models would do as well in human clinical trials.

Toxicogenomics aims at determining the genetic makeup associated with patient responses to chemotherapeutic agents and help identifying the patients at high risk for severe toxicity or those likely benefit from therapy with a particular regimen.[2] Several genetic variations like single nucleotide polymorphisms in drug metabolizing enzymes, transporters and molecular targets along with gene transcription may contribute to a variable drug response.[3] Thioprine methyl-transferase polymorphism (TPMT) is well defined in literature. Three TPMT alleles, TPMT*2, TPMT*3A, TPMT*3C account for nearly 95% of TPMT deficiency cases. Mercaptopurine is a purine antimetabolite used in the treatment of leukemia . Patients carrying TPMT polymorphisms are at increased risk of hematologic toxicity when treated with 6 Mercaptopurine as these polymorphisms decrease the rate of 6 MP metabolism. Recent studies have also focussed on irinotecan pharmacogenetics due to polymorphisms in UDP-glucuronosyltransferase 1A1 (UGT1A1). The role of genetic variations in UGT1A1 is suggested for the interpatient variability in SN-38G formation, which is the active metabolite of irinotecan. Similarly, polymorphisms in the enzyme dihydropyrimidine dehydrogenase (DPD), which is the rate limiting step in 5FU metabolism, increases systemic exposure to fluorodeoxyuridine monophospahte (FdUMP) and the incidence of adverse effects to 5FU. DPD activity is completely or partially deficient in up to 0.1% to 3%-5% individuals in general population. Genotyping methods have established for molecular diagnosis of TPMT deficiency which help in deciding a safe starting dose of 6MP therapy.[4] Polymorphisms in drug transporters like P glycoprotein, drug targets like thymidylate synthesis, MDR 1 etc. are some other well defined examples. P-glycoprotein is overexpressed in multidrug resistant cancers. Genetic variations in MDR! Gene has been correlated with the drug exposure of some commonly used drugs like digoxin and fexofenadine.

Drugs used in chemotherapy are cytotoxic and have several dose limiting side effects. Identification of targets unique to cancer cells, for example the over expression of epidermal growth factor receptor (EGFR) in breast cancer led to the discovery of Transtuzumab. As a result, the use of transtuzumab in HER2 positive patients reduces the time and costs, improving success rates. Dr. Roy has discussed these examples in details in the manuscript. The role of DNA polymorphisms in drug development has also been highlighted. The use of genotype screening in drug development may help prevent toxicity of drugs by appropriately correlating with the phenotyping of patients.

To summarize, in this post genome era, utilizing the molecular markers, screening techniques and genotyping, it is time to incorporate

pharmacogenomics and toxicogenomics as a useful tool for target validation and drug discovery in clinical oncology. Various pharmacogenomic technologies like proteomics, expression profiling, genotyping, immunohisto-chemistry, gene arrays etc can be used for candidate genetic markers. Selective gene typing may be applied in stratifying the trial population to achieve better treatment success in clinical trials. The true aim of pharmacogenomics, i.e individualizing drug therapy would then truly be achieved.

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Y. K. Gupta, Veena Gupta & Madhur Gupta
Department of Pharmacology,
All India Institute of Medical Sciences,
New Delhi-110029.