INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

REVIEW ON BIOMARKERS: TOOL FOR DIAGNOSIS OF A DISEASE AND DRUG DEVELOPMENT

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ARTICLE INFO

Article history
Received 27/05/2019
Available online
30/06/2019

Keywords
Biomarker,
Diagnostic Biomarkers,
Surrogate Markers.

ABSTRACT

Biomarker measurements have become an essential component of oncology drug development, particularly so in this era of targeted therapies. Such measurements ensure that clinical studies are testing our biological hypotheses and can help make the difficult decisions required to choose which drugs to stop developing or de-prioritise. In this review we discuss the intrinsic properties of biological sample based efficacy measurements and how these relate to their implementation in oncology drug development by way of points to consider and examples. In short use of biomarker data will help us make the best possible use of precious human samples and maximize the chances of success of the most promising therapeutic approaches.

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*Please cite this article in press as **Mr. Sandip Sharad Kshirsagar** et al. Review on Biomarkers: Tool For Diagnosis of A Disease And Drug Development. Indo American Journal of Pharmaceutical Research.2019:9(06).*

INTRODUCTION

Biological markers (biomarkers) have been introduced by 'Hulka and colleagues' as cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids." More recently, the definition has been broadened to include biological characteristics that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic Intervention. In practice, biomarkers include tools and technologies that can aid in understanding the prediction, cause, diagnosis, progression, regression, or outcome of treatment of disease. Biomarkers of all types have been used by generations of epidemiologists, physicians, and scientists to study human disease. The application of biomarkers in the diagnosis and management of cardiovascular disease, infections, immunological and genetic disorders, and cancer are well known. Their use in research has grown out of the need to have a more direct measurement of exposures in the causal pathway of disease that is free from recall bias, and that can also have the potential of providing information on the absorption and metabolism of the exposures.

Definition: A Biomarker, or Biological Marker

- In general a substance used as an indicator of a biological state.
- It is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.
- Anatomical, physiological, biochemical or molecular parameters associated with the presence and severity of specific disease states.
- Detectable and measurable by a variety of methods including physical examination, laboratory assays and medical imaging

Examples of biomarkers - CA 125 (ovarian cancer), CA 15-3 and 27-29 (breast cancer), CEA (ovarian, lung, breast, pancreas, and gastrointestinal tract cancers), and PSA (prostate cancer).

Need of Biomarkers

For chronic diseases, whose treatment may require patients to take medications for years, accurate diagnosis is particularly important, especially when strong side effects are expected from the treatment. In these cases, biomarkers are becoming more and more important, because they can confirm a difficult diagnosis or even make it possible in the first place. A number of diseases, such as Alzheimer's disease or rheumatoid arthritis, often begin with an early, symptom-free phase. In such symptom-free patients there may be more or less probability of actually developing symptoms. In these cases, biomarkers help to identify high-risk individuals reliably and in a timely manner so that they can either be treated before onset of the disease or as soon as possible thereafter. In order to use a biomarker for diagnostics, the sample material must be as easy to obtain as possible. This may be a blood sample taken by a doctor, a urine or saliva sample, or a drop of blood like those diabetes patients extract from their own fingertips for regular blood-sugar monitoring. Naturally, the detection method for a biomarker must be accurate and as easy to carry out as possible. The results from different laboratories may not differ significantly from each other, and the biomarker must naturally have proven its effectiveness for the diagnosis, prognosis, and risk assessment of the affected diseases in independent studies.

History of Biomarkers

The idea of using biomarkers to detect disease and improve treatment goes back to the very beginnings of medical treatment. The practice of uroscopy — examining a patient's urine for signs of disease — dates back to the 14th century or earlier, when practitioners would regularly inspect the colour and sediment of their patient's urine. Philadelphia chromosome: In 1960, researchers discovered that some patients with chronic myelogenous leukaemia (CML), a form of adult leukaemia in which there is a proliferation of myeloid cells in the bone marrow, have a specific genetic change associated with their cancer, a shortened version of chromosome 22. This abnormality, known as the Philadelphia chromosome, is caused by a translocation between chromosomes 9 and 22. The consequence of this genetic swap is the creation of the BCR-ABL 'oncogene'; this cancer-causing gene produces a protein with elevated tyrosine kinase activity that induces the onset of leukemia12. Researchers were able to use the Philadelphia chromosome as a biomarker to indicate which patients would benefit from drug candidates (tyrosinekinase inhibitors) specifically targeting the rogue protein. The end product was the drug imatinib (Gleevec), which decreases the proliferation of Philadelphia chromosome cells and slows the progression of the disease. As a postscript to this story, researchers further found that specific mutations in the BCR– ABL gene were biomarkers that predicted resistance to imatinib, leading to the development of newer tyrosine-kinase inhibitors dasatinib and nilotinib[1-2].

Classification Biomarkers

Biomarkers can be classified based on different parameters.

Biomarkers based on drug development

Diagnostic biomarkers: They provide the means to define a population with a specific disease. (i.e., cardiac troponin for the diagnosis of myocardial infraction.)

Prognostic biomarkers: They correlate with outcomes. For example, over expression of Her-2/neu in breast cancer or EGFR expression in colorectal cancer indicates poor prognoses.

Predictive biomarkers: They define populations that might respond more favorably to a particular intervention from an efficacy or safety perspective.

Biomarkers based on their characteristics

Molecular biomarkers: It can be used to refer to non-imaging biomarkers that have biophysical properties, which allow their measurements in biological samples (example, plasma, serum, cerebrospinal fluid).

Pharmacodynamic (PD) biomarkers: those used in decision making in early drug development.

Biomarkers based on genetic and molecular biology methods

Type 0- Natural history markers: A marker of natural history of a disease and correlates longitudinally with known clinical indices.

Type 1- Drug activity markers: A marker that captures the effect of a therapeutic intervention in accordance with its mechanism of action.

Type 2- Surrogate markers: A marker intended to substitute for a clinical end point; a surrogate end point is expected to predict clinical benefit or lack of benefit on the basis of epidemiology, therapeutic, Pathophysiological or other scientific evidence.

Merits of Biomarkers

- Identification of individuals destined to become affected or who are in the "preclinical" stages of the illness,
- Reduction in disease heterogeneity in clinical trials or epidemiologic studies,
- Reflection of the natural history of disease encompassing the phases of induction, latency and detection,

Demerits of Biomarkers

- Biomarkers can be difficult to validate and require different levels of validation depending on their intended use.
- If a biomarker is to be used to measure the success of a therapeutic intervention, the biomarker should reflect a direct effect of that intervention.

A Useful Characteristics of Biomarkers

- If the biomarker is to be used as a diagnostic test, it should be sensitive and specific and have a high predictive value.
- can be measured reproducibly by means of a reliable and widely available assay
- conveys information about the disease that is meaningful to the physician and the patient

Challenges in developing clinical biomarkers

- Tissue availability
- Assay methodology
- Clinical validation

General Uses Of Biomarkers

Diagnostic Biomarkers

Tumour markers such as PSA, CEA and CA-125 are well established as diagnostic biomarkers.

In Medicine

A biomarker can be a traceable substance that is introduced into an organism as a means to examine organ function or other aspects of health. For example, rubidium chloride is used as a radioactive isotope to evaluate perfusion of heart muscle. It can also be a substance whose detection indicates a particular disease state, for example, the presence of an antibody may indicate an infection. A biomarker indicates a change in expression or state of a protein that correlates with the risk or progression of a disease, or with the susceptibility of the disease to a given treatment.

In Cell Biology

- A biomarker is a molecule that allows for the detection and isolation of a particular cell type. For example, the protein Oct-4 is used as a biomarker to identify embryonic stem cells.
- In genetics, a biomarker (identified as genetic marker) is a DNA sequence that causes disease or is associated with susceptibility to disease.

In Exposure Assesment

A biomarker can also be used to indicate exposure to various environmental substances in epidemiology and toxicology. In these cases, the biomarker may be the external substance itself (e.g. asbestos particles or NNK from tobacco), or a variant of the external substance processed by the body (e.g. metabolite) [3-5].

Prognostic Biomarkers

A biomarker that provides information on the likely course of the cancer disease in an untreated individual. As most cancer patients are offered some kind of post-surgical treatment (adjuvant treatment), many "prognostic" studies will nowadays include patients who received systemic anticancer treatment, which may influence the natural course of the disease. However, we still have early stage cancer patients, e.g. low risk breast cancer patients and stage I and stage II colorectal cancer patients, who do not receive adjuvant treatment on a routine basis even though some of these patients will experience recurrence of their cancer disease. In these cases, evidence based prognostic markers would be extremely helpful in selecting patients for adjuvant systemic treatment.

Predictive Biomarkers

A biomarker which can be used to identify subpopulations of patients who are most likely to respond to a given therapy. With predictive biomarkers it should be possible to select the therapy with the highest likelihood of efficacy to the individual patient. Thus, predictive biomarkers are the basis for individualized or tailor-made treatment. Examples: predictive biomarkers being used in the daily clinical oncology practice is estrogen and progesterone receptors to predict sensitivity to endocrine therapy in breast cancer, HER2 to predict sensitivity to Herceptin treatment and KRAS mutation to predict resistance to EGFr antibody therapy. New predictive biomarkers such as assays for Topoisomerase 2α DNA aberrations may turn some types of conventional chemotherapy into targeted drugs [6-8].

Difference between Predective & Prognostic Biomarkers: (Table 1, Figure 1)

The figure illustrates the difference between a prognostic and a predictive biomarker. **Prognostic biomarker:** Indicates the likely course of the disease in an untreated individual. **Predictive biomarker**: Identifies subpopulations of patients who are most likely to respond to a given therapy. The blue patient is the one who will benefit from treatment individual. Blue and red patients are those who will relapse [9].

Table 1. Diffrence between Predictive and Prognostic Biomarkers.

Predictive biomarkers	Prognostic biomarkers
Associated with the indicated drug.	Biomarkers used for dose adjustments
Differential effectiveness and/ or toxicity.	Biomarkers are not identified precisely in the therapeutic indication
Indicate the like hood of response to a specific therapy.	Indicate the likelihood of outcome (tumour recurrence or patient survival) regardless of the specific treatment the patient receives.
Decides which treatment is best.	Helps to guide treatment.
Rapid access to most appropriate therapy	

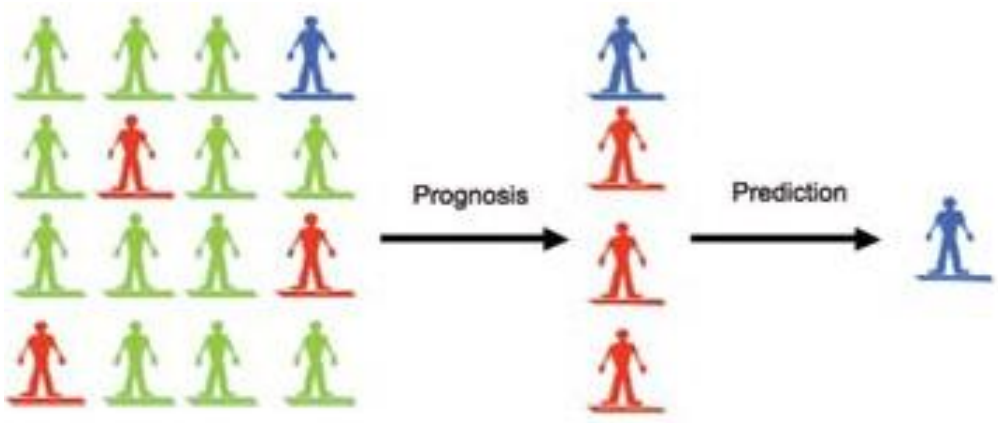

Figure 1. Stages of Biomarkers.

Biomarkers Used In Disease

Biomarkers depicting prodromal signs enable earlier diagnosis or allow for the outcome of interest to be determined at a more primitive stage of disease. Blood, urine, and cerebrospinal fluid provide the necessary biological information for the diagnosis. In these conditions, biomarkers are used as an indicator of a biological factor that represents either a subclinical manifestation, stage of the disorder, or a surrogate manifestation of the disease. Biomarkers used for screening or diagnosis also often represent surrogate manifestations of the disease. Gene signatures and specific disease markers that are identified by microarray-based gene expression profiling are validated by approaches such as RTQ-PCR, immunohistochemistry, and gene knockout methods such as RNA interference.

Table 2. Examples of Various Biomarkers Used In Diseases.

Therapeutic area	Biomarkers	Drugs
Breast neoplasms	HER2 over expression/ gene amplification	trastuzumab (Herceptin) lapatinib (Tyverb)
	oestrogen receptor	toremifene (Fareston) fulvestrant (Faslodex)
Cancer Ascites	EpCAM expression	catumaxomab (Removab)
Carcinoma, Non-Small-Cell Lung	EGFR mutations	gefitinib (Iressa)
	EGFR expression	erlotinib (Tarceva)
Colorectal Neoplasms	EGFR expression	cetuximab (Erbitux)
	KRAS mutation	panitumumab (Vectibix)
Gastrointestinal Stromal Tumors	Kit (CD 117) positive	imatinib mesilate (Glivec)
Leukemia, Myelogenous, Chronic, BCR-ABL Positive	Philadelphia chromosome	imatinib mesilate (Glivec) dasatinib (Sprycel) nilotinib (Tasigna)
Stomach Neoplasms	HER2 overexpression/ gene amplification	trastuzumab (Herceptin)
HIV infection	CCR5 tropism	maraviroc (Celsentri)

The goal is to provide comprehensive support during the drug discovery and development lifecycle, with the following objectives:

- Drug efficacy predictions
- Validation of cell and animal models
- Clinical proof of principle
- Monitoring intervention
- Create scientific interest

Cardiac Biomarker:

Cardiac biomarkers are substances that are released into the blood when the heart is damaged. Measurement of these biomarkers is used to help diagnose, evaluate, and monitor patients with suspected acute coronary syndrome (ACS). The symptoms of ACS are associated with heart attacks and angina, but they may also be seen with non-heart-related conditions. Increases in one or more cardiac biomarkers can identify patients with ACS, allowing rapid diagnosis and appropriate treatment of their condition. Cardiac biomarker tests are ordered to help detect the presence of ACS and to evaluate its severity as soon as possible so that appropriate therapy can be initiated. It is important to distinguish heart attack from angina, heart failure, or another condition because the treatments and monitoring requirements are different. For heart attacks, prompt medical intervention is crucial to minimize heart damage and future complications. Cardiac biomarker tests must be available to the doctor 24 hours a day, 7days a week with a rapid turn-around-time. Some of the tests may be performed at the point of care (POC) in the Emergency Room or at the patient's bedside. Serial testing of one or more cardiac biomarkers is often done to ensure that a rise in their blood levels is not missed and to estimate the severity of a heart attack. The current biomarker test of choice for detecting heart damage is troponin. Other cardiac biomarkers are less specific for the heart and may be elevated in skeletal muscle injury, liver disease, or kidney disease. Many other potential cardiac biomarkers are being researched, but their clinical utility has yet to be established (Table 3).

Table 3. Cardiac Biomarker.

Marker	What it is	Tissue source	Reason for increase	Time to Increase	Time back to normal	When/how Used
CK	Enzyme- 3 different isoenzyme exist	Heart, brain, and skeletal muscle	Injury to muscle and/or heart cells	4 to 6 hours after injury, peaks in 18 to 24 hours	48 to 72 hours, unless due to continuing injury	Performed in combination with CK-MB
CK-MB	Heart-related isoenzymes of CK	Heart primarily, but also in skeletal muscle	Injury to heart and/or muscle cells	4 to 6 hours after heart attack, peaks in 12 to 20 hours	24 to 48 hours, unless new or continuing damage	Less specific than troponin, used when troponin is not available
Mayoglobin	Oxygen-storing protein	Heart and other muscle cells	Injury to muscle and/or heart cells	2 to 3 hours after injury, peaks in 8 to 12 hours	Within one day after injury	Performed with troponin to provide early diagnosis
Cardiac troponin	Regulatory protein complex.	Heart	Injury to heart	4 to 8 hours	Remains elevated for 7 to 14 days	Diagnose heart attack

Cancer Biomarkers:

Cancer biomarkers are employed across the entire healthcare spectrum from the cancer biological research laboratory to patient monitoring in the clinic. Cancer biomarker's applications include the identification of novel therapeutic targets in cancer drug discovery and uses of cancer biomarkers as surrogate markers for drug efficacy in clinical trials. This report describes a number of factors providing the driving forces behind cancer biomarker growth and commercialization. Emerging cancer biomarker types and the increasing interest in circulating tumour cells, as well as data on potential DNA, RNA, and protein biomarkers under study, includes Oncogenes, Germline inheritance, Mutations in drug targets, Epigenetic changes.

Measurements of Biomarkers

Biomarker assays are characterised in terms of their sensitivity, specificity, limit of detection, limit of quantification and variability. We would argue that a thorough consideration of the characteristics listed in Table below; of both phenotype and candidate biomarker can also save considerable time and effort in a biomarker development program by aiding study design, interpretation and decision making [10-12].

Level of attribution

In order to correlate a phenotype and a biomarker, consideration should be given to the level at which the measurement is attributed. The level at which a marker is measured is often not the same level to which it is attributed or acted upon. Germline genetic variants are measured at the level of the individual but the results may have consequences at the level of the family. Conversely, individuals are diagnosed with cancer but conceptually the diagnosis may often be attributed to one of a pair of organs subject to identical genetic and environmental influences (and data from the contra-lateral ''twin'' may provide clues as to the impact of these influences) Generally speaking clinical endpoints of interest, such as survival or progression free survival, are usually measured or attributed at the level of the patient or subject. Many biomarker measurements are also at subject level (heritable genotypes, circulating PSA). However, some are attributes of a thin cross-section of a narrow core of tissue from one of potentially many tumours within an individual. It is rare that efforts are made to validate the assumption that the measurement made at one level can be attributed to another by measuring within subject inter-biopsy or inter-lesion variability and yet it is a prerequisite for correlation with subject level measurements and hence utility.

Dynamism

Conceptually similar to an appreciation of measurement level is consideration of temporal variation. Cancer is a phenotypically and molecularly progressive disease in which tumours evolve over time. Tumour biomarkers are likely to do the same, and even if they do not, the molecular context in which they find themselves almost invariably does. Therefore timing of measurements and sufficient longitudinal granularity can be critical both in assessing or predicting the impact of a therapeutic intervention (see later). The routine assumption of the reproducibility of a patient's response to a therapeutic intervention is elegantly discussed by Senn (2004).

Technical and biological reproducibility

Similar arguments can be applied to the process validity that leads from data acquisition to subject level attribution of phenotypic or biomarker data. How do the results differ if a different lesion is chosen for measurement of length or biomarker levels? If a different pathologist selects the region of tumour to be macro dissected? If a different panel of experts reviews images or if the same panel reviews them a second time? Such information on phenotypes is vital in setting realistic expectations for the possible sensitivity and specificity of a biomarker as these are unlikely to be higher than the repeat concordance of the phenotypic measurement itself.

Analyte abundance

Traditional assay development methods apply well to assays in homogenous media where analyte abundance is such that it can be described by the statistics of populations. Historically, we have struggled to make tissue based assays such as tumour immunohistochemistry anything more than semiquantitative. Newer, highly sensitive nucleic acid technologies take us into the realms of stochastic variation where a signal may disappear because the analyte is physically not present, rather than reaching the detection limit of an instrument. In such situations extreme care must be taken to discriminate between positive, negative and unknown results.

Nature and number of variables

Data may initially be univariate or multivariate, continuous or categorical. Ultimately the goal is to deliver a single ‘‘result’’ or index via a pre-defined set of rules and although this can remain a continuous value for practical purposes a cut-off may need to be set as the intended use will usually be to influence a categorical decision. The criteria for RECIST evaluation offer a good example of a multivariate approach to deliver a categorical patient level result and the Oncotype Dx assay is a good example of a multivariate continuous index. In general it is a good idea to maintain data in their continuous form until the final step of data processing as continuous data can always be made categorical but the reverse is not true. Further, we make a clear distinction between data for which the biology is intrinsically categorical (eg genotype) and data which is rendered categorical by the means of measurement such as ER, PR levels – continuous by radio ligand and RTPCR and categorical by IHC, respectively. In summary, it is important to understand the intrinsic biology and assay platform, validate the level to which a measurement may be attributed, take samples at appropriate times and examine how good the biomarker and phenotype are at predicting themselves on repeat biological or technical measurement (Table 4).

Table 4. Property Variables of Biomarkers.

Property	Explanation	Examples and impact
Dynamism	Measurements can be static or dynamic.	A static biomarker cannot correlate with a dynamic phenotype if the dynamic phenotype cannot predict itself on repeat measurement. Heritable genotype is a static measurement; tumour somatic mutation status is dynamic. Genotype is a good example of a static (subject level) biomarker which is often proposed to impact on plasma pharmacokinetics a dynamic (subject level) phenotype.
Level	Measurements can be made at the level of a thin slice of tissue, single lesion or subject level	Many candidate predictive tumour markers are measured on single sections of tumour tissue and the results automatically extrapolated as a patient level attribute.
Average, single molecule Detection	Classical assay development methods were established on analytes present at the level of billions of molecules. Recent technologies, especially using nucleic acids, take us down orders of magnitude to levels where stochastic variation must be considered.	The Jak2 mutation assay from Ipsogen is suggested to be used at an input of 25 ng of DNA. This corresponds to 10000 copies of the genome (5000 cells).
Univariate versus multivariate Markers	Composite measurements should have clear rules to derive a final easy to interpret multivariate index	The rules governing the combination of target, non-target and new lesions for determining RECIST measurements are a good example of deriving a single patient level index for decision making. Similarly the development of the Oncotype Dx assay is a good example for molecular biomarkers
Continuous/categorical	Measurements can be intrinsically continuous or discrete. Continuous measurements can be made discrete via cut-offs.	Decisions are invariably discrete and therefore care must be taken to provide a clear message with output from continuous markers.

Biomarkers in Drug Development

The role of Biomarkers in the drug development process

Biomarkers can provide their discoverers with tangible benefits by speeding up and focusing the development of associated treatments for the disease they indicate. Therefore most biomarker research is proprietary, and biomarkers are themselves commodities just like the drugs they help to bring to market. Biomarker research, too, follows a similar pipeline to drug research: from discovery, through initial documentation, exploratory use in pre-clinical and clinical development, to publication and regulatory approval, and ideally onward into widespread adoption in the clinics. There is undoubtedly as high an attrition rate in biomarker development as in drug development. The end point for a biomarker researcher may not simply be to support drug development, but to establish and manufacture diagnosis kits and software, with all the licensing opportunities that suggests.With the increasing cost and complexity of drug development, biomarkers play an increasing role in the early phases of drug development. Biomarkers can be classified into target, mechanistic, or outcome with varying degrees of linkage to disease or treatment effect. They can be used to determine proof of concept by characterizing the efficacy or safety profiles, or determining differentiation from any competitor drugs. Clinical validation of the biomarker has a direct influence on the clinical utility and therefore on the label of the co-developed product. As shown in (Figure 2) [13].

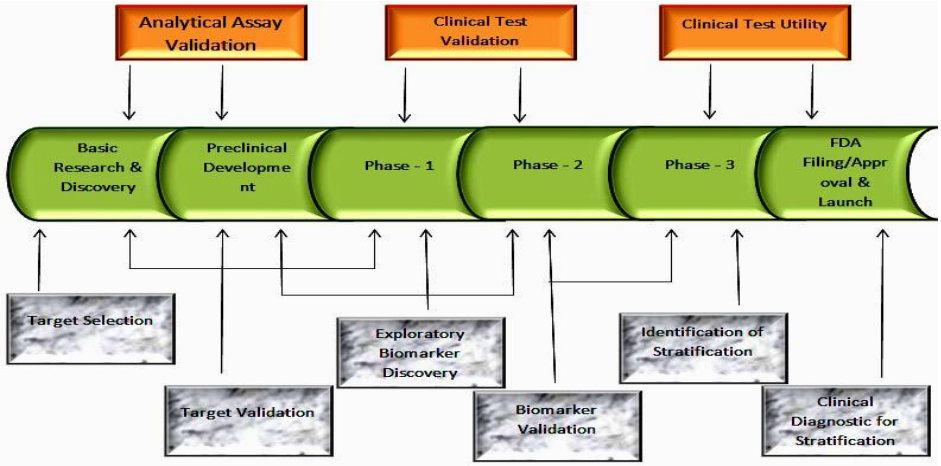

Figure 2. Flow chart showing the importance of biomarkers in drug development process.

Microarray Technology

A microarray is a multiplex lab-on-a-chip.

Microarray technologies now enable the simultaneous interrogation of the expression level of thousands of genes to obtain a quantitative assessment of their differential activity in a given tissue or cell. The development of these technologies has also motivated interest of their use in clinical trials and diagnosis. For instance, a key aim of many investigators is to identify genomic factors that are prognostic for survival or relapse-free survival, and that predict those patients who respond to treatment. Typically, such experiments investigate on the order of dozens of samples from different patients.

DNA Microarray

cDNA microarray technology is considered one of the most important and powerful tools used to extract and interpret genomic information. The cDNA microarray experiment requires to isolate Ribonucleic Acid (RNA) from both control (known) and experimental (patient) samples. The reverse transcription process is used to convert the extracted RNAs into cDNAs, which are further labeled with fluorescent probes, usually Cy3 for the control and Cy5 for the experimental channel. After subsequent hybridization and washing procedures, cDNA microarrays are scanned at the ~540 nm (green) for the control and ~630 nm (red) for the experimental channel respectively. The scanning procedure produces two 16-bit monochromatic images, which are further registered into a two-channel, Red-Green image. Analysis of cDNA microarray data helps in monitoring the expression levels of thousands of genes simultaneously and provides information relevant to cell activity. Therefore, cDNA microarrays have found applications in toxicological research, gene and drug discovery, and disease diagnosis (e.g., cancer, diabetes, and genetic diseases) (Figure 3).

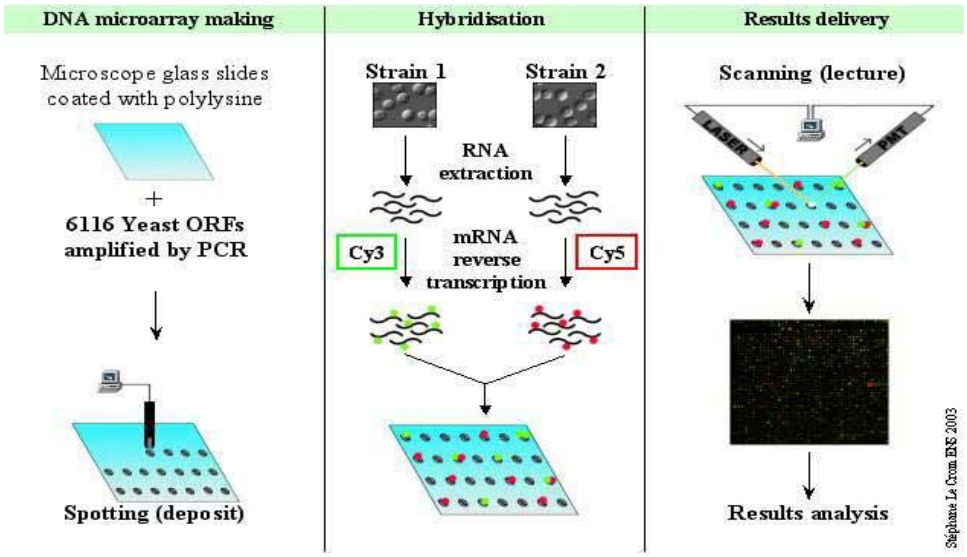

Figure 3. DNA Microarray.

General Overview of DNA Microarray Process

Microarray production process

DNA fragments amplified by PCR technique are spotted on a microscopic glass slide coated with polylysine prior to spotting process. The polylysine coating goal is to ensure DNA fixation through electrostatic interactions. PCR fragments are in our case the expressed part (ORF) of the 6200 Saccharomyces cerevisae genes (baker yeast). Slide preparation is achieved by blocking the polylysine not fixed to DNA in order to avoid target binding. Prior to hybridisation, DNA is denatured to obtained a single strand DNA on the microarray, this will allow the probe to bind to the complementary strand from the target.

Target preparation

RNA is extracted from two yeast cultures from which we want to compare expression level. Messengers RNA are then transformed in cDNA by reverse transcription. On this stage, DNA from the first culture with a green dye, whereas DNA from the second culture is labelled with a red dye.

Hybridization

Green labelled cDNA and red labelled ones are mixed together (call the target) and put on the matrix of spotted single strand DNA (call the probe). The chip is then incubated one night at 60 degrees. At this temperature, a DNA strand that encounter the complementary strand and match together to create a double strand DNA. The fluorescent DNA will then hybridize on the spotted ones.

CONCLUSION

It is an exciting time to be involved with biomarker research in oncology drug development. This technology help to maintain data, to bioassay and tackled problem arises because of way of treatment in cancer. In future biomarkers helps to prepare such history by beholden on scientists in the area to maintain a continual dialogue with customers; those who must take decisions on the basis of our data. A clear line of sight to the intended use of biomarker data will help us make the best possible use of precious human samples and maximize the chances of success of the most promising therapeutic approaches.

ACKNOWLEDGEMENT

Authors are thankful to Prof. T. J. Sawant for providing necessary facility, Authors are also thankful to Savitribai Phule Pune University for providing library facility.

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