

Biomarker as essential part of clinical development

Renuka Chinthapally, Cytel Statistical Software & Services Pvt. Ltd., Hyderabad, India

ABSTRACT

Biomarkers play an important role in the clinical development due to their ability to predict efficacy of drug earlier and accurately. Biomarkers are used as surrogate endpoints as they are cheaper than the true endpoints. Biomarkers are used as biological indicators for disease progression, effect of therapeutic intervention and drug induced toxicity. They reduce the overall cost of drug development. FDA estimates that just a 10% improvement in the ability to predict drug failures earlier in clinical trials could save US\$100 million in development costs per drug.

Biomarkers also offer the potential to inform treatment decisions, whether to move forward to the next phase of clinical development or not. The paper also emphasizes on prognostic and predictive biomarkers with examples. The growing need for biomarker evaluation in medical practice, has implemented the statistical methods to evaluate diagnostic accuracy. The receiver operating characteristic (ROC) curve is the standard analytical tool for evaluating diagnostic tests. However, implementing biomarkers in clinical practice is still a process filled with lots of pitfalls and limitations.

INTRODUCTION

Biomarker is a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition. It is a parameter that can be used to measure the progress of disease or the effects of treatment. Biomarkers may take the form of cellular characteristics, metabolites (e.g. sugars, lipids and hormones), molecular variations, or physical features (e.g., clinical symptoms). Biomarkers can be discovered using genomics, proteomics technologies or imaging technologies.

The global biomarkers market is expected to grow at a compound annual growth rate (CAGR) of 28.5 percent from \$0.63 billion in 2004 to \$2.90 billion in 2008. The clinical trials phase currently accounts for over 60 percent of drug discovery and development cost, and it may take up to \$1.7 billion to get a drug to market.

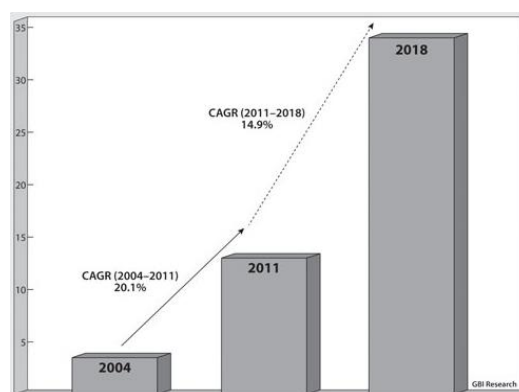


Figure 1 Biomarkers in Drug Discovery: Global Biomarkers market

DEFINITION OF BIOMARKER

Biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological process in the body, a pathological process, pathogenic processes, or pharmacologic responses to a therapeutic intervention. The response of the body to a therapy - may offer information about the mechanism of action of the drug, its efficacy, its safety and its metabolic profile.

A biomarker may be used to assess or detect:

1. A specific disease as early as possible – diagnostic biomarker (HCV RNA after infection)
2. The risk of developing a disease – susceptibility/risk biomarker (BRCA1-breast cancer)
3. The evolution of a disease (indolent vs. aggressive) – prognostic biomarker (HER-2-breast cancer) – but it can be predictive too.
4. The response and the toxicity to a given treatment – predictive biomarker (EGFR/NSCLC/gefitinib, DPD(dihydropyrimidine dehydrogenase)-gastrointestinal cancer/fluoropyrimidines)

HISTORY OF BIOMARKER

The idea of using biomarker to detect disease and improve treatment has started from the beginning of medical treatment. Uroscopy, the examination of urine for color and sediment is practiced even before 14th century for observing the signs of disease. Biomarkers have been used in pre-clinical research and clinical diagnosis from a long time. Body temperature is a well-known biomarker for fever. Blood pressure is a surrogate endpoint biomarker where change in biomarker can act as substitute for clinical observation. Blood pressure is used to determine risk of stroke.

Over the last four decades, the importance of biomarkers in clinical trials and patient management has increased exponentially. In 1960, Philadelphia chromosome is used as a biomarker to indicate the benefit of drug (tyrosine kinase inhibitors) in chronic myelogenous leukemia (CML) patients. In the late 1980's, scientists discovered that HIV viral load could be used as a marker of disease progression, and subsequently, as a measure of antiretroviral treatment efficacy. Most famous biomarker in recent drug development history is the HER-2 gene and receptor, discovered in the mid 1980's. 20–30% of breast cancer patients show an over-expression of the HER-2 receptor on their cancer cells.

The following graph (Figure 2) shows the number of publications listed on PubMed. The number of hits increased from 2 to 6089 for biomarker & clinical trial. This data indicate the significant growth in interest in biomarkers and their association with clinical trials over time.

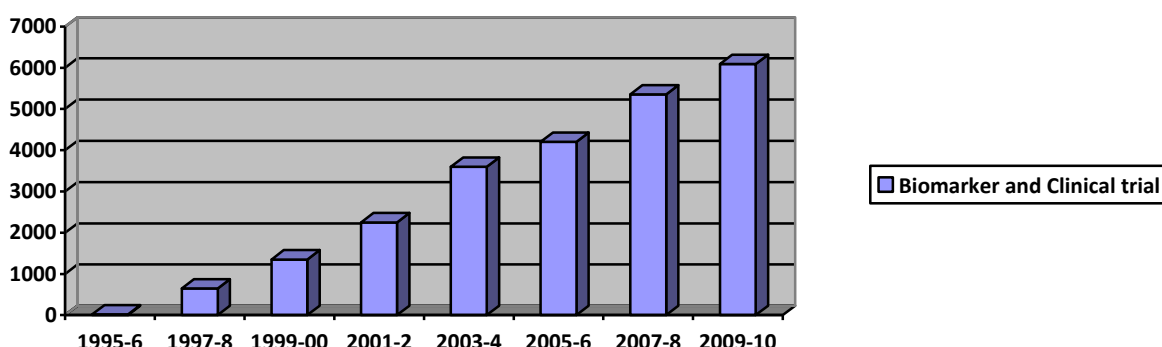


Figure 2: Number of publications listed on Pubmed

BIOMARKERS TODAY

Today you not conceive of developing a new drug without simultaneously looking for biomarkers for efficacy and safety of the drug. Biomarkers can confirm difficult diagnosis in chronic diseases such as Alzheimer's disease or rheumatoid arthritis.

Pre-clinical biomarkers in toxicology studies are used to predict safety of drug. These markers also help to select drug candidates that are more likely to be tolerated in humans, helping us to understand the risk-benefit of the drug earlier. The field of oncology is leading the way in the use of biomarkers in drug development. One example is circulating tumor cells (CTCs), a prognostic biomarker present in the blood of cancer patients gives early signal of efficacy.

The need for biomarkers to guide clinical research and prevent drug development disaster is highlighted in recent drug development failures. 34 drugs were withdrawn from the market, mainly as a result of hepatotoxic or cardiotoxic effects. Anti-inflammatory drug rofecoxib (Vioxx) due to its increased risk of heart attack and stroke was withdrawn, and more recently TGN1412 a monoclonal antibody to treat B cell chronic lymphocytic leukemia and rheumatoid arthritis, has been withdrawn due to extreme serious adverse effects in the phase I clinical trial.

Post marketing biomarkers can predict serious and rare adverse events of the drug after marketing, ximelagatran was withdrawn from clinical development due to rare, idiosyncratic adverse events involving elevations in alanine aminotransferase (ALAT) levels.

TYPES OF BIOMARKERS ASSOCIATED WITH DISEASE AND DRUG

Biomarkers can be disease-related and drug-related. Disease related biomarkers indicate the threat of disease (risk indicators or predictive biomarkers). If disease already exists (diagnostic biomarker), and how such disease may develop in individual case (prognostic biomarker). In such context biomarkers indicate whether a drug will be effective in a specific patient and how the patient body will process it.

Two studies involving prognostic and predictive biomarkers are discussed in this paper.

A phase III study was performed in subjects with metastatic medullary thyroid cancer. The study included

- i. Pretreatment period – screening assessments performed within 28 days of randomization
- ii. Treatment period - Each cycle of the treatment Period includes 4 weeks of daily administration of drug or placebo
- iii. Post-Treatment Period: patients had an end-of-treatment assessment at 30 days after the last dose of study treatment. The investigator obtained follow-up information quarterly.

The primary objective of the study is to evaluate progression free survival (PFS). Secondary objective of the study

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includes changes in serum levels of calcitonin and carcinoembryonic antigen (CEA) as prognostic biomarkers. The serum calcitonin level is a sensitive disease marker. Levels of the tumor markers calcitonin and CEA can decrease because of drug-mediated inhibition of their synthesis and secretion.

Serum levels of calcitonin and carcinoembryonic antigen are evaluated as prognostic markers in these subjects. It is noted that in this exploratory analysis there is significant change in biomarker level. There were 78% and 64% of the patients with available baseline and week 12 CEA values. Among these available CEA data at week 12, the drug administered arm had 38 % decrease from baseline and the placebo arm had 38% increase from baseline. Similarly for the calcitonin level, with over 35% missing data, there were 60% decrease and 23% increase from baseline for the drug and placebo arm, respectively. Both results show nominally significant difference between treatment arms. Table below shows the summary of tumor marker data, change from baseline at week 12. Based on Wilcoxon Rank Sum Test ; nominal p-value from exploratory subgroup

	Drug XXX N=219 Median (q1 q3)	Placebo N=111 Median (q1 q3)
CEA µg/L[n (%)]	170 (78%)	71 (64%)
Baseline	120.7 (33.5,422.7)	153.1 (32.3,478.2)
W12	56.4 (21.4, 260.9)	221.8 (69.5, 962.7)
Change from baseline	-23.7 (-143.1, -3.2)	35.6 (4.1, 269.6)
Percent Change from baseline	-38.0 (-56.1, -11.5)	38.0 (8.9, 104.0)
Pvalue	<0.0001	
Calcitonin pmol/L[n (%)]	140 (64%)	61 (55%)
Baseline	2298.1 (544.5,5754.0)	3886.0(792.0,9237.4)
W12	584.8 (177.3, 2671.5)	4968.0 (1219.0,11716.0)
Change from baseline	-1188 (-3071.0,-135.4)	322 (-0.5, 3941.3)
Percent Change from baseline	-60.2 (-81.7, -29.5)	22.7 (-2.3, 67)
Pvalue	<0.0001	

Table 1: Summary of Tumor Marker data, Change from Baseline at week 12

The box-plots shown in the following figure also demonstrated greater percent reduction from baseline in the drug arm as compared with that from the placebo arm at week 12. Three patients who had extreme values of the change from baseline of CEA/calcitonin level were not plotted in the box plot for a better presentation of the graph. The corresponding percent change from baseline of the CEA levels for these patients are 1257.58, 677.40 and 530.16 (all from the placebo arm). Similarly, the corresponding percent change from baseline of the calcitonin levels for these patients are 537.651, 433.913 and 535.922 (all from the placebo arm).

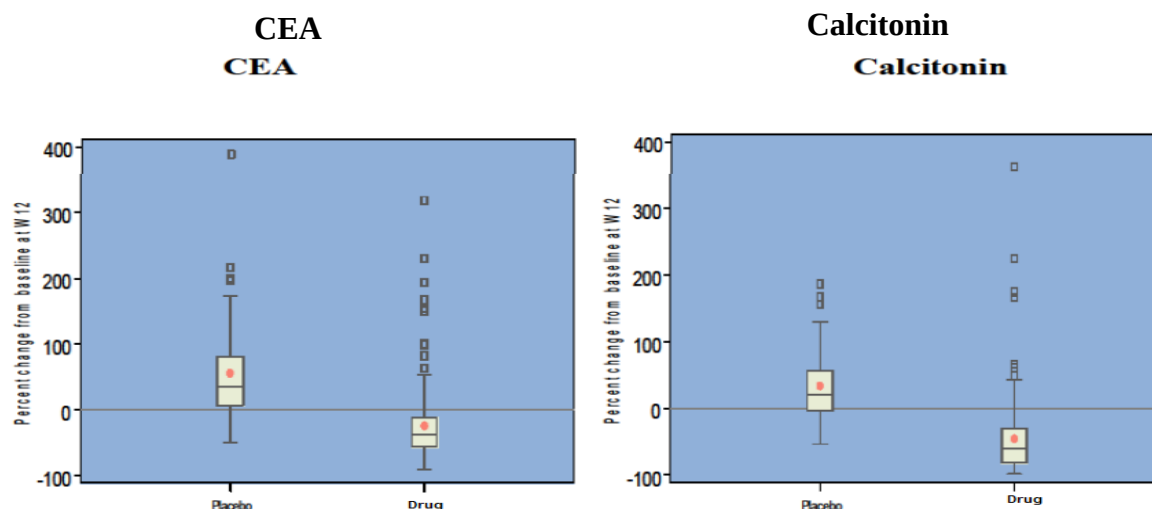


Figure 5: Percent change from Baseline in CEA and Calcitonin level at Week 12

Based on exploratory biomarker analyses, the drug treated arm appears to have significant reduction of the CEA and calcitonin levels as compared with the placebo arm.

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Cognitive biomarkers are used as predictive biomarkers to predict conversion of mild cognitive impairment (MCI) to Alzheimer disease. Cognition, a behavioral marker may be considered a surrogate for neural systems function, may also be related to regional pathologic characteristics and disease stage. In this study, the differences in cognitive marker values are evaluated among healthy controls and MCI and AD individuals.

Cognitive performance is obtained using both the Mini-Mental State Examination and the Clinical Dementia Rating. Verbal episodic memory was assessed by the Alzheimer Disease Assessment Scale-Cognitive using a composite score for the memory tests (word recall test, delayed recall, memory domainA, non-memory domainA). Knowledge was assessed by Clock Drawing Test

Variable	Controls Mean (SD)	MCI Nonconverters Mean (SD)	MCI Converters Mean (SD)	Pvalue
Logical Memory, immediate recall	14.03 (3.44)	7.56 (3.00)	6.20 (3.16)	<0.001
Logical Memory, delayed recall	13.23 (3.48)	4.34 (2.65)	2.61 (2.26)	<0.001
ADAS memory domainA	8.15 (3.89)	14.39 (5.11)	18.09 (4.25)	<0.001
ADAS non-memory domainA	1.19 (1.22)	2.88 (2.24)	3.83 (2.55)	<0.001
Clock Drawing Test	4.70 (.61)	4.35 (0.86)	3.87 (1.12)	<0.001

Table 2: Cognitive Test Scores at Baseline

Comparison between the groups on cognitive biomarker related data were analyzed by means of chi-square test or analysis of variance. All groups significantly differed in scores from each other compared with healthy controls. Therefore cognitive biomarkers are good predictors for conversion of MCI to Alzheimer's disease.

STATISTICAL TECHNIQUES FOR BIOMARKER EVALUATION

SENSITIVITY AND SPECIFICITY

The diagnostic accuracy of a biomarker is most commonly measured by calculating its sensitivity and specificity. Sensitivity is the proportion of patients who are correctly categorized as having disease among those who truly have the disease (high proportion of true positive rate). Similarly, specificity is the proportion of patients who are correctly categorized as not having the disease among all patients who truly don't have the disease (high proportion of true negative rate).

Most diagnostic biomarkers (CA19-9 tumor marker in serum) provide results for continuous scale, the sensitivity and specificity of the test depends on specific threshold selected. For example, for CA19-9 biomarker in the sample dataset, value of 37 U/ml is considered as threshold for positive result for pancreatic cancer. Sensitivity and specificity can be calculated for this threshold by constructing a 2 x 2 table with the FREQ procedure:

```
PROC FORMAT;
  VALUE tholdfmt 0 - 37 = "<37 U/ml"
  'Cancer'; 37 - high = "37+ U/ml";
  VALUE pcafmt 0 = 'No Cancer'
  1 =
  RUN;
PROC FREQ DATA=panca ORDER=formatted;
  FORMAT y1 tholdfmt. d pcafmt.;
  LABEL y1='CA19-9' d='Pancreatic Cancer';
  TABLES y1 * d / NOROW NOPERCENT;
run;
```

In the Pancreatic Cancer data set, d is a dichotomous variable for the patient's cancer status and y1 is a continuous variable of the biomarker CA19-9. For the 2 x 2 analysis, y1 is dichotomized for the appropriate threshold. The code produces the following output:

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Table of y1 by d

y1(CA19-9)		d(Pancreatic Cancer)	
Frequency			
Co1	Pct	Cancer	No Cancer
37+ U/ml		68 75.56	5 9.80
<37 U/ml		22 24.44	46 90.20
Total		90	51
			141

Figure 4: Output for 2 x 2 analysis using FREQ procedure

The percent values on the Cancer column are used to determine sensitivity and the percent values on the No Cancer column are used to derive specificity using the CA19-9 threshold of 37 U/ml. Among the 90 cancer patients, 68 tested positive, giving us a sensitivity of 76%. Likewise, 46 of the 51 non-cancer patients tested negative for a specificity of 90%.

When the threshold is predefined, confidence intervals for sensitivity and specificity can be calculated using standard statistical methods for binomial data. Using FREQ procedure CI are generated separately for sensitivity and specificity using WHERE statement. 95% CI are generated for sensitivity of the CA19-9 marker in the Pancreatic Cancer using the below code

```
PROC FREQ DATA=panca ORDER=formatted;
  FORMAT y1 tholdfmt. d pcafmt.;
  LABEL y1='CA19-9' d='Pancreatic Cancer';
  TABLES y1 / BINOMIAL;
  EXACT BINOMIAL;
  WHERE d=1;
run;
```

SAS generates confidence intervals for the proportion in the first row of the output (in this instance, 75.56%). Therefore, we have to make sure that the proportions are listed in the order that places sensitivity (or specificity) in the first row. The code above uses ORDER=formatted to instruct SAS to use formatted values for ordering. The BINOMIAL option in the TABLES statement along with the EXACT BINOMIAL statement produces confidence intervals using the normal approximation of the binomial distribution (asymptotic standard error or ASE) and the exact binomial distribution. Output is shown in the below figure

CA19-9				
y1	Frequency	Percent	Cumulative Frequency	Cumulative Percent
37+ U/ml	68	75.56	68	75.56
<37 U/ml	22	24.44	90	100.00

Binomial Proportion for y1 = 37+ U/ml	
Proportion (P)	0.7556
ASE	0.0453
95% Lower Conf Limit	0.6668
95% Upper Conf Limit	0.8443
Exact Conf Limits	
95% Lower Conf Limit	0.6536
95% Upper Conf Limit	0.8400

Test of H0: Proportion = 0.5	
ASE under H0	0.0527
Z	4.8488
One-sided Pr > Z	<.0001
Two-sided Pr > Z	<.0001

Exact Test	
One-sided Pr >= P	6.246E-07
Two-sided = 2 * One-sided	1.249E-06

Sample Size = 90

Figure :4 Output to generate confidence intervals using FREQ procedure

ROC ANALYSIS

The empirical ROC curve is the plot of sensitivity on the vertical axis and 1-specificity on the horizontal axis for all possible thresholds in the study data set. It is used to explore thresholds for the application of a new biomarker in clinical practice or to visually assess the overall performance of the biomarker. ROC curves can be generated using standard ODS

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STATISTICAL GRAPHICS and simple LOGISTIC procedure statements. The code below generates an ROC curve for the Pancreatic Cancer data.

```
ODS GRAPHICS ON;
PROC LOGISTIC DATA=panca PLOTS(ONLY)=ROC;
MODEL d(EVENT='1') = y1;
RUN;
ODS GRAPHICS OFF;
```

The PLOTS (ONLY)=ROC directs ODS STATISTICAL GRAPHICS to plot an ROC curve without plotting other standard graphs associated with PROC LOGISTIC. The MODEL statement is constructed using the standard PROC LOGISTIC syntax (dependent variable = covariates) with EVENT='1' specified as the outcome we want to predict. The code generates the ROC curve shown in Figure.

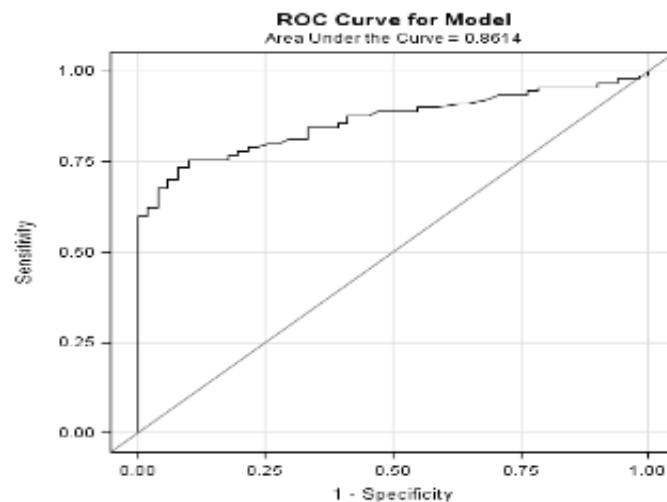


Figure 2: ROC curve for biomarker CA19-9

THE AREA UNDER THE CURVE

The area under the ROC curve (AUC) is the average sensitivity of the biomarker over the range of specificities. It is often used as a summary statistic representing the overall performance of the biomarker. A biomarker with no predictive value would have an AUC of 0.5 (also represented by the diagonal "chance" line above), while a biomarker with perfect ability to predict disease would have an AUC of 1. In the figure CA19-9 biomarker has an AUC of 0.86 for the diagnosis of pancreatic cancer in the sample population.

FUTURE CHALLENGES

Discovery of new biomarkers is one of the major challenges. In order to meet the huge clinical need for biomarkers the fundamental issue we have to deal with is a better understanding of pathophysiology of disease for developing better biomarkers. Usage of biomarkers is practically in every therapeutic area. Estimates show the total number of biomarkers of interest at about 1,133,00020.

Selection and clinical validation of biomarker is a challenge. Before biomarker is introduced into market, it passes through three phases:

1. biomarker discovery where biomarkers are identified;
2. prototype development, where immunological assays are created; and
3. Commercialization, where assays are readied for clinical use.

Each of these phases holds considerable pitfalls which need to be overcome using advanced technologies of proteomic and genomic technologies.

PITFALLS AND LIMITATIONS

The transfer of biomarkers from discovery to clinical practice is still a process filled with lots of pitfalls and limitations, mostly limited by structural and scientific factors.

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The following are the major pitfalls:

1. Lack of making different selections before initiating the discovery phase. The selection of biomarkers depends on parameters like sensitivity, specificity and predictive value of biomarker, hence the biomarker has to be carefully assessed before selection.
2. Lack in biomarker characterization/ validation strategies.
3. Robustness of analysis techniques used in clinical trials. Advanced technologies have to be used for characterization of biomarkers.

CONCLUSION

The ultimate vision is to have access to biomarkers in all therapeutic fields for which you need industry, academia and clinicians working together. Biomarkers could have such a huge impact, because you could reduce the time of your trials and improve internal decision making.

The pharmaceutical companies get to conduct their drug development programs in a much more expedited fashion and get to the business of getting the drug on to the market, and the patients get the medicines they need more quickly as well.

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CONTACT INFORMATION

Your comments and questions are valued and encouraged. Contact the author at:

Author Name: Renuka Chinthapally
Company: Cytel Statistical Software & Services Pvt. Ltd
Address: Hyderabad, India
Email: Renuka.Chinthapally@cytel.com
Web: <http://www.cytel.com>