Paper DH03

Increasing Trend of Biomarker Analysis in Clinical Trials

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ABSTRACT

Clinical trials are often designed to confirm efficacy of the investigational drug or to answer key development questions. In most trials, the data are subjected to exploratory analysis. This exploratory analysis could serve as a basis for explaining or supporting their findings, for improving the design of new trials, and for generating hypotheses for later research. One of the key exploratory analyses that is increasingly used in clinical trials is biomarker. In clinical research, biomarkers are used for multiple purposes like, to explore whether markers can be identified that will be predictive for the effect of a drug treatment, or to help elucidate the mechanism of action of a drug and the biology of the disease. Some clinical biomarker assessments are intended for more immediate impact or decision making. Combinations of clinical and research biomarker are used throughout the conduct of Phase I-III trials. Biomarker analysis is driving oncology research and commercialization of targeted cancer therapies. As part of the health care community, it is very important to understand and optimize biomarker strategy, be conscious of the challenges of the data and use appropriate statistical assessments to interpret and visualize the data in the most efficient manner, that helps to inform a clinical trial.

INTRODUCTION

A biomarker is a measurable indicator that predicts disease presence, severity, or response to treatment. Levels of biomarkers can be clinically useful by guiding disease diagnosis, or by revealing the pharmacodynamics of drug treatment. This paper talks about the rationale behind the increasing trend of biomarker analysis in clinical trials. It also describes what a biomarker is, the different categories of biomarkers, biomarker assessment types and at their clinical utility. It also outlines a statistical plan for a biomarker analysis in a trial along with related outputs and reporting of biomarker results.

1. STATISTICS DEPICTING THE INCREASE IN ONCOLOGY BIOMARKER TRIALS

Drug development is a long, high risk, and an expensive process. Out of 10 compounds only one compound reaches the market after an average of 14 years with a cost of $2.7 billion. An oncology compound that is entering phase I has about a 5% chance of ultimately achieving FDA approval. A major challenge today is to develop ways to improve oncology clinical trial success rates and reduce risks. In this context, biomarkers are increasingly used in oncology. Biomarker use has shown promising rates of success in oncology trials.

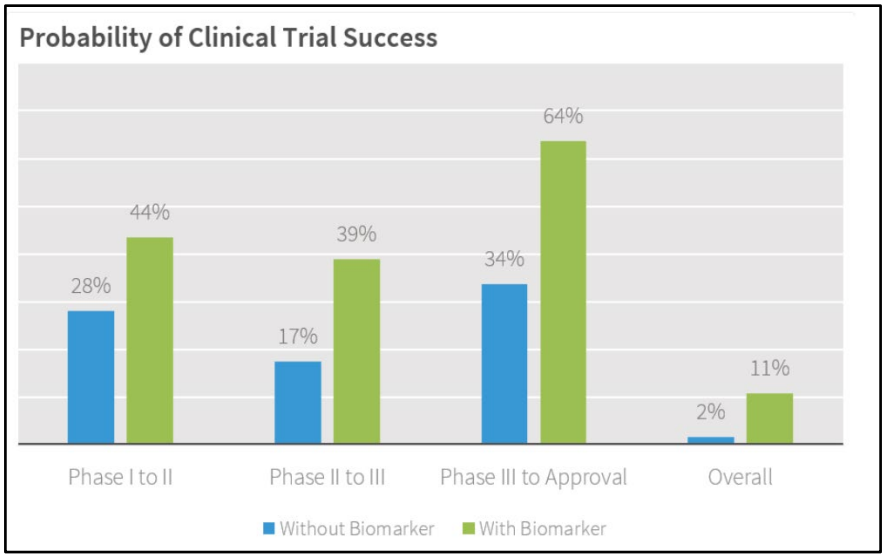


Figure 1. Probability of clinical trial success with and without a biomarker in oncology

Source: Using Protein Biomarkers Increases the chances of Success in Clinical Trials | Technology Networks

Trials that use biomarkers are much more likely to proceed to the next stage of development. For example, 10.7% of trials for new cancer drugs that use biomarkers obtain market approval, compared with just 1.6% of treatments tested on unselected patients.
Recent studies show that biomarker testing may improve outcomes for patients with cancer types such as digestive cancers, lung, and breast. Nearly 60% of all cancer drugs approved in the last 5 years require or recommend biomarker testing before use. Biomarkers may guide doctors' treatment decisions by providing clues about whether patients will respond to standard treatment options. The number of targeted therapies that require biomarker testing is increasing rapidly and cancer clinical trials are increasingly driven by biomarkers and the development of targeted therapies. *Source: Using Protein Biomarkers Increases the chances of Success in Clinical Trials | Technology Networks*

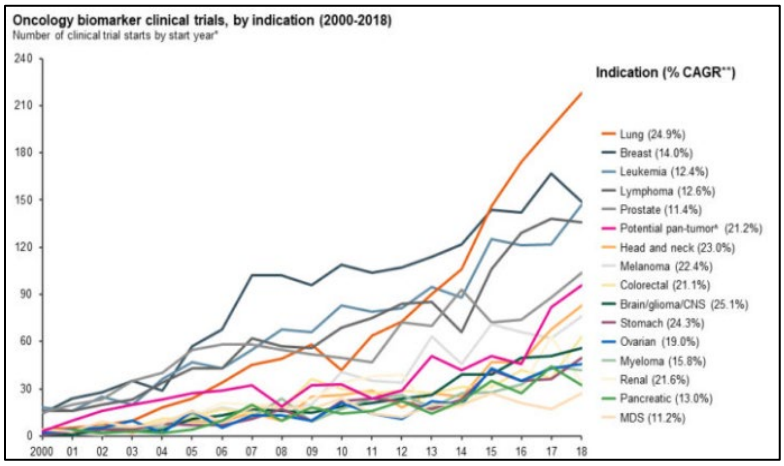


Figure 2. As we can see there has been an increasing trend of biomarker in clinical trials. This data is taken from L.E.K biomarker database. In the last 2 decades the no. of oncology trials using biomarker has nearly doubled.

Source: AACT; L.E.K. biomarker database

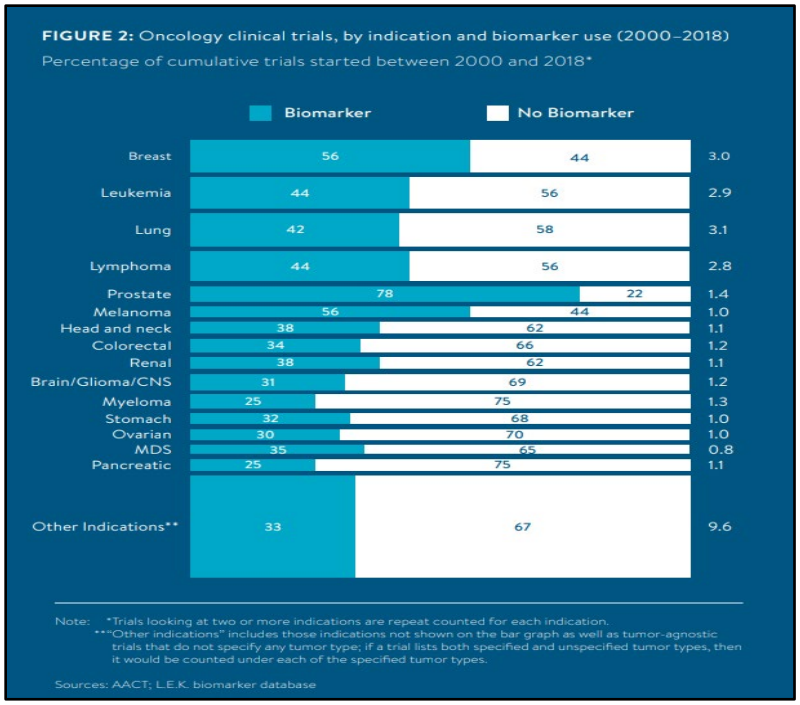


Figure 3. In terms of percentage, we can see that the biomarkers are being explored across all cancers, ranging from 25 percent to 60 percent of trials from 2000 to 2018. The highest trend is observed in breast, leukemia, lung, lymphoma, prostate, melanoma, and head and neck cancers.

Source: AACT; L.E.K. biomarker database

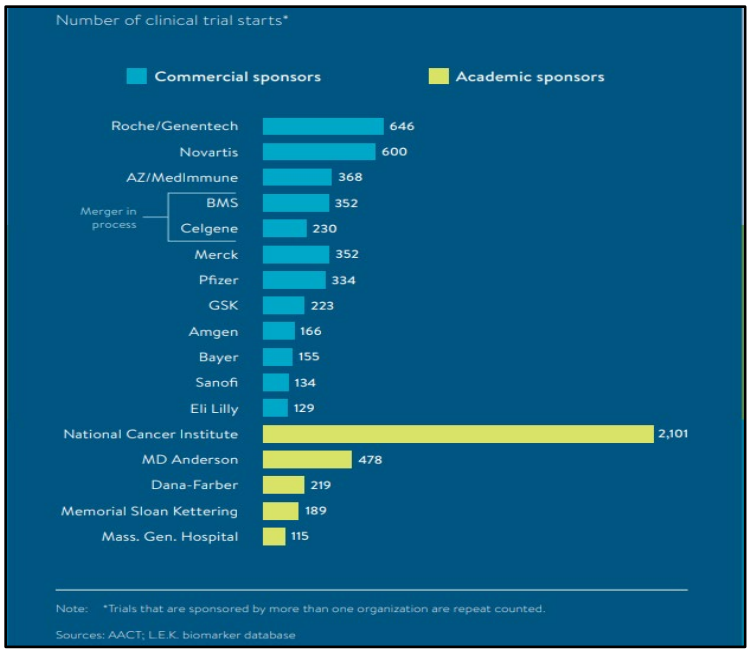


Figure 4. Biomarker testing has become a critical component in cancer research, clinical development, and treatment management. Seventeen organizations, including five academic centers, have sponsored or co-sponsored 100-plus oncology trials involving biomarker from the year 2000-2018.

Source: AACT; L.E.K. biomarker database

These trends clearly reflect advances in the use of biomarkers to guide personalized drug development than the

traditional approach.
However, they also come with its own challenges, for example, increased trial complexity and unknown patient outcomes for exploratory biomarkers. There are studies to show that biomarker-driven trials might impact drug approval timelines and increased cost and resources. Therefore, improved understanding of how biomarkers work would help to take informed decisions on which clinical trials would be worth pursuing, especially considering the extensive efforts required to conduct these trials.

2. BIOMARKERS AND IT's ROLE

2.1 HALLMARKS OF CANCER

There are some key hallmarks of cancer at the molecular level. Cells react to certain events occurring in their immediate environment, which in turn trigger events within the cell. These events are called signaling pathways because they give a 'signal' to the cell that it should do something.
When certain pathways are activated, a cell's behavior can be influenced by either the turning on or turning off of certain genes and their proteins. Cancerous cells also have no limit to the number of times they can replicate.
There are two common ways that cancers could evade an immune response in tumor microenvironment:

- Some tumors effectively exclude the immune system by hiding from it.
- Some tumors allow an initial dialogue with immune system, and then suppress it.

These processes are seen as the hallmarks of cancer, and by targeting the pathways involved in these processes, it may be possible to develop treatments that are effective for a large group of cancers.

2.2 DEFINITION OF A BIOMARKER

The **National Cancer Institute** defines a biomarker as 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. Biomarker is a characteristic which is objectively measured. It refers to a measurement of a particular analyte in tissue (for example protein expression level or gene mutation status in tumor tissue), or within a component of peripheral blood (for example protein levels in serum or plasma; also called soluble biomarkers); or the quantity of specific cell types (for example number of circulating tumor cells).

2.3 RATIONALE FOR USING BIOMARKER IN CLINICAL TRIALS

Findings from the biomarker analysis could be used to:

- Understand clinical pharmacology of the drug.
- Help elucidate the mechanism of action of a drug and the biology of the disease.
- To predict the effect of a drug in terms of both efficacy and safety in each population. We can identify patients who will respond to a particular therapy.
- Prognostic of disease progression. It can be used to forecast how aggressive the disease process is and/or how a patient may expect to fare regardless of therapy.
- It helps in the diagnosis of a disease.
- Some of these markers may enter clinical practice, for example, as a companion diagnostic test

Biomarkers also provide early identification of efficacy of the drug, its side effects and even early detection of the disease, which in the downstream also reduces the cost and resources for a clinical trial. Therefore, all these factors contribute to why biomarkers are now used more and more in clinical trials.

3. DIFFERENT TYPES OF BIOMARKERS AND THEIR UTILITY IN DIFFERENT STAGES OF CLINICAL DEVELOPMENT

3.1 TYPES OF BIOMARKERS

- **Prognostic biomarker:** These biomarkers can help estimate the risk of a disease. It also helps in monitoring the disease and its reoccurrence. It helps in understanding disease progression in a patient or a population.
  Example: Prostate-specific antigen (PSA) is used as a prognostic biomarker for assessing disease progression in prostate cancer patients.
- **Predictive markers:** They are used to assess if patients with a particular molecular aberration are more likely to have an improved outcome or response to a specific treatment strategy. It helps to identify which treatments are likely to be effective in a particular population, guiding in early treatment decision making.
  Example: expression of the HER2/neu protein in breast cancer.

- **Pharmacodynamic Biomarkers:** Pharmacodynamic markers indicate the effect of drug on the target. Pharmacodynamic biomarkers can help explain, whether the drug will hit or modulate its intended target. As seen from Figure 4 (c). a change in the biomarker level can be observed when test treatment is given as compared to standard.
  Example: Sweat chloride may be used as a pharmacodynamic biomarker when evaluating patients with cystic fibrosis, to assess response to cystic fibrosis transmembrane regulator (CFTR) potentiating agents.

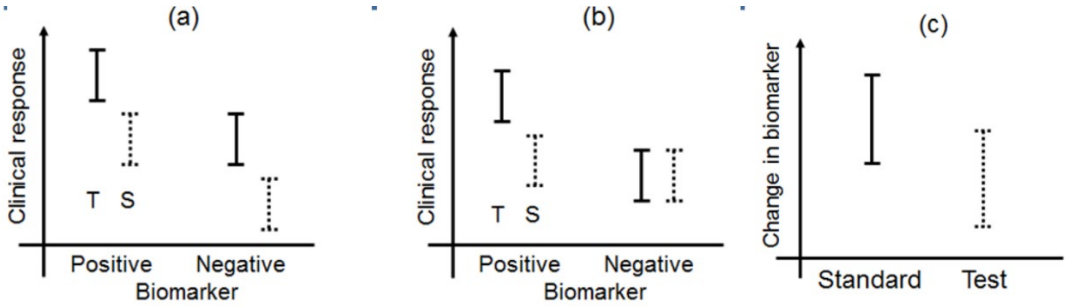


Figure 4: Biomarker types. (**a**) Prognostic biomarker, (**b**) predictive biomarker, (**c**) pharmacodynamic biomarker. 'S' and 'T' denote standard and test treatments, respectively.

- **Companion Diagnostic:** Companion diagnostic markers are used as a diagnostic test (usually molecularly based) that is specifically associated with a drug. A diagnostic is a medical device, a total system that needs to be fully comprehended in advance. It consists of:
  - Sample collection devices, transport, stability
  - Sample processing and assay reagents
  - Hardware and software

  Example: Bond Oracle HER2 IHC System (Leica Biosystems) for Breast Cancer – Tissue (ERBB2 (HER2))
- **Surrogate Endpoints:** A surrogate endpoint is intended to be a substitute for a clinical endpoint. It is expected to predict clinical benefit (lack of benefit or harm) based on therapeutic or other scientific evidence. In clinical trials, a surrogate endpoint (or marker) is a measure of the effect of a certain treatment that may correlate with a true endpoint but does not necessarily have a guaranteed relationship with it.

3.2 BASIS FOR THE SELECTION OF MARKERS TO MEASURE?

With respect to the clinical biomarker strategy there are two general approaches to determine which markers to measure: candidate-based or broad-based.

- Markers are selected using **a candidate-based approach** when something is already known about the marker, such as specific genes or proteins comprising the drug target signaling pathway.
- A **broad-based approach** does not rely on a biological hypothesis and is generally used in discovery. It allows to analyze different markers at once. For example, this approach is useful to explore entire pathways that may be associated with a particular compound.

3.3 TYPES OF BIOMARKER ASSESSMENTS WITHIN EACH LEVEL: DNA, RNA, PROTEIN

- **At the DNA-level:**
  - Different types of mutations, such as missense, nonsense, frameshift mutations, can have different consequences, such as changing or truncating a protein sequence and potentially affecting its function.
  - Gene Copy Number Variation occurs when there is amplification or deletion of a gene.
  - Chromosome rearrangements is a type of chromosomal abnormality where there is a breakage of DNA at two locations with a re-joining of the broken ends that results in a new chromosomal arrangement.
  - These are generally measured using the method of Next generation sequencing.
- **At the RNA-level:**
  - The gene expression level is measured.
  - Gene expression can be measured using a few different methods such as RT-PCR of specific gene(s) of interest.
- **Protein expression levels**:
  - Protein is the final product/consequence of gene expression, and these protein levels are measured using many different techniques.

- Immunohistochemistry will also allow us to see the detail of protein cellular localization, such as expression of the protein in the cytoplasm, in nucleus or in the membrane of the cell.
- ELISA measures soluble biomarkers in such samples as plasma, serum, and urine.

- **At the cellular systems level**:
  - Tumor cells after detaching from the primary tumor and circulating in blood stream can also be directly measured using techniques like Flow cytometry.

4. PLANNING FOR A BIOMARKER ANALYSIS FOR A CLINICAL TRIAL

There are a set of few questions that should be asked, before planning any kind of analysis. Knowing the answers to these questions allow us to better understand and handle the biomarker assessments and empower us to know what information is important for the various biomarker assessments.

- Studies to be included in the analysis: internal studies, external studies.
- What data (datasets and additional data derivations) are needed to answer the question, especially the definition of endpoints?
- Approach / Statistical analyses to address the question.
- Understand what type of data to expect with each marker type.
- Understand what tissue type is used most often to generate each marker type.
- Basic questions related to the technology used to generate the biomarker data.
- Gain insight into the limitations and potential challenges associated with each marker type.
- Basic understanding of the biological rationale behind why a particular marker is being collected.
- What standard shells are the most appropriate when analyzing each marker type.
- Do you foresee extensive post CSR assessments of these data?

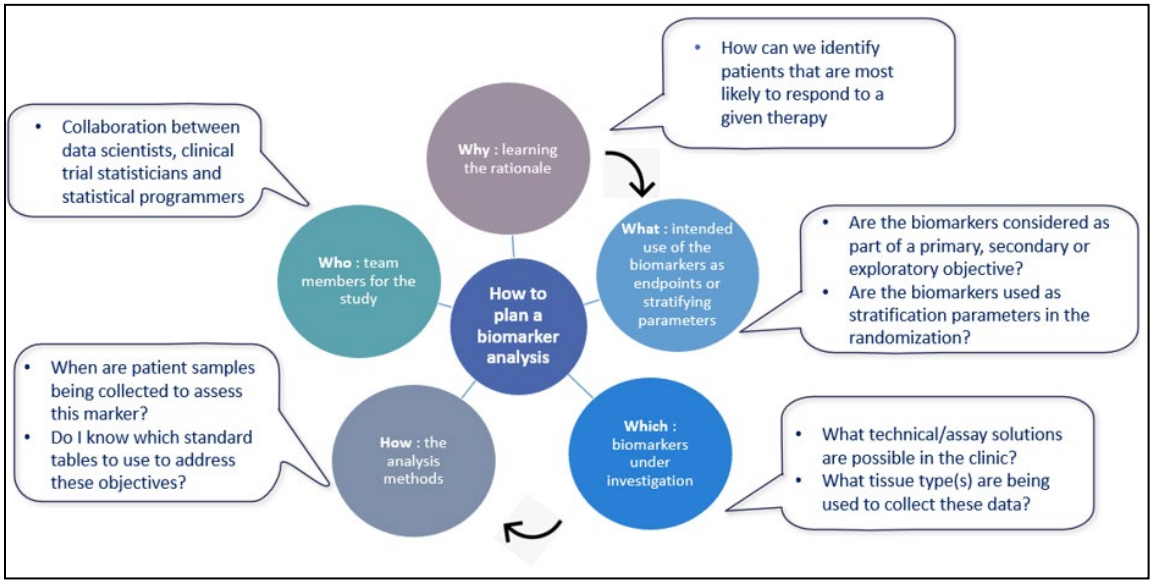


Figure 5: The W's and H's of planning a biomarker analyses.

5. STATISTICAL CONSIDERATIONS FOR BIOMARKER ANALYSIS

The analysis of biomarker data may differ from the analysis of other data collected during trials due to many different sources of variability, for example, differences in how samples are collected, handled, processed, preserved, and shipped, to differences in operator, instrumentation, timing, and temperature. The evolution in the technology of biomarker assessment is continuously improving. Biomarker data are obtained with the best tools we have but every assay has its pros and cons and, which result in specific opportunities and limitations when it comes to data analysis and result interpretation. The kind of assay performed will decide the way the data will be collected. These assays will decide the kind of statistical method/modeling method that can be applied to interpret the data in the most meaningful way. Below are some of the specificities of biomarker data.

5.1 MULTIVARIATE MODELING

- Along with the discovery of just one biomarker, sometimes a multivariate model using multiple biomarkers is also developed. The set of biomarkers of interest is commonly known as a signature. This generally involves building predictive models using both outcome and all the available data within a single study or across multiple studies.

- Predictive models usually involve a variable selection step to identify a subset of biomarkers that should be included in the final model/signature.
- Typically, the process of developing a biomarker signature entails the following steps - processing and normalization of the raw data, filtering out the most biologically/statistically relevant markers and then developing a predictive model on this subset.

5.2 META-ANALYSIS

- Meta-analyses will combine the results from several clinical trials to test a hypothesis which may require a larger or more diverse dataset than obtained from a single trial.
- For instance, studies may be combined within a project to assess the predictive value of a set of biomarkers. Alternatively, studies may be combined across several drug projects to assess the prognostic value of a set of biomarkers.

5.3 HANDLING MEASURES BELOW THE LOWER LIMIT OF QUANTIFICATION (LLOQ)

- When we encounter data with values lower than LLOQ, there must be proper defined rules and methods to analyze and report such data.
- Sometimes numerical values for the biomarker are reported, even if the values are below LLOQ. In this case, the imputation is needed. For example, If the proportion of a measure is below the provided pre-defined threshold, values will be imputed, and the standard set of summary statistics will be used. Sometimes the data will be only listed.
- Another example is when both baseline and post baseline values are below LLOQ, change from baseline will not be imputed and reported as missing. These are just some of the cases when we have data below LLOQ and how they can be handled.

5.4 UNSCHEDULED, MISSING AND SUMMARIZED MEASUREMENTS

- Unless otherwise specified, unscheduled and missing measurements will be handled in a similar way as laboratory data.
- The planned repeated measurements (e.g., multiple biological samples or technical replicates) can be done by averaging or linear mixed models for repeated quantitative measurement whereas a rule may be defined to handle inconsistent repeated qualitative measurements (e.g., a patient with mutated status from a fresh tumor sample and wild type status from an archival tumor sample will be considered as mutated).
- Missingness of biomarkers can be informative, like, biochemical assays where the quantity of analyte in the sample is classified as below LLOQ or above ULOQ.
- It can also be uninformative, like, poor image quality due to technical reasons, assuming that this is not linked to the subject's disease or drug related characteristics. Imputation strategies should be clearly defined for such cases.

5.5 LOGARITHMIC TRANSFORMATION OF BIOMARKER DATA

- Biomarker measurements are generally done via instruments which provide non-negative values for concentration of the analyte/biomarker as measured from a biological sample.
- These data are generally right skewed i.e., most of the data are on the lower end of the scale with fewer samples on the higher end of the scale.
- Another common feature of biomarker data especially those related to luminescence / fluorescence technologies is that the scale difference between the lower percentiles and higher percentiles can be many orders of magnitude apart.
- This phenomenon of the data poses many statistical and other practical challenges. Depending on the level of skew of the data, the visualization of the data can be very difficult, which can be made easier by visualizing the data in the log scale or equivalently visualizing the log transformed data.
- Another challenge, working with the raw right skew data is that the extreme observations have a large influence on the estimates making them more prone to sampling error.

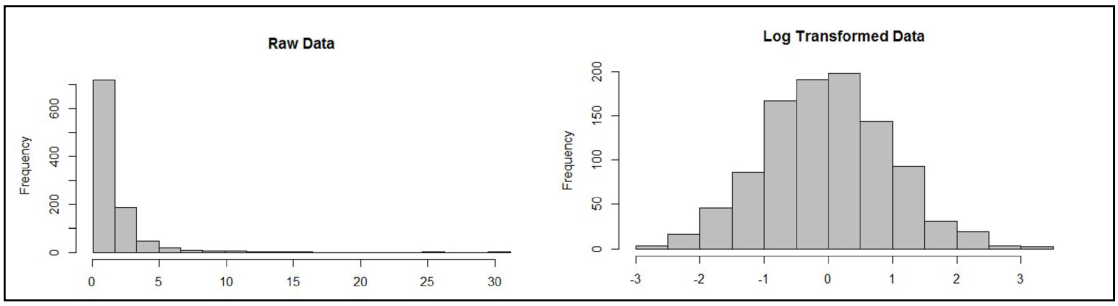


Figure 6. Visualization of raw data and log-transformed data

Implementation and Interpretation of the log transformed Data
The log transformation may make the data meet the requirements for using many standard statistical models. However, one needs to be cautious about the interpretations of the estimates from such transformations. The point estimates and confidence intervals derived from log transformed data cannot be interpreted in the same scale as the raw data.

6. OUTPUTS GENERATED FOR VISUALIZING BIOMARKER DATA

Not every kind of statistical method or assessment can be used on every kind of data. The type of outputs that are produced to visualize the biomarker data and the methods used for analysis, would depend upon the kind of assays done to collect the biomarker data. For example, if data is collected via ELISA, one might expect a larger proportion of data being below LLOQ and appropriate rules must be applied for the best representation of the. Here are few examples of the kind of outputs generated for biomarker analysis.

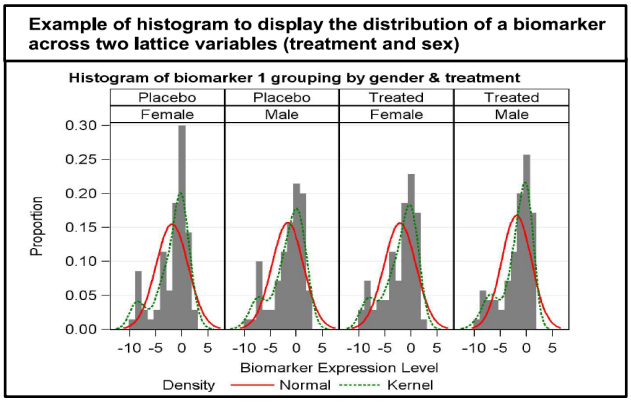
6.1 Tables: All patients or for subgroups defined by the selected baseline characteristics. Examples:

- Summary table for continuous variables.
- Standard summary statistics for the level and fold-change from baseline where applicable.
- Summary table for categorical variables.
- Shift table for the changes from baseline for categorical variables or using normal ranges or pre-specified thresholds for continuous variables.

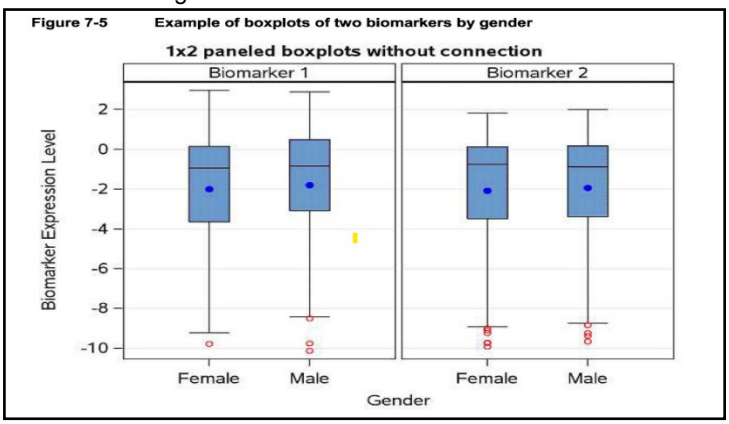
These tables are used to assess the relationship between categorical biomarkers and selected safety, efficacy, and PK endpoints.

6.2 Figures:

Histograms can be used to display the distribution of continuous variable. This figure can be used to detect if there is a shift of the central tendency between the groups.

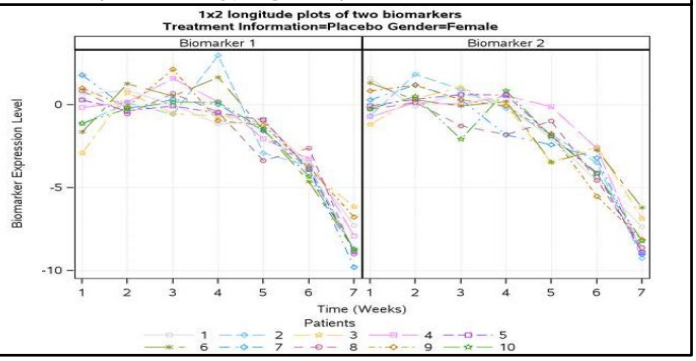


Boxplots can be used to display the relationship of biomarkers with categorical or ordinal variables when sample size is large, can be used to visualize the distribution of observed values in quantiles across another grouping variable including time.

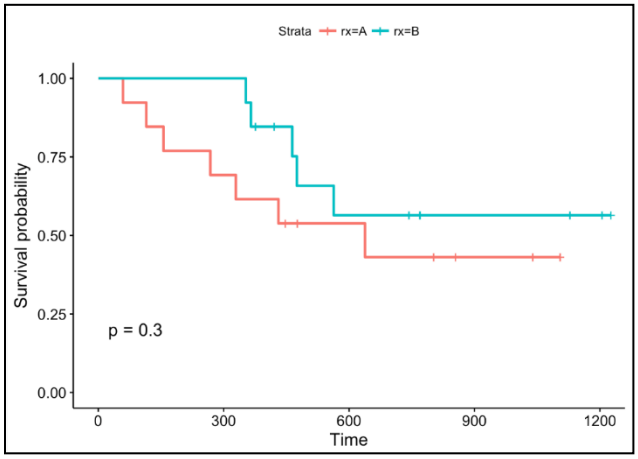


Longitudinal Plots are used to display subject/group level biomarker behavior over time. Used to visualize the variations over time within a subject/group or between/among groups. Example, pre and post treatment levels of the biomarker can be plotted for each patient as patient profiles over time to explore trends. Changes in biomarker levels over time, such as absolute change, percent change, or fold change from baseline, can be explored using longitudinal plots.

Example of longitudinal plots to display patient profiles (top) and mean profiles by treatment with 95% CI (bottom) across two lattice variables (biomarker type, gender)



Kaplan-Meier plot: can used to display time-to-event endpoints. This may be used with categorical biomarkers, such as mutation status.



Source: Survival Analysis in R: Kaplan Meier & Cox Proportional Models Tutorial | DataCamp

By convention, vertical lines indicate censored data, their corresponding x values the time at which censoring occurred. The log-rank p-value of 0.3 indicates a non-significant result (p <0.05 is considered to indicate statistical significance).

7. REPORTING BIOMARKER ANALYSIS

Biomarkers can be part of a primary, secondary, or exploratory objective in a clinical. The way they are handled and reported differs greatly depending on the scope of its analysis. Below are few examples when a biomarker analysis is a primary, secondary, or exploratory objective.

1. **Primary objective**: this happens when the biomarker is itself a primary endpoint and is used as a stratification parameter in the randomization or is used to define study entry.
2. **Secondary Objective**: For secondary objectives, there is usually strong prior evidence about the use of the biomarkers under investigation from either pre-clinical models or patient samples and based on that knowledge an interested biomarker specific to the drug can be studied.

Biomarker analysis, when part of a primary or secondary objective, is submitted as part of the CSR.

3. **Exploratory Objective:** An **additional exploratory biomarker analysis** can be submitted in the form of an abstract or HAQ's. The results are of interest and either complement the CSR or influence the clinical program. Additional analyses that may be performed after the completion of the end-of-study CSR can be documented in separate reports. These analyses may include combining or pooling of data from other studies or the analysis of biomarkers generated from samples collected during the study but analyzed after completion of the CSR.
4. **Exploratory analysis for additional related studies or research:** These are optional additional biomarker studies. The biomarker samples (e.g., bone marrow, peripheral blood, plasma) may be used for additional studies for research to help develop ways to detect, monitor or treat diseases. Those biological samples may be stored for up to 15 years after the end of the study and the decision to perform such exploratory biomarker research studies would be based on outcome data from this study or from new scientific findings related to the drug class or disease, as well as assay availability. T**he goal of these exploratory statistical analyses should be considered as the generation of new scientific hypotheses.** These hypotheses may be compared with results found in literature as well as verified with data derived from subsequent clinical trials.

CONCLUSION

Biomarkers are an integral part of drug development and have the potential to enhance screening and diagnosing disease, monitoring disease activity, or to predict the benefit of therapy. Combinations of clinical and research biomarker are used throughout the conduct of Phase I-III trials. Biomarkers have become highly valuable in driving oncology research and development and the commercialization of targeted therapies and will continue to have significant implications for all stakeholders across the health care. Especially in oncology, we are in the time when there is an evolving paradigm shift of personalized medicine. Biomarkers are used to optimize targeted therapy portfolios and make clinical development decisions. Despite the benefits there are still some challenges/limitations of using biomarkers in clinical trials on a larger scale. There needs to be large scale validation studies to confirm the clinical utility of biomarkers. It is important to maximize the value of biomarker data as it is often an expensive component of most clinical studies. Emerging technologies are expected to play a significant role in the discovery of new biomarkers and their application in clinical trial. Also, advancement in data analytics may improve the identification of biomarkers which will further result in the reduction of the time and cost associated with biomarker discovery and validation.

"Ultimately, biomarker-driven therapy approaches will lead to better patient outcomes by enabling early detection, identifying treatment responders and monitoring treatment, response, and targeted therapeutic effect. Further, we anticipate these methods will expand to other therapeutic areas. For the immediate future, all signs point to cancer care will be profoundly influenced by biomarkers to guide researchers and physicians at every stage, from drug development to disease management." *Source: The Evolution of Biomarker Use in Clinical Trials for Cancer Treatments, The Journal of Precision Medicine*

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- *Using Protein Biomarkers Increases the chances of Success in Clinical Trials | Technology Networks*

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