

# Fulfilling the promise of biomarkers in drug discovery and development

by Dr P. Goix

In 2004, the U.S. Food and Drug Administration released a white paper, now known as the Critical Path Initiative, which challenged the pharmaceutical industry to reduce the time (12-15 years) and expense (approximately \$1-2 billion) required to bring a drug to market [1]. Biomarkers were seen as key to meeting this challenge with a promise of providing more effective drug and target identification, safety and toxicity monitoring and patient identification and stratification. With unprecedented sensitivity and dynamic range, the technology described in this article helps fulfill the promise of biomarkers through its advances in single-molecule detection.

## Biomarker definitions

A biomarker is any objectively measured factor that predicts or signals a specific state, such as diseased or normal, or a response to therapy. Historically, the term biomarker has referred to a physical or physiological yardstick, such as blood pressure. Today, the term has come to represent molecular or biological biomarkers, which come in many forms, including human genes and genetic variations and expression differences in RNA, proteins and metabolites.

For immediate and clinically useful information, proteins and metabolites are held to be more dynamic and reflective of physiology. They also carry more diagnostic information compared to DNA and RNA because each gene can give rise to different proteins depending on splicing and post-translational modifications. Human plasma is regarded as the most accessible and comprehensive source of the human proteome giving insights into ongoing physiological and pathological processes. However the wealth of information in one sample of human plasma presents a challenge.

## Plasma proteome – technical hurdles

Proteins in plasma have an extraordinary dynamic range of more than 10 orders of magnitude in

concentration, with one million different protein molecules and twenty-two proteins making up 99% of the protein content. Translating biomarker discoveries into clinically relevant diagnostic assays, especially in the field of proteomics and metabolomics, has presented the molecular diagnostic industry with significant hurdles for the development of sensitive and selective diagnostic assays.

To allow detection at highly sensitive levels, technological advancements in immunoassays, such as those developed at Singulex and incorporated into the Erenna BioAssay System, are enabling the quantitative clinical detection of single molecule biomarkers at previously undetectable levels. This has, for the first time, provided researchers with the tools needed to measure biomarker levels rapidly in normal tissue, establishing a baseline for biomarker deviation that can indicate disease onset or toxicity problems earlier.

The Erenna BioAssay System, a direct molecular detection technology, is used with ultra-sensitive, customisable immunoassays with an extensive clinical dynamic range across multiple mammalian species. Currently, Singulex with pharma and academic partners has over 75 prototyped custom-made protein or metabolite bioassays. The required sample volume is small, and complex matrices such as serum can be assayed without

prior protein depletion. These advantages enable partners to develop scalable, clinically translatable assays rapidly.

## Biomarker discovery and validation

In the biomarker pipeline, the technologies that will enable the use of biomarkers in drug discovery and development vary depending on the research stage [2]. The first stage, biomarker discovery, depends upon mass spectrometry techniques to perform unbiased, semi-quantitative analysis of differences between diseased and healthy states. Due to the high false positive rate, the list of biomarker candidates generated in the discovery stage must be further verified through additional more targeted and quantitative mass spectrometry techniques, such as immunoaffinity peptide enrichment.

The second stage, biomarker validation and clinical assay development, depends upon extremely sensitive and specific high-throughput immunoassays. The single greatest challenge in discovering biomarkers with clinical utility is the sensitivity and specificity necessary to determine that a biomarker is truly positive for disease or toxicity, and can be used to detect deviations from normal.

In collaboration with researchers at the University of California, San Francisco, Singulex was the first to identify and quantify normal levels in human plasma of cardiac troponin I (cTnI), a protein biomarker used to detect cardiotoxicity and heart attacks. The lower limit of detection of today's leading commercial assay is 350 ng/L. The company's cTnI assay is able to detect well below this limit and can determine that the level of cTnI in healthy individuals is 7 ng/L [3]. The ability to detect the normal level and deviations from the normal enables earlier identification of patients at risk for adverse cardiac events. The technology extends the clinical utility of new and existing biomarkers by improving the limits of detection.

## Incorporating biomarkers into drug discovery and development

The ultimate biomarker would be able to indicate the diseased state and be altered by therapeutic intervention so that clinical outcome could be predicted. However, it is most unlikely that one biomarker could be used for all these purposes; different biomarkers must be used at different stages in drug discovery and development.

Biomarkers have been classified into three types. The first are markers of the disease state. The second type indicates the effects of a therapeutic intervention based on the mechanism of action for a drug, even though this may not be known to be associated with the desired clinical outcome. This type of biomarker is often used in preclinical screening of drug candidates. The third type of biomarker is used as a surrogate end-point because a change in that marker predicts clinical outcome.

Areas like oncology have progressed further than other areas in biomarker discovery and use, and have already proven successful with such examples as HER-2, a biomarker for a subset of more aggressive breast cancers. HER-2, a human epidermal growth factor, not only identifies patients that will benefit from Herceptin but is also the target of the drug.

Not all diseases will be amenable to biomarker discovery and use. Biomarkers will be easier to use in diseases that are well defined with more homogeneous patient populations. However, the more that is learned about disease mechanisms and genetic differences among populations, the more biomarkers will be derived from this research.

## Preclinical stage

Incorporating biomarkers early in the discovery process when a new therapeutic target is being identified is of utmost importance especially if the biomarkers can reflect mechanism-based intervention. To find these discovery biomarkers, researchers can look to their pre-clinical experiments, such as animal and cell culture models. Analysis of expression changes in target versus non-target tissues in treated animal models may provide possible information to establish biomarkers for a potential therapeutic.

High-throughput screening targets may also be used as an initial source of potential discovery biomarkers. It is important to keep in mind that these discovery biomarkers which are used pre-clinically may not be the same biomarkers used after the drug is selected to enter clinical trials.

In preclinical studies, biomarkers are used to determine if the drug is hitting the target, after which additional biomarkers must confirm that hitting the target actually alters the pathophysiological mechanism, and that altering this mechanism affects clinical outcome. Biomarkers can also reveal other drug targets as well as optimise the selection of molecules that interact with these targets for further development.

## Clinical stage

For one successful drug, there are 60 in discovery, 20-30 in early development, and 5-8 in clinical programmes. One of the biomarker development goals is to assess drug safety and efficacy accurately, thereby reducing attrition of drugs during clinical phases of development and hence reducing the overall cost of drug development.

Singulex' new, more sensitive technologies developed for protein and metabolite biomarker detection provides a significant benefit for monitoring established safety and toxicity biomarkers, such as troponin I for cardiotoxicity, throughout clinical trials. The current cardiotoxicity tests can only detect large-scale damage, which halts drug development. The benefits of more sensitive assays are not only the detection of mild toxicity, repeated exposure to which could lead to major problems, but also in giving the green light for further development. Being able to detect normal levels of biomarkers, especially for toxicity, but for any deviation from normal provides the total information needed to make accurate decisions on whether to proceed further in drug development. Another goal, as the drug and biomarkers move through the drug development phase and into clinical trials, is to identify and stratify patients to maximise the signal in early proof-of-concept trials. If biomarkers could identify patients whose response rate could be double or higher, then clinical trials could involve half the current number of subjects yet yield sufficient proof of efficacy.

## Companion diagnostics

The use of biomarkers to identify and stratify patients in clinical trials would ultimately create drug/diagnostic combinations, such as Herceptin/HER-2, and fully deliver the promises of personalised medicine. Today's drugs which have been withdrawn from market or Phase 3 development due to serious adverse events may have had a different fate if combined with a companion diagnostic to identify responders or to closely monitor toxicity.

## Partnering strategies

The development and use of biomarkers in drug discovery and development has fallen into a gray area where no one knows who is responsible for developing the technology and assays within a company. For effective biomarker integration into drug discovery and development, pharmaceutical companies are seeking collaboration, not only within their company but also with new companies and technologies.

Singulex provides the technology to validate and deliver highly sensitive, customisable assays for almost any protein or metabolite biomarker. With numerous collaborations in leading pharma and academic institutions, assays for cardiac and liver toxicity have been developed as well as for biomarkers for diseases such as pancreatic cancer and Alzheimer's disease. To request an assay, apply to enter the Early Technology Access Program, or for more information, please visit [www.singulex.com](http://www.singulex.com).

## References

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## The author

Philippe Goix, Ph.D.  
Singulex  
Hayward, CA, USA.  
[pgoix@singulex.com](mailto:pgoix@singulex.com)

Hotline: [www.drugplusinternational.com](http://www.drugplusinternational.com) & tick 30858