



Biomarkers in the Pharmaceutical Industry

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Although biomarkers have been the essence of laboratory medicine since its inception, they are relatively new tools in the development of pharmaceutical compounds. The main utility of biomarkers for pharmaceutical companies has been in making drug development a more efficient and a cost-effective process. This is primarily the case because of the drastic and perhaps alarming increase seen in the development cost of new drugs in the last few decades, which has been accompanied by a decrease in the number of drugs obtaining regulatory approval. Furthermore, the Critical Path Initiative of the US Food and Drug Administration has identified biomarkers as important tools that may help to correct this imbalance. As will become apparent in the discussion below from 4 biomarker groups in leading companies, a large number of these biomarkers have been successfully used for internal decision-making, yet they may have never left the pharmaceutical space. At present, biomarkers, at a growing number, are being codeveloped along with the therapeutic compound as companion diagnostics; this is part of the Precision Medicine paradigm of delivering the right medicine to the right patient. This development will undoubtedly move biomarkers beyond the pharmaceutical space and into the clinical laboratory, thus influencing the practice of laboratory medicine. It will also become apparent that biomarkers developed by the pharmaceutical industry must be evaluated and validated using rigorous procedures. Clinical chemists can certainly assist in this endeavor. This may, in turn, lead to a larger number of useful biomarkers, not only in drug development but also in the clinical laboratory, as companion diagnostics.

Biomarkers are extensively used throughout the process of drug development. In your experience, what are the

most prominent or illuminating examples of how biomarkers directly impacted the development of a drug?



Sanofi team: Biomarkers have played critical roles in the development and ultimately clinical use of a number of medications. For example, biomarkers used for evidence of clinically relevant activity include LDL-cholesterol for statins and ezetimibe and hemoglobin A_{1C} for long-acting insulins, insulin sensitizers, and insulin secretagogues. In addition, in the age of targeted therapy in oncology, biomarkers have been used to select patients for treatment with drugs that inhibit the drivers of cancer, including BCR-ABL fusion for Gleevec, *HER2* [v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 (*ERBB2*)⁸] amplification for Herceptin, *EML4-ALK* (echinoderm microtubule associated protein like 4-anaplastic lymphoma receptor tyrosine kinase) translocation for Xalkori, and *BRAF* (B-Raf proto-oncogene, serine/threonine kinase) mutation for Zelboraf. These biomarkers were

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⁸ Human genes: *ERBB2* (also known as *HER2*), v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2; *EML4*, echinoderm microtubule associated protein like 4; *ALK*, anaplastic lymphoma receptor tyrosine kinase; *BRAF*, B-Raf proto-oncogene, serine/threonine kinase; *EGFR*, epidermal growth factor receptor.

used to enrich for patients most likely to show clinical response.



Michael E. (Ransom) Burczynski: We have used many target engagement, pharmacodynamics, and predictive biomarkers to progress drug candidates to registered pharmaceutical therapies. However, one of the most prominent ways in which we have used biomarkers was to “kill” programs

rather than to advance them. Patient stratification biomarkers are also very useful in pharmaceutical development. One specific example in which a patient stratification biomarker led to the approval of a drug is *BRAF V600E* for Zelboraf.



John A. Wagner: Compelling go/no-go decisions and patient selection are among the most important uses of biomarkers in drug development. Sitagliptin, a first-in-class dipeptidyl peptidase-4 (DPP-4)⁹ inhibitor, benefited from a robust target engagement and well-qualified disease-related biomarkers

in achieving proof of concept. Target engagement biomarkers included the inhibition of plasma DPP-4 activity and disease-related biomarkers included plasma glucose, in both the preclinical and clinical environments. The tandem use of these biomarkers facilitated the design of clinical efficacy trials while streamlining dose focus and optimization, the net impact of which reduced overall cycle time.

Patient selection biomarkers, codeveloped as companion diagnostics, are also striking examples of how biomarkers directly impact drug development. Pharmacogenetic biomarkers, including *BRAF V600E* for vemurafenib in melanoma, *EML4-ALK* for crizotinib, and *EGFR* (epidermal growth factor receptor) for erlotinib and gefitinib in non-small cell lung cancer, as well as Kirsten rat sarcoma viral oncogene homolog (*KRAS*)

against the use of cetuximab and panitumumab in colorectal cancer, are all prominent examples.



Scott D. Patterson: Pharmacodynamic biomarkers are often used to assess target engagement in the early phases of drug development. We had one example in which a program was halted on the basis of a pharmacodynamic response. Biomarkers revealed that the molecule, which was a dual receptor

antagonist, completely blocked only one receptor and not the other. In the absence of that data, the molecule might have progressed to phase 2, where the lack of efficacy would have eventually been discovered. This would have occurred with significant expenditure of time and resources.

Another use of biomarkers having obvious drug development impacts is as companion diagnostics. An updated list of companion diagnostics may be found in the following link: (<http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm>).

What are the major pitfalls in the application of biomarkers to drug development? What is an illustrative example worthy of further study by the readership?

Sanofi team: The first pitfall of any biomarker discussion is whether we are using the same definition. Biomarkers that predict disease progression are rare, yet these are often the biomarkers to which most experts refer.

A deep understanding of the clinical specificity of the biomarker for the disease is really important. However, biomarkers may not always predict a meaningful clinical response, even when they seem to be closely tied to clinical outcomes. For example, niacin raises HDL-cholesterol but does not reduce clinical events; ezetimibe decreases LDL-cholesterol but at this time has not definitively been shown to reduce clinical events.

Another potential pitfall is the variability in the robustness of analytical parameters of the assays and the lack of rigor in designing studies to associate biomarkers with a phenotype. For example, studies identifying gene expression signatures predictive of response to chemotherapy or pathway activation were published in top journals, widely cited, and even began to be used as a way to assign patients to therapy. However, due to unclear methods and design issues, these signatures were ulti-

⁹ Nonstandard abbreviations: DPP-4, dipeptidyl peptidase-4; NGS, next generation sequencing; LDT, laboratory-developed test.

mately deemed unusable and the supporting publications were retracted.

Michael E. (Ransom) Burczynski: The top pitfall is the published reports of biomarkers that are not accurate. For example, a published paper detailed several translocations of a gene in a tumor type of our interest. The data were compelling enough for us to pursue further validation of these findings, only to discover that the prevalence of the translocations were vastly exaggerated in the initial literature report. This meant that the translocations would not be a good predictive marker of potential responders. We now work with groups like Compendia or utilize information in The Cancer Genome Atlas to quickly evaluate reported literature results in silico before pursuing them further.

John A. Wagner: The most serious pitfall is the misuse of validation and qualification studies that result in biomarkers that are not fit for purpose. Also, biomarker discovery, development, validation, and qualification are time-consuming activities. Thus, lack of appropriate planning of these activities creates missed opportunities and delays.

Scott D. Patterson: Defining causality in patient selection biomarkers is important for their success but often challenging to determine. Prospective clinical trials may be of great help in delineating causality, but the timely execution of these trials is sometimes not possible. Prospective–retrospective analyses can prove useful in these cases. Here, hypothesis testing with samples from previously completed trials is undertaken using analytically validated assays together with predefined statistical analysis plans.

Many potential biomarkers arise from academic work, yet few candidates prove useful. What can be done to enhance the downstream utility of academic findings?

Sanofi team: There are multiple aspects that could be addressed to increase the utility of biomarker findings. Methodological issues can potentially lead to initial identification of biomarkers that are not subsequently confirmed (e.g., inadequate replication, too small sample size, unclear methods, variation in reagents), especially in studies that explore large numbers of biomarkers. Areas that do not receive as much attention are sample collection, storage, and chain of custody, as well as the quality of the technical validation. In addition, markers identified in animal models may not translate to human disease. Larger experiments with more replication, a clear methodology, and readily available reagents, as well as careful selection of preclinical models relevant to the disease and/or pathway of interest, may enhance downstream utility.

Validation of biomarkers in the clinical setting requires well-designed clinical trials with sufficient power to conclusively demonstrate a correlation of the biomarker with key clinical parameters such as morbidity and mortality. Standards of evidence used in drug development should also be applied to biomarker development.

Michael E. (Ransom) Burczynski: This is a very important point. The National Biomarker Development Alliance, of which I am a member, is dedicated to establishing standards that can provide guidelines for academic investigators, journals, and other participants in the publication process to enhance the validity of published biomarker reports. Other groups are also trying to develop guidelines for biomarker discovery protocols, including the one led by John Quackenbush. We believe that strengthening the analytical validation of the biomarker assays on the front end, and strengthening the data analysis specifics on the back end, will greatly enhance the accuracy of reported biomarker findings in the literature.

John A. Wagner: In order for academic work to gain more traction, greater attention must be paid to biomarker validation and qualification. Because of the plethora of opportunities, it will be important to identify priority areas on the basis of high unmet medical need and lack of clinically tractable endpoints. Development of a framework for an objective, cost–benefit model to evaluate and prioritize biomarkers to pursue for qualification would be helpful. It would then be important to incentivize academia to develop focused efforts to provide the level of evidence necessary to qualify biomarkers.

Scott D. Patterson: This issue may likely represent poorly designed studies and a lack of application of rigorous assay qualification standards. Standards applied to “research” level assays, both analytical validation and sample collection procedures, often result in data that are insufficient to draw from statistically robust conclusions. Ensuring that grant-funding agencies and journals demand and facilitate clear descriptions of the analytical validation of assays and sample collection procedures will go a long way to addressing these concerns.

What is the main driver for the codevelopment of drugs and companion diagnostics? How do you envision companion diagnostics affecting the practice of laboratory medicine?

Sanofi team: As our knowledge of biology and technology advances, there will be many situations in which a specific drug will have a favorable benefit–risk profile in only a defined subset of patients with a particular disease. That is, patients will be selected on the basis of who is most

likely to show clinical improvement or not experience serious adverse events. Companion diagnostics will be critical for identifying these subsets, and codevelopment of the drug and diagnostic is generally the most effective path. For example, β -blockers may work only for heart failure patients with specific β -adrenergic receptor genetic polymorphisms. Details regarding testing, clinical trial design, regulatory, and commercialization aspects of companion diagnostics are still evolving. Because companion diagnostics play an increasing role, clinical laboratories will need to accommodate new tests and their corresponding platforms.

Michael E. (Ransom) Burczynski: The main driver is personalized or stratified medicine. I prefer the term stratified medicine because what we are really hoping to do, by combining a drug with a diagnostic, is to simply increase the likelihood that the patient will respond favorably to administered therapeutics on the basis of the molecular details of their disease. Companion diagnostics is almost certain to become a standard practice of laboratory medicine. Next generation sequencing (NGS) and whole genome sequencing, once stabilized, will greatly impact the field of codiagnostics.

John A. Wagner: The primary driver for companion diagnostics is to enable safe and effective innovative therapies in segmented patient populations, whereas unselected populations would not enjoy the same benefits. Companion diagnostics should become an increasingly important component of the practice of laboratory medicine.

Scott D. Patterson: Diagnostics codeveloped with therapeutics are often (although not exclusively) used to measure a biomarker that can identify the patient population most likely to benefit (or potentially be harmed) from the therapeutic intervention. Being able to identify the patient population who can gain the most benefit from a therapy is the primary driver. The impact on laboratory medicine can vary depending on the platform upon which the biomarker is measured and whether assays for the measurement of that biomarker already exist as a laboratory-developed test (LDT). A further complication would occur if another companion diagnostic existed to measure the same biomarker, but on a different platform and with a different cutoff value.

There is currently a great effort directed at the qualification of biomarkers for use in clinical trials, such as the different initiatives from the C-Path Institute of the NIH. How do you envision these efforts enabling clinical trials in the future?

Sanofi team: Biomarkers that are well qualified will enhance their value to predict clinical response and, hence, increase their clinical utility. Well-qualified biomarkers

can be used to identify patient responses to medications in trials in which the length of therapy or the number of patients is not sufficient to show a statistically significant change in a clinical endpoint. These efforts can provide transversal benefits across multiple programs and therapeutic areas. Clinical qualification of genomic, proteomic, metabolomic, and imaging technologies, as well as optimal clinical trial design, will have major impacts on enabling clinical trial data to be most effectively used both for later-stage trials as well as for decision-making in earlier clinical studies.

John A. Wagner: Qualified biomarkers in priority areas will drive faster and more effective clinical trials, resulting in better and innovative therapies. Qualifications resulting in surrogate endpoints, which satisfy regulatory requirements, as well as companion diagnostics, will drive approval of new therapies.

Scott D. Patterson: The qualification of biomarkers for a specific context of use that allows more rapid evaluation of the benefit of a particular therapy will have a significant impact on drug development. Qualification of surrogate endpoints (a biomarker that substitutes for a clinical endpoint) will obviously positively impact drug development.

Pharmacogenomics has been helpful in understanding the difference in metabolism of certain drugs, such as warfarin. What role do you think pharmacogenomics will play in the identification/segmentation of patients who are most likely to benefit from a pharmaceutical intervention?

Sanofi team: Pharmacogenomics will have a greater focus on the identification of patient subsets that show differential response to a drug (e.g., polymorphisms in β -adrenergic receptors for β -blocker response). Many of these variants will be related to disease susceptibility and the target/pathway of the drug. There is a need to recognize and understand outliers in responses.

Michael E. (Ransom) Burczynski: It is not perfectly clear how pharmacogenomics may play a role in identifying responders/nonresponders to biologics, but it certainly has the potential to help us identify patients at risk for idiosyncratic drug reactions in the future.

John A. Wagner: Pharmacogenomics is experiencing an increasing role in patient selection as evidenced by the inclusion in drug labels for required or recommended use, resulting in a large number of personalized medicine therapies. Multivariate “signatures” will likely be increasingly used, opening up new possibilities for patient selection biomarkers.

Which biomarker platforms do you believe will be the most disrupting/impactful in the future of drug development?

Sanofi team: NGS already is, and will continue to be, a very disruptive platform. All classes of DNA alterations (mutations, amplifications, deletions, rearrangements) and RNA changes (gene expression profiling, expressed fusions, alternative splicing) can be detected on one platform. The affordability of NGS has led to its implementation on a huge scale across academia, biotechnology, and pharmaceutical companies, and it is now penetrating the diagnostics industry. Beyond targeted therapy, NGS could impact the emerging immunotherapy field by T-cell–receptor locus sequencing and other applications. Plasma microRNAs constitute another exciting new biomarker platform that may be particularly useful because of its sensitivity and specificity. Proteomics, lipidomics, and metabolomics, as well as advanced imaging-based technology, may also provide important advances.

Michael E. (Ransom) Burczynski: The highly “plexed” platforms, which could prove useful in anticancer immunotherapies. We have made a great progress in the genomics platforms, but other areas such as flow cytometry (e.g., chip-based flow cytometry, CyTOF) and immunohistochemistry (e.g., multiomyx approaches) are starting to emerge and likely to be disrupting and impactful in the future of drug development.

John A. Wagner: Emerging platforms include omics technologies that have enabled the use of simultaneous measurement of “signatures,” or patterns of co-occurring genetic sequences, peptides, proteins, or metabolites as biomarkers. It is also possible to use signatures across different platforms. For these multivariate

platforms to find their way into the clinical arena, issues including method validation and qualification of evolving algorithms must be resolved.

Scott D. Patterson: Platforms that are reasonably priced, have a small footprint, and do not require extensive expertise. NGS in clinical laboratories will enable comprehensive genetic analysis to be undertaken in a more cost-effective manner and certainly qualifies as a disruptive technology. Highly multiplexed and cost-effective technologies for analysis of circulating tumor DNA that may be used as substitute for tissue-based analysis would be truly disruptive and beneficial to patients and their healthcare providers.

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