

The Use of Biomarkers in Drug Discovery and Personalised Medicine

a report by

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The challenges faced by the pharmaceutical industry in terms of maintaining delivery of new medicinal products in an environment of escalating research and development (R&D) costs have been well documented. One of the key initiatives of the US Food and Drug Administration (FDA) in response to this pipeline problem, the Critical Path Initiative,¹ identified six priority challenges for targeted research to increase efficiency, predictability and productivity in the development of new medicinal products. At the top of the list was the development of biomarkers to address the issues of predictive medicine, allowing the prediction of a potential product's performance as early as possible with the greatest degree of certainty. This recognition by regulatory agencies of the importance of biomarkers for the future success of drug discovery and development is reflected both by their prevalence within the literature – a simple search for the term 'biomarker' in Medline giving in excess of 350,000 hits – and in the recent approvals of clinically validated molecular diagnostics such as the Oncotype and MammaPrint multivariate diagnostics for predicting breast cancer relapse.

What Are Biomarkers and What Use Are They?

The word biomarker is frequently used, often very generically, to cover a wide range of measurements that are applied in a large number of ways. This inherently wide scope of the term biomarker has been a source of confusion in this area. Varying formal definitions for biomarker can be found: these usually involve the concepts of an objective measurement of a characteristic as an indicator of a biological process, whether a normal process, a disease process or a response to treatment. Often, the measurement is implicitly or explicitly thought of as being biochemical in nature, but biomarkers are not limited to this definition; for example, biomarkers based on imaging technologies are proving very powerful in a diverse range of applications, from tumour progression to joint narrowing.

A useful first distinction can be made between two broad classes: surrogate and predictive biomarkers. Surrogate biomarkers are measured characteristics that can be used as substitutes for other characteristics, but that are easier to measure due to cost, time or ease of observation. This is not a new concept; for example, raised cholesterol has been used as a surrogate for heart disease for many years. A closely related concept is that of the surrogate clinical end-point; however, for a biomarker to add value it does not need to demonstrate the same levels of validation or strength

of relationship as are required of a surrogate end-point. Predictive biomarkers, on the other hand, are measured characteristics that can be used to predict the likely outcome for a patient in terms of either disease progression (prognostic biomarkers) or response to treatment (predictive biomarkers), which can then guide clinical treatment. Predictive biomarkers can work in identifying both patients who would respond positively to a drug (efficacy biomarkers) and those who should not be administered the drug as they would be at increased risk of an adverse event (safety biomarkers). This forms the basis of personalised medicine: selecting the right drug for the right patient. Clearly, while these various classes of biomarkers have similarities, and a particular biomarker may fulfil multiple roles, there are also differences in the ways in which they need to be analysed and interpreted and in how they can be integrated into the drug discovery and development process. For example, an efficacy biomarker will be most effectively co-developed with a drug, while it may be possible to develop a safety biomarker only at a late stage in development – or even post-registration – once a safety issue has been identified.

Many applications of biomarkers can be considered as enabling improved decision-making by increasing the knowledge and confidence of researchers and thereby improving assessment of risk profiles. This may be at a patient level, using a personalised medicine approach to aid treatment decisions based on an individual's predicted efficacy benefits and safety risks; it may also be at a drug programme level. Within drug discovery, biomarkers may be used to screen out compounds with potential toxicity issues or prioritise compounds based on likely efficacy. In the early phases of clinical development, biomarkers may provide rapid confirmation of the activity of compounds and allow early assessment of dose selection. If these decisions can reliably be made as early as possible in the discovery and development process, they will lead to a substantial reduction in both expenditure and time by focusing resources on those compounds, doses and indications most likely to succeed. The whole biomarker discovery process can also be highly valuable within the wider drug discovery process; for example, newly discovered biomarkers may also be candidate drug targets, and the identification of biomarkers is closely related to understanding the mechanism of action of drugs.

New Technologies

While biomarkers themselves are not a new concept, much of the current interest surrounding them can be attributed to recent developments in technologies that allow simultaneous measurement of many hundreds or thousands of molecules from small biological samples. The highest-profile such technologies are genomics, transcriptomics, proteomics and metabonomics, which measure entire complements of DNA, messenger RNA (mRNA), proteins and metabolites, respectively. Each of these can be performed using a number of underlying technologies, each with its own characteristics. This technology development is still continuing, as



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demonstrated by the emergence of comparative genomic hybridisation (CGH) to measure genomic copy numbers and the promise of low-cost whole-genome sequencing in the near future. As a biomarker progresses through its life-cycle, it will be measured on a number of technologies. Typically, high-coverage techniques are used in biomarker discovery, but in the validation and application stage a more focused, lower-coverage technology may be applied to more rapidly and cheaply generate more reliable results. Biomarker discovery may be performed on a high-density microarray platform, moving the selected genes onto a lower-density reverse transcriptase–polymerase chain reaction (RT–PCR) platform for validation. This movement between platforms could also be extended to moving between -omics; for example, a biological pathway may be identified on a transcriptomics platform, but if in application it is easier to measure a protein from the same pathway, a proteomics technology may be used to assess the biomarker in practice. Given the great potential provided by the development of new technologies, the big challenge for the pharmaceutical industry is how to best identify and exploit biomarkers.

Lots of Data – An Opportunity and a Danger

The great benefit and strength of these new technologies is their ability to generate a huge amount of measurements. Conversely, this is potentially also their greatest risk: given this wealth of information, it is easy to jump to erroneous conclusions. As a starting point, one absolute must throughout the biomarker discovery, development, evaluation and application process is high-quality data. This runs right through the process: from study design, sample collection and storage through to using the technology. The Microarray Quality Control Project² is an FDA initiative that in its first phase demonstrated the high levels of agreement and reproducibility that can be achieved across different microarray platforms and sites. This provided powerful evidence that, in an idealised scenario, microarray technology could deliver reliable and reproducible results. The next step is to ensure that the same high standards of quality are regularly and widely achieved in practice. We have discovered that in the majority of a random sample of publicly available microarray data sets, the biggest source of variation or difference between samples was their underlying quality. If this issue is not minimised and dealt with appropriately both in the laboratory and in data processing, it has the potential to mask true signals and generate false conclusions.

The availability of new technologies simultaneously measuring many features within a single assay has led to the extension of the traditional univariate concept of biomarkers (a single measurement) to multivariate biomarkers, combining multiple measurements into a single result for each patient. This can cover a range of complexity, from a simple sum of the expression of a few genes within a pathway to provide a more accurate measurement of the overall levels of activity within that pathway, to a 'black-box' modelling technique combining information from many hundreds or thousands of distinct genes. Clearly, a multivariate model based on a clear biological hypothesis and mechanism would be preferable, but if a biomarker can be shown to be beneficial and deliver clinical benefit without an underlying hypothesis, this should not prevent its adoption. Additional specific issues related to multivariate diagnostics have been recognised by the FDA in a recently released draft guidance document³ covering the regulation of *in vitro* diagnostic multivariate index assays

(IVDMIAs). The robustness of any multivariate model in practice should also be considered. Experience with complex multivariate models in other fields, e.g. financial credit ratings, has shown that while increasing the complexity of models can produce small short-term improvements, in the longer term a simpler, more robust approach will generally give more reliable results.

The complexity of the data generated by many of the new technologies, particularly their extremely high dimensionality, makes their interpretation far from easy, with great potential for spurious claims. With such complex data, incorrect analysis that fails to account for either the high dimensionality or the underlying structure of the data arising from a combination of the underlying biology, the technology and aspects of the processing such as batch effects can easily lead to false-positive conclusions. In a review of published microarray-based gene expression profiles, Dupuy and Simon⁴ found that half of the publications contained at least one of three basic flaws – inadequate control for multiple testing; spurious claims of clusters and clinical outcome; and incorrect cross-validation – each of which can give rise to false claims.

Given the potential for erroneous conclusions, study and experimental design takes on new levels of importance. With the difficulties in obtaining samples that are sometimes faced, there is a temptation to pool data from samples taken from disparate collections. However, there is a huge difference between this approach, where the data may hide all manner of biases and imbalances, and a properly designed study collection, where these effects can be minimised or at least be fully recognised and accounted for in the analysis. An excellent example of how these biases can arise and how they can inflate effect sizes can be found in Akey et al.⁵ However, it is important to remember that biomarkers are unlikely to provide the whole story by themselves. In practice, clinicians will have a host of other clinical information available to them in terms of known risk factors, which will be used in combination with any biomarkers to make a clinical decision; this needs to be borne in mind when developing biomarkers.

New Ways of Working

Integrating biomarkers into drug discovery and development presents a number of challenges to traditional approaches. For a diagnostic to be co-developed with a drug requires adaptations of the clinical programme and the design of individual trials to incorporate the diagnostic development alongside the drug development. On an organisational level, one feature of applying biomarkers is the need for multidisciplinary teams and greater levels of integrated working across different disciplines, bringing together biological, numerical and analytical skills. Another feature is the blurring of the traditional boundary between discovery and development, requiring the sharing of information both up and down the pipeline.

The Future

Technological developments have opened up a huge range of opportunities for biomarkers throughout pharmaceutical research, and there is little doubt that these technologies will continue to develop, generating new and wider ranges of information from a more extensive range of samples. In their early days, overenthusiastic misapplications of these new technologies damaged their reputation. Hopefully, these lessons have now been learned and biomarkers can now start to deliver real benefits within the pharmaceutical industry and, ultimately, to patients. ■

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