Essential Tools

The growing utilisation of biomarkers throughout the drug development process allows for new and innovative practices in the treatment of many diseases, including Alzheimer's disease and cancer. These now essential tools could revolutionise the development of advanced therapeutics

Dr Mélanie Bodnar-Wachtel and Dr Tanja Schubert at Bioclinica Lab

Biomarkers play an important role in clinical trials, covering the whole spectrum of drug development from target identification and preclinical tests through Phase 1 studies into post-marketing. According to a recent report, around 12% of all trials registered in 2013 included biomarkers in outcome measures and/or inclusion criteria (1). They are primarily used in Phase 2 studies, helping to prove that the investigated product has the expected biological effect. Phase 1 trials assess the safety and tolerability of a new investigational drug and, although they are a starting point to identify the most valuable biomarkers for a subsequent Phase 2 study, the trial population is generally limited. Phase 3 studies usually include well-developed and characterised biomarkers, very often selected during Phase 2.

According to the FDA-NIH Biomarker Working Group definition, a biomarker is "a characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions" (2). They can be molecular, histologic, radiographic or physiologic, including chemical and biochemical analyses from fluids and tissue biopsies, physiological assessments and medical image analysis. Each individual biomarker is part of one or more of the seven categories below, as defined by the Working Group:

- Diagnostic
- Monitoring
- Pharmacodynamic (PD)/response
- Predictive
- Prognostic
- Safety
- Susceptibility/risk

Each category can have several contexts of use, describing the manner and the purpose of use for a given biomarker during development. For example, monitoring biomarkers can indicate drug-induced toxicity or detect changes in the extent of a disease, while those in the prognostic category could be used for enrichment strategies.

With the progress in companion diagnostics, biomarkers have greatly assisted as an advance for precision medicine, an approach for disease prevention and treatment at the individual patient level. Indeed, companion biomarkers are able to predict the response to a specific therapy and help to classify patients into responders and non-responders. Usually co-developed with drugs, companion biomarkers provide essential information on the safe use of therapies.

Validation and Qualification

Hundreds of papers are published on biomarkers in drug development each month. Most of these biomarkers are developed and assessed in one laboratory, making it difficult to evaluate their quality. However, reliable, accurate and reproducible biomarkers are critical to assure the safe conduct of drug development, especially when they are being used to guide treatment and identify patients. In addition to their clinical utility, analytical validity is a crucial component for use in clinical trials to assure the delivery of high-quality research data. This is assessed by measuring the performance characteristics of the assay, including:

- Sensitivity
- Selectivity
- Accuracy
- Precision
- Stability

Analytical validation should meet the requirements of the intended use of the biomarker, increasing in rigour from a biomarker used as exploratory parameter to a surrogate endpoint. Thus, a 'fit-for-purpose' approach has been developed to address the need for a continuously evolving validation strategy (3). Testing labs are challenged to understand the intended use of a biomarker in any given development programme and to determine the level of assessment and analysis needed to meet the requirements.

The analytical validation of an assay is a necessary step for a further FDA qualification, a process facilitating the integration of biomarkers in drug development (4). This pathway aims to qualify the biomarker for a particular context of use in multiple programmes through the FDA's Biomarker Qualification Program by gathering robust and reliable scientific evidence on the link of a biomarker with biological procedures and clinical endpoints to ensure utility for a particular context of use.

The process is quite lengthy, lasting 2-3 years according to the FDA – and requires a variety of information, such as raw analytical data and protocols. This public process aims to aid recognition and adoption of new biomarkers by the scientific community and is also a step towards standardisation as all of the analyses are considered, gathered and shared.

Surrogate Endpoints

Biomarkers directly measure biological processes. As such, they are independent to how a subject feels, functions or survives, which is the classical definition of a clinical outcome. These are the most reliable, and are very often considered as primary endpoints in trials. The effect of a drug on such an endpoint should provide the evidence of a clinically meaningful benefit for the patient – like improved survival, for example. Sometimes, the integration of clinical outcomes into trials can be challenging and impractical – this might be too long, too costly or require too many participants. To overcome this situation, surrogate endpoints can be used as a substitute for a meaningful clinical one. A surrogate endpoint does not directly measure the clinical outcome, but is expected to predict it, based on scientific evidence.

Biomarkers can be considered as relevant surrogate endpoints as they are able to predict drug efficacy more quickly than conventional clinical ones and are independent of patient choices, judgments or motivation. For example, the blood levels of HbA1c are considered as a substitute marker for risk of microvascular complication in type 2 diabetes mellitus (5).

Between 2010 and 2012, around 45% of new molecular entities have been approved by the FDA on the basis of surrogate endpoints (5). To reliably use biomarkers in this way, they must perfectly predict, or correlate with, the true clinical endpoint. Additionally, the biomarker has to be in the causal pathway resulting in the clinical endpoint (6). Even with a clearly demonstrated causality link between the surrogate and the clinical endpoint, surrogates have been shown to overestimate the treatment effects (7).

To avoid the approval of drugs that have no clinical benefit or could be harmful to the patient, the use of surrogate endpoints must be tightly regulated. Surprisingly, there remains no consensus on how to evaluate these markers. In light of this, a group of researchers and health officials has proposed a three-step framework to validate them (8). Briefly, the level of evidence supporting the relationship between the surrogate endpoint and the final outcome has to be considered. Then, the strength of the association should be assessed using statistical analysis from all randomised trials of a treatment and, finally, the relationship should be quantified. Such validated surrogate endpoints will facilitate and accelerate development programmes with confidence but cannot reflect the overall treatment benefit and/or risk if used alone.

Bone Biomarkers

PD markers are used "to show that a biological response has occurred in an individual who has been exposed to a medical product or an environmental agent" (2). A change in the

Figure 1 : Biomarkers in clinical trials - Categories and contexts of use

level of a PD marker can provide early evidence of the effect the drug has on its target or on a clinical endpoint, and can guide dosing. They therefore provide insights into proof-of-mechanism and proof-of-concept.

Circulating biomarkers are commonly used as PD biomarkers in trials in rheumatoid arthritis (RA). The inflammatory environment imbalances bone turnover biological steps – namely bone resorption and formation – favouring its progressive degradation. To monitor disease progress or the effect of a drug, biomarkers of bone and cartilage degradation have been developed and validated. Most are direct products of bone resorption like C-terminal telopeptide of type 1 collagen CTX-I, and formation such as osteocalcin released in the blood stream or in the synovial fluid. As an example, these biomarkers have proven useful in better understanding the effect of an anti-IL-6Rα on joint damages in RA patients (9). This study revealed that serum levels of bone resorption biomarkers (among others) were decreased in the treated group compared to placebo, underlying a limitation of joint damage and a putative improvement in disease activity. Similar results were observed with the IL-6R antibody, tocilizumab (10).

Genomic Biomarkers

During the past decade, next-generation sequencing technologies revolutionised cancer research by allowing comprehensive characterisation of the molecular landscape of cancer types. This technology confirmed the complexity of human cancers by improving the understanding of genetic heterogeneity. As an example, a study performed by The Cancer Genome Atlas Research Network demonstrated that 18 genes were significantly mutated in lung adenocarcinoma, which is only one type of lung cancer (11).

Genomic biomarkers can be used as susceptibility/risk, prognostic and predictive biomarkers. Patients harbouring breast cancer gene 1/2 mutations have an increased risk of developing breast and ovarian cancer compared to non-carriers, and detection of these mutations can also be used as prognostic biomarkers by assessing the likelihood of a recurrence of breast cancer after therapy (12). Genomic biomarkers also appear to be essential to prediction. Many cancer therapies directly target 'drivers mutations', which confer a growth advantage to tumour cells, such as activating epidermal growth factor receptor (EGFR) mutations in lung cancer. For anti-EGFR trials, screening patients for EGFR mutation status will predict their sensitivity to the drug and could allow a selection of patients more likely to respond to the therapy.

Genomic biomarkers for solid tumours are mainly quantified from tissue extracts, thus requiring biopsies. In order to minimise these invasive procedures, many researchers are focusing on identifying biomarkers from peripheral blood, called 'liquid biopsy'. Circulating free DNA and tumour cells are blood sources of tumour DNA and have been shown to reflect characteristics of both the primary tumour and metastasis. These cells are of particular interest as they can be detected very early in the disease process (13).

Alzheimer's Disease

The two main pathological hallmarks of Alzheimer's disease (AD) are the aggregation of amyloid-β (Aβ) proteins into extracellular plaques and neurofibrillary tangles, primarily composed of tau proteins. The core biomarkers of AD – namely Aβ1-42, total tau and phosphorylated-tau – directly reflect these two pathological hallmarks and assess their levels in the cerebrospinal fluid (CSF) of patients revolutionised AD diagnosis by allowing the discrimination of AD from non-AD dementias. These biomarkers also appear to be essential tools for early detection of AD patients as their CSF levels begin to demonstrate abnormality, usually 15-20 years before the onset of clinical symptoms. Identifying AD patients early in the disease progress and having these individuals in trials may improve capability to demonstrate the efficacy of new candidate drugs.

In addition to Aβ and tau, new biomarkers are investigated to monitor other AD molecular events such as synaptic loss and axonal damages. The dendritic protein neurogranin is described as a novel marker of synaptic dysfunction in AD. Neurogranin CSF levels are increased in AD patients compared to normal ageing controls, and are associated with disease intensity (14). Interestingly, this increase in neurogranin concentration is AD-specific and not found in other types of dementia, suggesting a possible use in differential diagnosis (15).

Even if CSF is the most relevant matrix to analyse brain biochemistry, it remains difficult to obtain due to the need of a lumbar puncture. Blood biomarkers rationally appear as a good alternative as they are less invasive and more cost- and time-efficient. Researchers are currently working to find new relevant blood biomarkers of AD and adapt the analytical techniques for quantification currently used in CSF.

Biomarkers are powerful tools to accelerate drug development and approval. Although biomarker research is moving fast, finding the relevant one for a specific context of use is anything but easy. Indeed, it can take decades of research to determine the role of a biomarker in a biological or pathological process.

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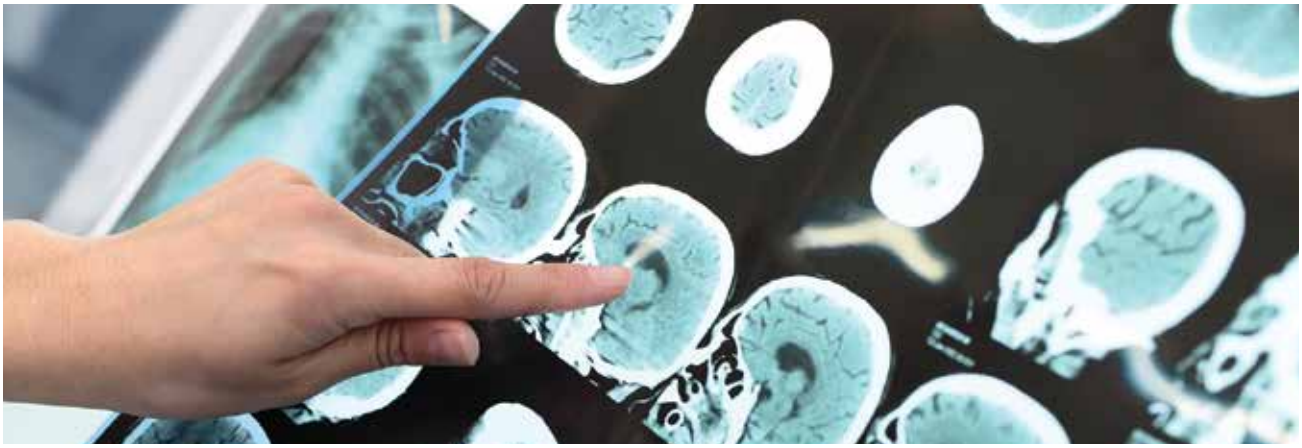

Moreover, despite their apparent ease-of-use, biomarker assessments have to be tightly regulated – especially when used as surrogate endpoints. Regulatory agencies, pharmaceutical companies and academia are working hand-in-hand to establish clear and concise biomarker validation and qualification programmes, ensuring the reliability of data and providing clear evidence of biomarker utility.

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About the authors

Dr Mélanie Bodnar-Wachtel, PhD, is Scientific Expert for Biochemical Markers at Bioclinica Lab and is responsible for neurosciences and oncology projects. Drawing on her experience in academic and industry research, she is dedicated to bringing innovative solutions to the development of new technologies for biomarker assessment and in expanding Bioclinica Lab's biomarker portfolio. She earned a PhD in Sciences, speciality Molecular, Integrative and Cellular Biology from Université de Lyon, France, and a Master's degree in Biology and Health, speciality Oncogenesis, from École Pratique des Hautes Études, France.
Email: melanie.bodnar-wachtel@bioclinicalab.com

Dr Tanja Schubert, PhD, is Vice President and General Manager of Biochemical Markers at Bioclinica Lab. She leads the Molecular Marker Lab division of the company and is responsible for all operational activities, including lab processes, service quality, study management, process improvements and regulatory compliance. She holds the accreditation of the College of American Pathologists for Bioclinica Lab and is a trained Pharmacist, with a PhD in Natural Sciences from Ludwig-Maximilians-Universität München, Germany.
Email: tanja.schubert@bioclinicalab.com