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Biomarkers and translational research

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ABSTRACT: Biomarkers have the potential to play important roles in diagnosis and in the identification of patient populations that could benefit from targeted therapy. They also serve as markers of drug efficacy and could be used to monitor treatment effectiveness, drug toxicity, and development of resistance. One example of a successful biomarker development is represented by the testing for Her-2/ERB2 over expression. Tissue sampling is crucial for the definition and validation of new biomarkers. In general, biomarker and its corresponding assay must be validated before phase III to be useful in reducing trial size.

Keywords: Biomarkers; Tissue sampling; Gefitinib development; Surrogate marker

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DEVELOPMENT OF BIOMARKERS: WHAT ARE THE SCIENTIFIC HURDLES?

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For next-generation therapies drug development by chance is moving toward cancer therapies that are molecularly targeted. Nevertheless, drug development still relies on the same basic strategies used for cytotoxic drugs. In the new era of cancer treatment, instead of applying the concept of maximum tolerated dose (MTD) as does cytotoxic drug therapy, we must think in terms of the optimal biologically active dose (OBAD) and, perhaps, the maximum tolerated economic cost. Only administration of molecularly targeted drugs at OBAD can demonstrate their optimum therapeutic efficacy.

Biomarkers could play important roles in disease diagnosis and in the identification of patient populations that could benefit from targeted therapy. They also serve as markers of drug efficacy and could be used to monitor treatment effectiveness, drug toxicity, and development of resistance. Moreover, some biomarkers appear to be surrogates for clinical benefit; as such, they have the potential to serve as endpoints in clinical trials. To use biomarkers to maximum advantage, several scientific hurdles must be surmounted. For example, a need exists to differentiate molecular and therapeutic targets, determine which targets to block to achieve tumour control, overcome resistance mechanisms, and identify patients who need treatment and are potential responders.

Many techniques – genomics, proteomics, interactomics, peptidomics, and degradomics – offer a spectrum of analytical possibilities. In the early days, procedures involved removing most proteins, e.g. albumin, prior to analysis. That approach, unfortunately, eliminates the opportunity to investigate interactomics involving the small peptides bound to large molecules. To identify potential biomarkers and study their roles in the disease pathway, it is necessary to look for small molecules in serum (peptidomics) as well as protein-degradation products (degradomics). Many peptides have excellent cancer signatures. Reverse-phase protein microarray provides a map of known cell-signalling proteins.[1]

Biomarker development should follow different pathways depending on the stage of drug development. For early stages of clinical development, biomarkers can identify or confirm molecular targets, help optimise dose schedules for the anticancer agent, and might correlate with clinical benefit. Identifying clinically relevant targets is challenging; in numerous examples, the intended target was found to be irrelevant. As not all molecular targets are legitimate therapeutic targets, however, biomarkers can provide a means of determining which target(s), when inhibited, correlate with tumour control. In the case of some anticancer agents (e.g. cetuximab, gefitinib, farnesyl transferase inhibitors, and inhibitors of vascular endothelial growth factor [VEGF]), it appears that the original molecular target is not the only therapeutic target.

In the later stages of clinical development, identified markers could be used to select the patients most likely to respond to the targeted agent. Any biomarker used as a basis for patient selection must demonstrate excellent sensitivity and specificity; otherwise, the risk of not treating patients who might benefit would be

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unacceptably high. Identified biomarkers could also be a basis for monitoring treatment and identifying resistance mechanisms.

One example for the need to identify biomarkers is immunotherapy. It is important to bear in mind that immune-mediated anti-tumour reactivity is the result of a well-orchestrated interaction of multiple factors and multiple pathways. Redundant pathways and interactions present a set of challenges when designing immunotherapies or identifying surrogates. Studying any of the multiple immune mediators in isolation offers little about efficacy because of these interactions. Therefore a set of biomarkers is expected to more and more replace single ones. Methods such as proteomics need to be better standardised and validated as they may speed up the identification of biomarkers and thus drug development as a whole.

As soon as biomarkers have been fully validated, they have the potential to be developed to surrogate markers. Surrogate endpoints should be based on functional parameters of critical importance for cancer control. Although functional parameter techniques have been around for many years, they can provide very useful information for molecularly targeted drug development. Such parameters include cell proliferation, cell death, inflammatory infiltration, and tumour regression.

For example, the immediate preoperative anastrozole, tamoxifen, or combined with tamoxifen (IMPACT) trial relied upon a measure of proliferation – immunohistochemical assessment of the nuclear antigen Ki-67 – as an endpoint.[2,3] The method was highly reproducible and predictive of therapeutic efficacy. Mohsin et al.[4] used another functional parameter – apoptosis – in a trial of the neoadjuvant trastuzumab in 35 patients with locally advanced Her-2/neu overexpressing breast cancers. They found that induction of apoptosis correlated with tumour regression.

Tumour biopsies can be examined for inflammatory lymphocyte migration as another means of monitoring treatment efficacy using functional parameters, as shown in one study involving stage III melanoma patients treated with interferon-alpha.[5] Patients whose tumours demonstrated $\geqslant$2% CD4+ tumour-infiltrating lymphocytes (TIL's) had prolonged time to progression and improved overall survival compared with patients whose tumours had < 2% TIL's. Finally, tumour regressive changes correlate with long-term survival. Overall survival in metastatic melanoma patients has been shown to correlate with tumour regressive changes.[6]

Trials that rely on functional parameter endpoints obviously depend on the availability of tumour biopsies. Obtaining such samples is critical for developing molecularly targeted therapies. Stated otherwise, 'No tissue – no trial'.[7] For example, with biopsy specimens, it would be possible to compare all phosphorylated proteins in the tumours before and after treatment to observe potential changes. Moreover, it is becoming more possible to predict response to immunotherapy based on tumour biopsies. Therefore prognostic biomarkers also provide means for identifying patients who might be at high risk of disease recurrence after radical surgery and might be candidates for adjuvant therapy.

Predictive biomarkers could be used to discern which patients would be more likely to respond to a particular therapy. Interleukin-6 (IL-6) production by peripheral blood mononuclear cells (PBMC's) preoperatively collected from patients with primary colorectal cancer predicts survival. Eight of 13 patients with >5000 pg/mL IL-6 died from cancer within the 54-month follow-up period, whereas no cancer-related deaths were recorded among 21 patients with 5000 pg/mL IL-6 or less. A multivariate Cox regression analysis, stratified for tumour and node stage, identified IL-6 production as an independent prognostic factor.[8]

In conclusion, molecularly targeted treatment of cancer is sometimes criticised for poor therapeutic efficacy. Among the reasons that it has not met with greater success are reliance upon suboptimal dosing, the fact that molecular targets are not always the therapeutic target, pathway redundancy, and resistance mechanisms. However, molecularly targeted treatment of cancer is still at a very early stage; there is a great need to identify relevant therapeutic targets and establish molecular and functional surrogate endpoints. The techniques are available the time to design the respective clinical trials is now.

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HOW TO SELECT BIOMARKERS FOR MULTITARGETED THERAPIES

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