the challenges of targeted therapy of cancer

Due to the increasing understanding of the mechanisms relevant to the genesis of cancer, we are experiencing a transition from disease- to target-oriented therapy. This is the challenge of bringing new therapeutics to clinical application and registration now focuses on the molecular target expressed by the malignant cell rather than treatment of the histopathological entity itself.

The new concepts, however, bring along major problems for clinical drug development. One major issue is the limited availability of predictive in vitro models on the one hand, and the need to perform early clinical trials usually in heavily pretreated patients with a considerable tumor load on the other.

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 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 tumor control, overcome resistance mechanisms and identify patients who need treatment and are potential responders.

definition of biomarkers and surrogate markers

Several definitions of relevant terms have been proposed by the Biomarkers Definitions Working Group of the National Institutes of Health (NIH) and the US Food and Drug Administration (FDA):

A biological marker (biomarker) is 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention' [1].

A clinical endpoint is 'a characteristic or variable that reflects how a patient feels, functions, or survives' [1].

A surrogate endpoint [2] is 'a biomarker that is intended to substitute for a clinical endpoint. A surrogate endpoint is expected to predict clinical benefit or harm (or lack of benefit or harm) based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence. Although all surrogate endpoints can be considered biomarkers, it is likely that only a few biomarkers will achieve surrogate endpoint status' [1].

The goal of incorporating biomarkers into cancer treatment and clinical trials is to manage a patient’s disease by administering effective and well-tolerated therapies, based on an understanding of the patient’s unique genetic and molecular profile. For targeted therapies that might only benefit a proportion of patients, failure to select patients correctly has the potential to dilute trial outcomes.

development of biomarkers

There are many types of biomarker of potential interest in the field of targeted anticancer therapy. These can mainly be divided into those that present in histopathological tissues and blood-borne biomarkers. Significant advances in imaging [e.g. positron emission tomography (PET) scans] have also improved the ability to monitor treatment effects.

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 to optimize 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 tumor control. In the case of some anticancer agents [e.g. cetuximab, gefitinib, erlotinib and inhibitors of vascular endothelial growth factor (VEGF)], it appears that the molecular target is the 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 unacceptably high. Proper patient selection enables efficient clinical trial design for targeted therapies and ensures that the number of individuals exposed to the risks of anticancer therapy is minimized.

examples of successful biomarker development

Patient selection can be facilitated through the use of systems that enable selection of patients more likely to benefit from targeted therapy. Herceptest was the first such system developed. It is used to identify patients whose tumours overexpress Her-2/ERB2 and, therefore, who would be most likely to respond to treatment with trastuzumab (Herceptin). Her-2/neu is an example of an efficacy target. Validating the target–biomarker–antibody relationship involved a great deal of effort because the initial diagnostic test was somewhat ineffective. Once the marker was validated, however, only patients whose tumours overexpressed Her2/neu (~20–25% of invasive breast cancers) were enrolled in the phase III trial. Consequently, only 470 patients were required; if subjects had been accrued from the general patient population, an estimated 2200 subjects would have been necessary. Significant benefit was demonstrated in 1.6 years of follow-up instead of ~10 years. The response rate in this subpopulation was 50% compared with ~10% in the overall patient population [3].

The antibodies used for the test system must work on different types of tissue. This needs to be confirmed by testing in multi-tissue arrays to make sure that background staining is not problematic. The final step is standardization of the assay to ensure consistency across laboratories. The keys to successful development of antibodies for use in patient selection are high quality—in terms of specificity, functionality and sensitivity—and standardization of reagents (no batch-to-batch variation), automated protocols and use of imaging as a means of interpreting the response.

Regulatory authorities throughout the world strongly advocate standardization of testing to minimize the number of patients who experience adverse side-effects from treatment. Proper patient selection can also optimize treatment expenditure by selecting the patient population most likely to respond.

The clinical development of gefitinib, an orally available epidermal growth factor receptor tyrosine kinase inhibitor (EGFR TKI) is a more complex example of biomarker development. Phase I and II development of gefitinib showed dramatic and unexpected tumor regressions in ~10% of patients with advanced non-small-cell lung cancer (NSCLC) but data from early-phase trials did not show a clear correlation between patient outcome and EGFR expression in archived tissue [4]. Subsequently, however, data emerged indicating that EGFR mutations and increased gene copy number, as measured by fluorescence in situ hybridization (FISH) are associated with clinical response to gefitinib treatment [5]. One of the challenges in the development of gefitinib was that knowledge of potential biomarkers emerged during the conduct of the pivotal trials. Indeed, increased EGFR gene copy number measured by FISH was shown to be a prognostic biomarker for outcome after surgery in patients with NSCLC [6], and subsequently shown to be predictive of response to gefitinib [7]. Later EGFR mutations also emerged as predictors of response to EGFR TKIs in patients with advanced NSCLC [8].

Evolution of biomarkers during the conduct of large randomized trials might become the rule rather than the exception. Although initial candidate biomarkers are evaluated early in development, knowledge increases exponentially as research and clinical experience become more widespread and increased clinical data with which to correlate the translational work become available.

Another example of a biomarker used as a safety target exists. Irinotecan (Campto), which is approved for treating metastatic colorectal cancer, was found to cause grade 4 neutropenia in ~8% of the general patient population. Subsequent data have shown that uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) affects the drug’s metabolism and, therefore, its toxicity profile [9]. The UGT1A1*28 polymorphism, characterized by an additional TA repeat in the TATA sequence of the UGT1A1 promoter, was associated with greater toxicity [9]. Consequently, the drug’s label was modified to reduce the starting dose for patients homozygous for the polymorphism.

importance of tumor biopsies

Before attempting to show that a novel compound has benefit in the clinic, a major step in cancer drug discovery is demonstrating that the target is hit (proof of mechanism). Traditional physician examination and imaging are no longer adequate to confirm a ‘hit’ in the new molecular era. Despite active research to increase the utility of blood and other body fluids [10,11], identification of biomarkers to assist in the understanding of drug mechanism and effect still relies heavily on tissue samples. In turn, this reflects the fact that the microscopic examination of tissue samples—histopathology—remains the ‘gold standard’ for cancer diagnosis worldwide.

Acquisition of tissue samples is increasingly central to the research of molecularly targeted therapies. Trials that rely on functional parameter endpoints obviously depend on the availability of tumor biopsies. Obtaining such samples is critical for developing molecularly targeted therapies. Stated otherwise, ‘No tissue—no trial’ [12]. For example, with biopsy specimens, it would be possible to compare all phosphorylated proteins in the tumors before and after treatment to observe potential changes. Therefore protocols should be developed with tissue collection and analysis in mind. Pathologists are critical partners in clinical trials to ensure that samples are properly fixed, labeled and shipped.

The situation is complex because security is a key concern to pathologists when providing samples to investigators outside their institutions. Therefore, every measure should be taken to ensure that samples are promptly returned to pathologists. A study-initiation visit should be undertaken to offer pathologists clear guidance on what is needed, with an emphasis on the importance of biomarker work. Routine clinical practice does not necessarily generate the samples required for biomarker analysis.
Future trials aimed at patient selection outcomes will need usable tissue from almost all patients, as well as a protocol design and statistical plans that can accommodate the evolution of science during the life of the trial. Patients entering trials are generally prepared to consent to tissue sampling after an appropriate discussion with their treating physician. Most patients seem willing to allow diagnostic biopsy tissues to be used for research, but obtaining biopsies purely for research purposes is more challenging. Ethics boards may object to the banking of tissues for future undefined studies and focusing on the pathways of interest, and careful consideration to the wording of the informed consent can be very helpful in this regard. What is clear is that this is a rapidly moving and important field and consultation between academia, industry, regulators, ethics boards and patient advocates will be crucial to improving the acquisition of samples for future biomarker research, ultimately leading to better targeted therapies.

**challenges of using biomarkers in clinical trials**

Drug development is based on different types of biomarker in the context of drug development.

*Diagnostic biomarkers* provide the means to define a population with a specific disease.

*Prognostic biomarkers* correlate with outcomes. For example, overexpression of Her-2/neu in breast cancer or EGFR expression in colorectal cancer indicates poor prognoses. Such prognostic markers are frequently the basis for establishing inclusion criteria for a clinical trial or for defining a patient population.

*Predictive biomarkers* define populations that might respond more favorably to a particular intervention from an efficacy or safety perspective. They can be used to stratify patients for subgroup analyses.

*Surrogates* are biomarkers that correlate with clinical benefit and changes in the marker correlate with alterations in outcome. Examples include response rate or progression-free survival in oncology or bone mineral density in osteoporosis prevention and treatment. If validated, a surrogate may serve as a primary endpoint in a pivotal registration study and could support approval of an anticancer agent.

The Critical Path Initiative (Figure 1) of the FDA aims to stimulate and facilitate a national effort to modernize the scientific process through which a potential human drug, biological product or medical device is transformed from the discovery or proof-of-concept stage into a standard therapeutic or diagnostic product. The focus of this initiative is to update the evaluative tools currently used to assess the safety and efficacy of new medical products, including the validation and use of biomarkers in clinical trial patient selection and as surrogate endpoints [13].

From a regulatory point of view, biomarkers can be an indicator of efficacy as a surrogate marker but cannot serve as a marker of clinical benefit *per se*. Ideally, validation should be carried out prospectively, although initially, retrospective studies may be undertaken to identify surrogate markers. Validation should be carried out in another, complementary population that does not include the original subpopulation found to be positive for the biomarker.

The best sort of trial would be a randomized prospective trial which provides information not only on true endpoint differences between the two treatment arms, but also on the degree of correlation between surrogate endpoint and true endpoint.

**assessment of protein profiles (proteomics) as biomarkers**

Given the shortcomings of single tumor markers and the complexity of cancer biology, multiple/composite biomarkers are increasingly relied on to assess the safety and efficacy of novel anticancer drugs. The global analysis of cellular proteins has been termed proteomics/proteome profiling and is a key

![Figure 1.](image_url)
area of research that is developing in the post-genome era. It involves the simultaneous separation, identification and characterization of thousands of proteins present in a biological sample in a single procedure. Proteins are the main functional output and neither the genomic DNA code of an organism nor the amount of mRNA that is expressed for each gene product yields an accurate picture on the state of a living cell, which can be altered by many conditions. Especially post-translational modifications of proteins, such as phosphorylation or glycosylation, are very important in determining protein function.

With the use of proteomics a whole range of protein markers can be assessed at the same time. Currently, two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) followed by mass spectrometry is a core technology to separate proteins on the basis of charge in the first dimension and molecular mass in the second [14]. Changes in the expression of proteins can be identified by comparing the protein spots by computer-assisted photometric evaluation. This technique allows direct comparison of protein expression and is used to identify proteins that are differently expressed between normal or malignant tissue or during the course of therapy [15]. This strategy has also been shown to allow the detection of proteins that may be involved in the generation of resistance mechanisms [16].

Another technique with a much higher throughput is represented by matrix-assisted laser desorption/ionization (MALDI), a ‘soft’ ionization technique that most often uses a time-of-flight (TOF) mass spectrometer. The advantage of MALDI-TOF mass spectrometry (MS) over alternative approaches are ease of use, simple sample preparation and high throughput, rendering it an ideal tool for large-scale clinical application [17]. Using MALDI-TOF MS for clinical specimens can yield important information about disease state, drug response and/or efficacy, and drug toxicities. It can also be used to analyze biomolecules that could serve as classifiers to determine which patients will most likely respond to certain molecularly targeted therapies. The use of MALDI-TOF MS as a clinical tool has been overshadowed by some notable failures and is a complicated issue: variations in instrument settings, variations in sample preparation (freeze–thaw cycles) and pre-treatment (fractionation) can lead to non-reproducible data. Furthermore, biological and population variations (e.g. age, gender, race, digestive state) need to be considered, identified and quantitatively measured. The success of MALDI-TOF MS profiling hinges on sensitive and robust data analysis algorithms to render mass spectra comparable across different laboratories and instruments.

Recently, based on the MALDI-TOF system, a simple mass spectroscopy-based pretreatment patient selection system was established that is highly reproducible and capable of classifying patients by survival. It is specific for EGFR-TKI treatment and is capable of distinguishing patient subgroups with respect to co-variates [18]. Prospective trials are being planned based upon this technique.

**functional imaging for the development of anti-angiogenic agents**

One of the key questions relating to the development of anti-angiogenic agents is how to identify their optimum dose and schedule as a single drug and then proceed to combination trials. Therefore a large amount of work has been devoted to identifying and validating biological pharmacodynamic endpoints that could be used to optimize development of anti-angiogenic drugs. While measurement of serum levels of cytokines involved in angiogenesis was shown to be of minor relevance, potential effects of anti-angiogenic compounds can be monitored by functional imaging and various methods have been evaluated. The most widespread of these is the use of dynamic contrast-enhanced magnetic resonance imaging (MRI) as vascular permeability is a surrogate for angiogenesis and can be assessed by MRI scanning. With this imaging technique, a marked heterogeneity in drug distribution and clearance has been shown among different patients and even when deposits of tumor were compared in the same patient [19]. Given these significant biological differences in intratumoral drug pharmacokinetics and biological response, major effort and emphasis needs to be put on the investigation of functional imaging as a pharmacodynamic biomarker. This is of major clinical relevance as effective treatment of some patients requires higher doses than that of others as the optimal biologically active dose varies among individual patients [19].

**conclusion**

In summary, biomarkers represent a chance to allow proof of principle in early clinical trials in order to move rapidly to phase III and registration. Potential biomarkers can be based on biopsy material, functional imaging or proteomic approaches dependent on the kind of drug under development. They can help to define subpopulations of patients who profit or do not profit from therapy. Relevant markers may differ between the various types of targeted therapy and are under continuous development. Any biomarker used as a basis for patient selection must be validated and demonstrate excellent sensitivity and specificity as the risk of not treating patients who might benefit would otherwise be unacceptably high.

**disclosures**

No significant relationships.

**references**


