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USE OF BIOMARKERS AND SURROGATE ENDPOINTS IN DRUG DEVELOPMENT AND REGULATORY DECISION MAKING: Criteria, Validation, Strategies[1]

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■ **Abstract** In the future, biomarkers will play an increasingly important role in all phases of drug development, including regulatory review. However, only a few of these biomarkers will become established well enough to serve in regulatory decision making as surrogate endpoints, thereby substituting for traditional clinical endpoints. Even generally accepted surrogate endpoints are unlikely to capture all the therapeutic benefits and potential adverse effects a drug will have in a diverse patient population. Accordingly, combinations of biomarkers probably will be needed to provide a more complete characterization of the spectrum of pharmacologic response. In the future, pharmacogenomic approaches, including those based on differential expression of gene arrays, will provide panels of relevant biomarkers that can be expected to transform the drug development process.

INTRODUCTION

Biological markers (biomarkers) can serve many unique purposes, including confirmation of diagnoses, monitoring treatment effects or disease progression, and prediction of clinical outcomes. In this review, we focus on the current and potential uses of biomarkers as indicators of drug exposure. We take a broad view of exposure to include drug doses, dosing rates and duration of treatment, and systemic plasma concentrations. We evaluate how the relationship between exposure and the magnitude of biomarker response may be applicable for predicting the efficacy or safety of a drug or drug product.

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There is a high level of interest in biomarkers in the pharmaceutical industry, which is faced with the ever increasing cost of research and development, and with growing pressure to accelerate the rate of bringing new drugs to the marketplace. In this context, biomarkers show considerable promise for improving the efficiency and informativeness of drug development and regulatory decision making. For years, a limited number of biomarkers have been used by regulatory agencies as a basis for approval and market access of several drugs. There is a legal basis for this, as well as a common set of biomarker characteristics that provide regulatory authorities with a level of certainty sufficient to allow some biomarkers to be used as surrogates for definitive clinical endpoints. However, there continues to be extensive debate about widespread reliance on biomarkers as substitutes for more traditional evidence of clinical efficacy (1, 2). Accordingly, there is a vital need to establish consensus on the basic principles needed to properly develop, evaluate, and validate biomarkers.

DEFINITIONS AND BACKGROUND

At least some of the controversy surrounding the use of biomarkers as surrogates for clinical endpoints reflects ambiguity in the terminology used by members of the different disciplines that are concerned with the design, execution, analysis, and evaluation of clinical trials. A number of recent attempts have been made to clarify this terminology (3, 4). A synthesis of some proposed working definitions is as follows: (*a*) *biological marker* (biomarker)—a physical sign or laboratory measurement that occurs in association with a pathological process and that has putative diagnostic and/or prognostic utility; (*b*) *surrogate endpoint*—a biomarker that is intended to serve as a substitute for a clinically meaningful endpoint and is expected to predict the effect of a therapeutic intervention; and (*c*) *clinical endpoint*—a clinically meaningful measure of how a patient feels, functions, or survives. The hierarchical distinction between biomarkers and surrogate endpoints is intended to indicate that relatively few biomarkers will meet the stringent criteria that are needed for them to serve as reliable substitutes for clinical endpoints.

In fact, not all clinical endpoints are equally definitive and they can be further categorized as follows: (*a*) *intermediate endpoint*—a clinical endpoint that is not the ultimate outcome but is nonetheless of real clinical benefit; and (*b*) *ultimate outcome*—a clinical endpoint such as survival, onset of serious morbidity, or symptomatic response that captures the benefits and risks of an intervention.

In some cases, the clinical benefit of an intermediate endpoint such as exercise tolerance may be important to patients even though this benefit is not associated with improvement in the clinical outcome of increased survival. However, in other cases, when the ultimate outcome is considered, the clinical benefit of an intermediate endpoint is more than offset by the adverse effects of drug therapy.

For example, quinidine was used for many years to maintain normal sinus rhythm in patients who previously had atrial fibrillation. Maintenance of normal sinus rhythm was beneficial to some patients because it was associated with increased cardiac output and a decreased risk of systemic embolization from the

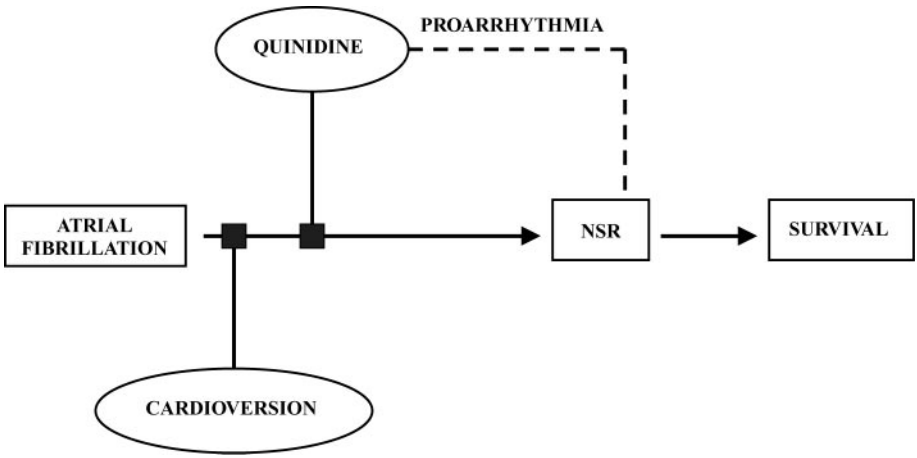

Figure 1 Path diagram illustrating the potential of the adverse proarrhythmic effects of quinidine therapy (broken line) to outweigh its potentially beneficial effects (solid line) in maintaining normal sinus rhythm (NSR) in patients with previous atrial fibrillation.

right atrium. Although meta-analysis confirmed that patients treated with quinidine remained in normal sinus rhythm longer than those who were untreated, it was found that quinidine therapy was associated with increased mortality (5). The path diagram shown in Figure 1 can be used to illustrate this apparent therapeutic paradox. This example deals with an intermediate clinical endpoint, but unanticipated adverse consequences of drug therapy are a frequent confounding factor when biomarkers are relied on as surrogates for definitive clinical endpoints. This limitation underlies much of the controversy surrounding the use of surrogate endpoints as the basis for regulatory evaluation of new therapeutic entities (1–3).

Biomarkers and surrogate endpoints in current use usually consist of either physiological or laboratory measurements. Several biomarkers and surrogate endpoints commonly used for a number of therapeutic drug classes are listed in Table 1, together with their corresponding clinical endpoints. Even these commonly used biomarkers vary with respect to their acceptance as surrogate endpoints. Thus, blood pressure and cholesterol are the only two cardiovascular biomarkers currently accepted as surrogate endpoints (3). The results of the CAST (cardiac arrhythmia suppression trial) study have shown that suppression of ventricular arrhythmias can no longer substitute for survival in evaluating the efficacy of antiarrhythmic drugs (6). Similarly, increases in bone mineral density do not necessarily reflect decreases in fracture rate in patients treated with fluoride (7), and decreases in serum levels of prostate-specific antigen may not correlate with a decrease in tumor growth (8).

Although the biomarkers listed in Table 1 differ with respect to their ability to substitute for definitive clinical endpoints, they all have some degree of clinical utility. Biomarkers such as these have traditionally been identified through

TABLE 1 Examples of biomarkers and surrogate endpoints[a]

Therapeutic class	Biomarker/surrogate	Clinical endpoint
Physiologic markers		
Antihypertensive drugs	↓Blood pressure	↓Stroke
Drugs for glaucoma	↓Intraocular pressure	Preservation of vision
Drugs for osteoporosis	↑Bone density	↓Fracture rate
Antiarrhythmic drugs	↓Arrhythmias	↑Survival
Laboratory markers		
Antibiotics	Negative culture	Clinical cure
Antiretroviral drugs	↑CD4 count, ↓viral RNA	↑Survival
Antidiabetic drugs	↓Blood glucose	↓Morbidity
Lipid-lowering drugs	↓Cholesterol	↓Coronary artery disease
Drugs for prostate cancer	↓Prostate-specific antigen	Tumor response

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studies of pathophysiology or epidemiology that have established their biological plausibility. Thus, clinical and epidemiological evidence indicated that high blood pressure was associated with an increased incidence of atherosclerotic cardiovascular disease, heart failure, stroke, and kidney failure (9). The mechanistic linkage between hypertension and cerebral hemorrhage and infarction was further established by pathophysiologic studies in man and in animal models (10). This linkage provided an initial basis of construct validity for believing that reductions in high blood pressure might be reflected in improved clinical outcomes (11).

However, experience acquired through well-controlled clinical trials is needed to provide the criterion validity that is the best support for a particular biomarker (11). Thus, a large clinical trial, in which over 4000 patients with elevated serum cholesterol levels and coronary artery disease were studied, was required to establish that cholesterol-lowering drugs could have a favorable impact on overall mortality as well as on the occurrence of cardiovascular events (12). However, there is a hierarchy in the level of criterion validity provided by clinical trials. Temple (3) has emphasized that one level of support for a biomarker is obtained when a number of drugs of the same pharmacologic class have consistent effects on the marker and on a relevant clinical endpoint. Even greater support is provided for a biomarker when this consistency can be demonstrated by drugs from different pharmacologic classes. As a result, both Rolan (11) and Temple (3) have concluded that biomarkers, paradoxically, are the least innovative, and thus in many situations the least useful, when their validity is best established.

USES OF BIOMARKERS IN DRUG DEVELOPMENT

Certainly a high level of stringency is required when a biomarker response is substituted for a clinical outcome and is proposed as the basis for regulatory approval of an application to market a new drug. However, biomarkers need not be validated

as rigorously in order to play other important roles, such as facilitating our understanding of disease mechanisms and natural history, expediting the development of new drugs, addressing regulatory concerns related to dose-exposure-response relationships, and even assisting with some aspects of clinical practice. For example, a few tumor markers, such as prostate-specific antigen and α-fetoprotein, are used to help diagnose and monitor the treatment response of patients with prostate and hepatocellular carcinoma (13). Despite their clinical utility, changes in these biomarkers would not constitute an appropriate regulatory basis for new drug approval unless accompanied by appropriate clinical evidence of disease response. Nonetheless, these and other biomarkers can play an important role in a number of phases of new drug development, and in the regulatory review of investigational new drugs (INDs) and new drug applications (NDAs).

Drug Discovery and Preclinical Development

Epidemiologic studies that link changes in a biomarker to pathophysiology can play an important role in identifying a suitable therapeutic target. For example, the association of elevated serum cholesterol levels with an increased incidence of coronary heart disease provides an underlying rationale for developing drugs that lower cholesterol by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (14).

Biomarkers also play an important role in the preclinical assessment of potentially beneficial and harmful effects of a new drug candidate. Screening tests in animals using biomarkers, such as blood pressure lowering, provide important demonstration that a compound is likely to have the intended therapeutic activity in patients. Biomarkers for potential toxicity play an equally important role. For example, a drug found to prolong the QT interval in animals may warn of potential cardiovascular risk in subsequent clinical studies.

Pharmacokinetic-pharmacodynamic (PK-PD) studies with biomarkers may be particularly useful (15, 16). In one instance, PK-PD studies showed good correlation between the hypotensive effects of an antiarrhythmic drug in dogs and humans (17). Blood levels measured when adverse events such as seizures occur in animal toxicology studies may help guide the design of dose escalation studies in humans and serve as a surrogate for preventing similar adverse events in humans (18). Breimer & Danhof (19) have provided additional examples in which whole-animal, mechanism-based PK-PD studies have been used to forecast the results of human PK-PD studies and to guide dose selection and dose escalation strategies.

Early Phase Clinical Development

Biomarkers are perhaps most useful in the early phases of drug development, when measurement of clinical endpoints may be too time-consuming or cumbersome to provide timely proof of concept or dose-ranging information. An example would be a study in which different doses of zafirlukast, a leukotriene antagonist, were

administered to asthmatic subjects to assess the efficacy of this agent in preventing leukotriene D_4-induced bronchoconstriction (20). Plasma concentration measurements made in conjunction with this study also demonstrated a plasma concentration threshold of 5 ng/ml that was required for therapeutic effects, supporting evidence of a receptor-mediated mechanism of action for this drug. Mildvan et al (21) have proposed a biomarker classification scheme for use in clinical trials of antiretroviral drugs and have emphasized the important role that $CD4^+$ T-lymphocyte counts and measurement of HIV-1 plasma RNA concentrations have played in the early clinical development of these drugs.

In some cases, a new biomarker is needed to facilitate the development of a novel compound. For example, the proportion of hemoglobin molecules modified to have a high oxygen-binding affinity (%MOD) was used in the early phase evaluation of tucaresol, a drug designed to prevent hemoglobin S (HbS) polymerization and subsequent hemolysis and painful crises in patients with sickle cell disease (11). The extent to which HbS is polymerized depends on the erythrocyte concentration of deoxygenated HbS, and the scientific rationale for this biomarker was based on the observation that HbS polymerization is inhibited when 20%–30% of hemoglobin is maintained in the oxy-conformation. Measurements of %MOD were included in the initial Phase I studies of tucaresol to demonstrate the oral dose range needed to obtain %MOD values of 19%–26% (22). This marker was also used to guide tucaresol dosage in the initial studies in patients with sickle cell anemia (23). Additional endpoints used in this study were lactic dehydrogenase and bilirubin concentrations as markers of hemolysis, and percentage of irreversibly sickled cells. Although the validity of these markers has not been confirmed in extensive clinical trials of antisickling therapy, this example illustrates the potential utility of even novel markers during early phase drug development.

Late-Phase Clinical Development

Several studies with simvastatin, an HMG-CoA reductase inhibitor, can be cited to illustrate the continued use of a biomarker throughout a clinical development program. Serum cholesterol measurements were used as a biomarker in a Phase II dose-ranging study (24). The efficiency of this study is indicated by the facts that only four study centers were needed to enroll the 43 patients who participated in the study, and the study duration was only 6 weeks. Although daily simvastatin doses of 80 mg were well tolerated, the study indicated that near-maximal effects were obtained with a daily dose of only 20 mg. The 20-mg/day dose was then selected as the starting dose for the subsequent placebo-controlled Phase III trial, in which 444 patients with coronary heart disease were followed in 94 centers for a median of 5.4 years (12). Serum cholesterol measurements were used in this pivotal trial as the basis for further simvastatin dose adjustments, the goal of treatment being to reduce serum total cholesterol to 3.0–5.2 mmol/liter (117–200 mg/dl). Two aspects of this Phase III trial merit particular emphasis. First, the primary endpoint of the study

was total mortality. By showing that the relative risk of death for patients receiving simvastatin compared with placebo-treated patients was 0.70 (95% confidence interval: 0.58–0.85, $p = 0.003$), the study provided the first strong evidence for advancing serum cholesterol measurements from biomarker to surrogate endpoint status. Second, by selecting for this study an initial simvastatin dose of 20 mg/day as the minimal dose for satisfactory effect (MDSE) rather than the maximally tolerated dose, the sponsor avoided the all-too-common pitfall of registering a starting dose that was subsequently found to be excessive (11). A final benefit from this Phase III study is that serum cholesterol measurements are now used routinely in clinical practice as a biomarker to guide simvastatin dose adjustments. Despite the current acceptance of cholesterol lowering as a surrogate endpoint, it should be pointed out that other drugs that lower cholesterol may have adverse effects that outweigh their benefit. For example, probucol, a drug structurally unrelated to statin HMG-CoA reductase inhibitors, has pronounced lipid-lowering effects but also prolongs the electrocardiographic QT interval and has caused *torsades de pointes* ventricular tachycardia in some patients (25). Accordingly, it cannot be assumed a priori that any cholesterol-lowering drug will have beneficial effects on survival.

Biomarkers that reflect disease prognosis may also be useful in developing eligibility criteria and stratification groups in late-phase clinical trials. Baseline plasma HIV-1 RNA concentrations and $CD4^+$ T-lymphocyte counts have been shown to be independent prognostic markers of clinical progression in patients receiving antiretroviral therapy for HIV-related disease (26). This supports the established practice of using $CD4^+$ T-lymphocyte counts as entry and stratification criteria for clinical trials of antiretroviral therapy (21). Although these biomarkers have also provided a basis for the accelerated approval of a number of antiretroviral drugs (27), federal regulations stipulate that accelerated approvals based on a surrogate endpoint are subject to the requirement of further studies to demonstrate clinical benefit (28).

Perhaps the most widespread application of surrogate endpoints in late-phase clinical development is in the substitution of drug concentration measurements for clinical endpoints in the registration of new drug formulations and generic drug products. Federal regulations state that measurement of either blood concentrations or urine excretion rates of a drug may be used to demonstrate that a new formulation has bioavailability comparable to that of the reference material (29).

EVALUATION AND VALIDATION OF BIOMARKERS

The scientific program for evaluating biomarkers must be planned as early as possible in the drug discovery and preclinical period of drug development with a blueprint to bring that biomarker into clinical trials and to establish the link between the biomarker and the clinical outcome. There is a critical need for rigorous assessment of the procedures and criteria used to evaluate or validate biomarkers in order for them to gain widespread acceptance. As emphasized above, the extent

of rigor depends on the intended use of the biomarker (11). Most of the emphasis and work in the biomarker field has been geared toward efficacy uses. This is generally because the primary goal of early phase drug development is to establish proof of concept by gathering short-term evidence in screening clinical trials. On the other hand, many adverse drug reactions have a relatively low incidence, and long-term drug exposure is needed to resolve safety issues and reveal infrequent but important adverse events. Nonetheless, increased effort needs to be expended on the development and evaluation of improved biomarkers for drug toxicity.

The ultimate value of a biomarker will depend on whether it is assessed in an exploratory or observational type of study, or in a definitive or confirmatory study. The most desirable paradigm for evaluation of biomarkers is provided by adequate and well-controlled clinical studies that (*a*) define standardized relationships between drug exposure and response, (*b*) test hypotheses regarding mechanism of drug action, and (*c*) provide estimates of the magnitude of benefit. The size and duration of the treatment effect are essential aspects of biomarker evaluation, but sample size and study design are also important.

Adequate and well-controlled studies to evaluate biomarkers are often not attempted or are not feasible during drug development. However, one should not focus too strongly on developing a biomarker just to serve as a surrogate endpoint. For example, the evaluation or validation of a biomarker intended to be examined in a Phase II proof-of-therapeutic concept study may be based on a well-controlled study with a relatively small number of subjects and a short duration of treatment. Such a study may be called observational because it lacks the study power to test a hypothesis, but it can provide valid data to assess the strengths and limitations of a biomarker. This may be acceptable for addressing such exploratory questions as proof-of-therapeutic concept or even certain regulatory questions about dose or dosage regimen changes, but it would be inadequate for establishing a biomarker as a surrogate for a clinical outcome.

Evaluation of Biomarkers

Evaluation of a biomarker can be based on an exploratory process of determining how many of the characteristics of an ideal biomarker are met relative to the context of its use. Characteristics of biomarkers that underpin their utility have been described by several authors (3, 30, 31). Ideally, the attributes of a biomarker should include the following.

1. Clinical relevance, in that the marker provides evidence to support a theoretically rational basis for use, such as the ability to reflect some measurement of, or change in, a physiologic or pathologic process or activity over a relatively short period of time. The marker is influenced by exposure to a drug and is believed or assumed to be related to the drug's presumed pharmacologic action or intended clinical effect. There should be a strong, mechanistic molecular or biochemical basis for the biomarker

in which it is positioned early or late in the causal chain of pathological events leading to the clinical endpoint. This obviously requires an understanding of the pathophysiology of a disease and of a drug's mechanism of action. However, one must recognize that diseases frequently have multiple causal pathways.

2. Sensitivity and specificity to treatment effects, defined as the ability to detect the intended measurement or change in the target patient population via a given mechanism, without interference from other pharmacologic or clinical effects of the drug unrelated to the drug's mechanism of therapeutic action. The caveat here is that, as shown in Figure 1, drugs have both intended and unintended actions.
3. Reliability, defined as the ability to measure analytically the biomarker or change in biomarker with acceptable accuracy, precision, robustness, and reproducibility. This refers to the quality and variability of the assay for quantitating the biomarker.
4. Practicality, defined as noninvasiveness or only modest invasiveness in order to obviate inconvenience and discomfort to healthy volunteers or patients
5. Simplicity, for routine utilization without the need for sophisticated equipment or operator skill, extensive time commitment, or low measurement cost. This is needed to facilitate widespread acceptance of the biomarker for use in drug development and in subsequent clinical practice.

Validation of a biomarker is a complex part of the evaluation process. The criteria for validation are defined by the nature of the question that the biomarker is intended to address, the degree of certainty that is required for the answer, and the assumptions about the relationship between changes in the biomarker and clinical endpoints. Validation has been described as not being an all-or-none (binomial) variable, such as the outcome of an efficacy trial, but a continuous variable that varies during the drug development process as new information and data are obtained (11). There are multiple dimensions to biomarker validation that encompass important elements of study design and data analysis, including statistical assessment. There are also multiple pathways to validation of a biomarker for an intended use, and validation data itself is likely to arise from the totality of evidence provided progressively by preclinical animal studies, early Phase I and Phase II clinical studies in healthy volunteers or patients, and late-phase efficacy and safety trials in patients with the targeted disease.

Typically, validation takes into account the following properties of a biomarker and criteria for validation.

1. Sensitivity, referred to as the ability of an appropriate biomarker or a change in biomarker to be measured with adequate precision, and with sufficient magnitude of change, to make it sensitive enough to reflect a

meaningful change in important clinical endpoints. Sensitivity also describes the quality of the relationship between the magnitude of change in the biomarker and the magnitude of changed in the clinical endpoint because a high level of correlation, unfortunately, does not necessarily prove a cause-effect relationship.

2. Specificity, referred to as the ability of a biomarker or a change in biomarker to distinguish patients who are responders to an intervention from those who are nonresponders in terms of changes in clinical endpoints. Specificity defines the extent to which a biomarker explains all or most of the changes in a clinical endpoint.
3. Bioanalytical assessment of the laboratory or test measurement of the biomarker in terms of accuracy, precision, reproducibility, range of use, and variability.
4. Probability of false positives, defined by situations in which a desired change in a biomarker is not reflected by a positive change in a clinical endpoint or, even worse, is associated with a negative change in a clinical endpoint.
5. Probability of false negatives, defined by situations in which no change or a small observed change in a biomarker fails to signal a positive, meaningful change in a clinical endpoint.
6. A PK-PD model that has been shown to predict future clinical outcomes or suitable dose adjustments based on biomarker measurement. This establishes the correlation between changes in the biomarker and changes in drug exposure, measured as plasma concentration or dose. One of the challenges here is to prospectively plan and properly implement the model and to determine which metrics of drug exposure and biomarker time course are best able to predict clinical outcomes.

There are some patient factors (e.g. age, gender, race, and genetics), disease factors (e.g. stage and progression), and drug factors (e.g. metabolism and protein binding) that may modify treatment effects on biomarkers but are not themselves directly affected by a drug. Many of these factors may necessitate adjusting treatment effects on biomarkers and, thus, may affect the validity with which a biomarker can be applied to all patients with a disease. For example, the cognitive status of elderly patients with Alzheimer's disease or the etiology of hypertension in African-American patients may influence the predictive value of biomarkers that are influenced by drug exposure.

Validation of Biomarkers as Surrogate Endpoints

A surrogate endpoint can be thought of as a biomarker that can be definitively substituted for a clinically meaningful endpoint in an efficacy trial. There is value in biomarkers as surrogate endpoints only to the extent to which they can predict

long-term clinical outcome and serve as confirmatory evidence of efficacy. Many authors have published their validation criteria for surrogate endpoints. The most rigorous standards are those of Fleming & DeMets (2), who stipulated that both of the following conditions must be satisfied: (*a*) The surrogate endpoint must be correlated with the true clinical outcome; and (*b*) as initially proposed by Prentice (32), the surrogate endpoint must fully capture the net effect of treatment on clinical outcome. More recently, Temple (3) has laid out examples of evidence that support and evidence that does not support the use of biomarkers as surrogate endpoints. Extensive clinical evidence is needed, and the process of rigorous scientific and statistical assessment can be time-consuming and expensive. In many cases, the time and effort needed to validate a surrogate endpoint, to the extent that it is accepted by regulatory authorities, may exceed the time and effort that would be expended in measuring the clinical outcome directly.

Our current state of knowledge and lack of public consensus on validation of biomarkers as surrogate endpoints makes it impossible to provide specific steps or guidelines that can be followed. However, many authors have suggested approaches to validating surrogate endpoints and several criteria can be summarized as follows (30–33).

1. Biological plausibility should provide a mechanistic basis for using the surrogate endpoint.
2. Epidemiological or survey studies of the natural history of the disease should support surrogate status by establishing the statistical relationship between the biomarker and the clinical endpoint under basal conditions (30).
3. Adequate and well-controlled clinical trials should provide an estimate of the expected benefit in terms of clinical endpoints that can be derived mathematically or mechanistically from an estimate of the change in the potential surrogate endpoint. Ideally, an appropriate dose- or exposure-response relationship would be established as supplemental support for surrogate status.
4. The analysis should include a consideration of potential adverse reactions unrelated to the clinical endpoints predicted by the surrogate endpoint.
5. An exposure-response model should be developed that mathematically describes and predicts relationships between drug doses or plasma concentrations, and surrogate endpoints and clinical outcomes. Verification of these predictions is important.
6. The development and validation of biomarkers and surrogate endpoints should be built into the drug development process, beginning with the preclinical phase.
7. It may be helpful to conduct a meta-analysis of multiple clinical trials to look across and within studies to determine the consistency of effects

following interventions with various drug classes and within different stages of the disease (33).

Although there are many useful biomarkers, only a few of them are validated as surrogate endpoints and used to document the efficacy of a drug. Hence, there is a risk that the potentially useful applications of biomarkers will be overlooked in ill-advised attempts to elevate them to surrogate status.

USE OF BIOMARKERS IN REGULATORY REVIEW

Observational and definitive clinical trials are conducted during the course of drug development to address a number of questions posed by the sponsor. The use of biomarkers for developing dose response or PK-PD relationships has been shown to increase the efficiency and informativeness of clinical studies (34). In particular, biomarker-based data can provide answers to questions related to dose and dosage regimens needed for the product's label. These are of considerable interest to sponsors, as they may help to assure success in the marketplace through product differentiation. Similarly, regulatory authorities can pose questions to the database submitted in an application or dossier and frequently rely on dose-response or PK-PD relationships to better understand the effects of a drug and to address their own questions related to drug dose and dose adjustments that might be needed in light of patient factors that introduce variability in average exposure-response relationships.

There have been several excellent publications that provide a regulatory perspective on the use, benefits, and risks of biomarkers and surrogate endpoints in regulatory decisions leading to market access of new drugs (1, 3). For ordinary approvals, there are relatively few, well-established surrogate endpoints. They include blood pressure and serum cholesterol for cardiovascular drugs, blood sugar and glycohemoglobin for antidiabetic drugs, plasma testosterone levels for prostate anticancer drugs, and tumor size for antineoplastic agents. Viral RNA load and $CD4^+$ T-lymphocyte counts are the most well-known surrogate endpoints used for accelerated approval of antiretroviral drugs. However, accelerated approval also has been based on reductions in tumor size and decreased rate of gastrointestinal polyp formation as surrogate endpoints (R Temple, personal communication).

Legal Basis

The Food and Drug Administration (FDA) has a legal basis for using surrogate endpoints in ordinary and accelerated drug approvals leading to market access of new drugs or drug products (28, 35). The standards for linking a biomarker to a clinical outcome are higher for ordinary approvals than for accelerated approvals. This difference is based on consideration of many factors, including the degree of scientific evidence needed to support biomarker surrogacy, public health needs, relative risk/benefit ratio, and availability of alternative treatments.

Surrogate Endpoints as Confirmatory Evidence

The FDA Modernization Act of 1997 (36) states that confirmatory evidence, when combined with evidence from one adequate and well-controlled study, can support effectiveness as required for ordinary drug approvals. This provision has generated significant interest in the potential role of biomarkers to function as surrogate endpoints in providing that confirmatory evidence.

The quantity of evidence needed to support effectiveness, other than two adequate and well-controlled clinical trials, is discussed in Section II of the *FDA Guidance for Industry*, entitled "Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products" (37). This guidance states that one adequate and well-controlled clinical efficacy study can sometimes be supported by evidence from a well-controlled study or studies using a pharmacologic effect, as a biomarker, that is not an established surrogate endpoint. Acceptance of this evidence of efficacy is based on (*a*) the quantity of evidence showing that there is a strong theoretical or mechanistic link between the pharmacologic effect and clinical outcome; and (*b*) the quantity of data showing that there is a strong link between the pharmacologic effect and clinical outcome based on prior experience with the pharmacological class, and a clear understanding of the pathophysiology and mechanism of drug action.

Further discussion is necessary with regard to study design and data analysis in order to clarify the nature and use of biomarker data, linked to dose and/or plasma drug concentrations, to serve as potential confirmatory evidence. To facilitate that discussion, the FDA is in the process of writing a guidance for industry that deals with exposure-response relationships.

Other Regulatory Uses of Biomarkers

Biomarkers that are imperfect surrogate endpoints for any of a variety of reasons often are useful in addressing regulatory questions, and sponsors are encouraged not to abandon attempts to bridge biomarkers to clinical outcomes once Phase III efficacy trials are underway. Obviously, these biomarkers should have a rational and reasonable link to clinical outcome, and there should be a hypothesis that supports their use and that makes them relevant to decision making by a regulatory agency. Aside from the use of surrogate endpoints in adequate and well-controlled clinical trials to support the effectiveness necessary for market access, biomarkers that do not meet standards for becoming surrogate endpoints have other value in regulatory decision making and may be used in analyses that complement the results of adequate and well-controlled efficacy trials. For new chemical entities, these biomarkers are frequently incorporated in observational studies that are conducted routinely in Phase I or Phase II drug development.

The *FDA Guidance for Industry*, entitled "Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products" (37), provides an important

perspective on the potential usefulness of biomarkers, surrogate endpoints, and PK-PD relationships. Some examples of these uses are provided below (37).

1. The most obvious example is the use of plasma drug and/or metabolite levels as surrogate endpoints for efficacy and safety in the approval of generic drug products.
2. For new chemical entities, biomarkers can support, but not replace, clinical outcome results from efficacy studies when they are proximal to the clinical outcome, or they can measure real clinical benefit to the patient, such as increasing exercise tolerance or improving pulmonary function.
3. Biomarkers that are more distal in the causal chain leading to the clinical outcome and were investigated in early clinical trials may be suitable for assessing the clinical significance of changes in systemic drug exposure due to intrinsic and extrinsic patient factors, such as age, gender, smoking habit, degree of renal impairment, and drug-drug interactions. Several clinical pharmacology regulatory guidances recommend that sponsors define therapeutic equivalence limits using PK-PD relationships for studies of drug-drug interactions, and the effects of renal or hepatic impairment to determine label claims and the need to adjust doses (38, 39). For example, HMG-CoA reductase inhibition or bleeding times may be used as biomarkers to assess drug-drug interactions with cholesterol-lowering statins and anticoagulants, respectively.
4. Biomarkers may be useful for subgroup analyses of efficacy or safety data from adequate and well-controlled clinical trials or from meta-analysis of several clinical studies to identify covariates that were expected to account for differences in response. The level of certainty provided by a subgroup analysis using a biomarker is dependent on many factors, including attributes of the biomarker, as described above, and whether the hypothesis of a subgroup difference was developed prestudy or post hoc.
5. Biomarkers are useful in providing adequate evidence to bridge from a preexisting database of efficacy to support an efficacy decision in new situations or settings. These include approval of drugs or drug products for different populations (e.g. pediatric and ethnic groups) when certain conditions are met, different dosage forms (e.g. a controlled-release product for an established immediate-release product), different routes (e.g. parenteral vs oral), and different dosage regimens (e.g. three times a day vs twice a day). The *ICH E5 Guidance*, entitled "Ethnic Factors in the Acceptability of Foreign Clinical Data" (40), addresses the use of biomarkers specifically for bridging efficacy and safety data across ethnic groups in various regions of the world (40).

Risks

Over the years, important lessons have been learned about the risks involved in assuming causal relationships between presumed surrogate endpoints and clinical

outcomes (6, 41). These risks arise when (*a*) treatment intervention affects the surrogate endpoint coincidentally, but not the desired clinical outcome because the causal pathways differ mechanistically; (*b*) many treatment interventions (e.g. different antihypertensive drugs) affect the same surrogate endpoint (e.g. blood pressure) but account for different changes in desired clinical outcome, such as the incidence of stroke, myocardial infarction, or congestive heart failure (42); (*c*) the prediction of clinical outcome following treatment intervention (e.g. propranolol) by a surrogate endpoint such as blood pressure is dependent on the demographic, environmental, disease, age, or genetic factors in the patient population (e.g. elderly African-American vs Caucasian or young African-American patients) (43); and/or (*d*) the proposed surrogate endpoint (e.g. ventricular premature beats) does not encompass other actions of the drug, in particular, those related to adverse reactions and safety. This was the case with the cardiac arrhythmia suppression trial (CAST) (6).

Not only are drugs likely to have adverse effects that are not reflected by changes in a single biomarker, but a single biomarker may not indicate the full therapeutic benefit of a drug. For example, there appears to be an inflammatory component of coronary heart disease that accounts for the fact that the combination of C-reactive protein, an inflammatory biomarker, and lipid measurements predicts the relative risk of myocardial infarction better than when either marker is used alone (44). Pravastatin has been shown not only to lower serum cholesterol levels but to reduce plasma concentrations of C-reactive protein (45). It is likely that this apparent antiinflammatory effect of pravastatin accounts for the fact that, in the West of Scotland Coronary Prevention Study, the incidence of coronary heart disease events in patients treated with this drug was lower than that predicted from their cholesterol levels and a combination of other risk factors that did not reflect inflammatory response (46).

It is clear that most biomarkers are unlikely to capture all the effects of a drug, and thereby fulfill the most stringent criterion for a surrogate endpoint, although it is desirable for the totality of evidence to lean in that direction. Consequently, there will probably be a trend in the future for clinical trials in this and in many other therapeutic areas to incorporate panels of biomarkers that can reflect more adequately the full spectrum of relevant potential therapeutic and toxic drug effects. For example, it seems likely that future clinical trials with statin HMG-CoA reductase inhibitors will incorporate both C-reactive protein and serum cholesterol as biomarkers.

FUTURE DIRECTIONS

The rapid expansion of genomic information has focused considerable attention on the potential influence of genetic polymorphisms on response to drug therapy and has led to the development of pharmacogenomics as an important new field of scientific endeavor. Advances in pharmacogenomics are likely to result in the development of biomarkers that will play important roles as entry, stratification,

or exclusion criteria for clinical trials and, subsequently, will guide the optimization of drug prescribing for individual patients. In the area of cholesterol-lowering therapy, for example, it was found in the Regression Growth Evaluation Statin Study (REGRESS) that the Taq1B polymorphism in the *CETP* gene that codes for cholesterol ester transfer protein (CETP) affects not only the rate of progression of coronary atherosclerosis but also the extent to which patients benefit from pravastatin therapy (47). Coronary atherosclerosis appears to progress more rapidly in patients who are homozygous for the Taq1B allele. However, these also are the patients in whom pravastatin seems to be most effective. Patients homozygous for the B2 allele showed the least progression of atherosclerosis over the two-year study period, but there was no difference in disease progression between patients who were treated with pravastatin and those who received a placebo. Disease progression and pravastatin response were intermediate in patients who were B1B2 heterozygotes. This experience indicates that the efficiency of clinical trials could be enhanced considerably by using pharmacogenomic biomarkers to guide patient enrollment and stratification.

To date, the potential role of pharmacogenomic biomarkers perhaps is best illustrated by the clinical development program and labeled indications for trastuzumab, a humanized monoclonal antibody against human epidermal growth factor receptor-2 (HER2) (48). HER2 is overexpressed in 25%–30% of patients with breast cancer and is associated with a more aggressive clinical course and shortened survival in these patients. One of the entry criteria for the more than 1000 women with breast cancer who participated in the Phase I, Phase II, and pivotal Phase III clinical trials of trastuzumab was that they have metastatic cancer that overexpressed HER2. Now that the new drug application for trastuzumab has been approved, the labeling states that trastuzumab therapy is indicated for patients with metastatic breast cancer whose tumors overexpress HER2 (49).

Despite the fact that measurement of HER2 overexpression has been central to the development and current clinical use of trastuzumab, only 20% of patients identified by this biomarker have responded to trastuzumab treatment (50). It is likely that additional biomarkers will be needed to increase this low predictive ability. In addition, even though FDA-approved immunohistochemical and flurorescence in situ hybridization (FISH) methods are now available for assessing HER2 overexpression, interpretation is operator dependent and quality-control programs are not in place to assess whether individual laboratories can perform the test accurately and reproducibly (50). Clearly, problems of this sort also will need to be surmounted as other pharmacogenomic biomarkers are incorporated in late-phase clinical development programs and in clinical practice.

On the other hand, age, sex, diet, and other environmental exposures are contextual factors that may affect the relationship between genetic substrate and disease susceptibility. For example, a common nucleotide substitution (C677T) in the N(5,10)-methylenetetrahydrofolate reductase (*MTHFR*) gene reduces enzyme activity and causes moderate elevations in plasma concentrations of homocysteine (51). Because there is evidence that homocysteinemia may lead to atherosclerosis in individuals whose dietary intake of folic acid is inadequate, this provides a

rationale for conducting studies in which the plasma homocysteine concentration-lowering effects of vitamin therapy are evaluated (52, 53). In these trials, plasma concentrations of plasma homocysteine will probably serve as a biomarker, and special attention may be focused on individuals who are homozygous for the C677T mutation in the *MTHFR* gene.

Because the pathophysiology of most common diseases is multifactorial, single-gene mutations have generally only a limited correlation with the occurrence of a disease or with its progression or response to therapy. Accordingly, future developments in pharmacogenomics are likely to focus on the use of microarrays to study the differential expression of as many as 10,000 genes in a single experiment (54, 55). The efficient identification and monitoring of relevant induced gene products has the potential to provide biomarkers that will be particularly useful in developing drugs for conditions that are difficult to track by currently available methods. Microarray techniques seem particularly well suited to monitoring disease progression or therapeutic response based on serial analysis of gene expression (SAGE) (56).

The large-scale study of gene expression marks the transition from structural to functional genomics and will focus increasing attention on the bioinformatic intrastructure needed to support microarray and other high-throughput methods of generating pharmacogenomic data (57, 58). The massive amount of data that will be collected will need to be stored, processed, and analyzed by standardized relational database management systems, and a number of these have been described recently (56, 59, 60). The use of advanced bioinformatic techniques will facilitate the serial transition of data to information and then to knowledge. Linked with pharmacogenomics, it can be anticipated to enhance the entire drug discovery and development process, from mapping disease genes, to stratifying patients and providing improved early-response monitoring in clinical trials, to ultimately making allele-specific therapeutics a clinical reality (59).

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