Strategic Approach to Fit-for-Purpose Biomarkers in Drug Development

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Key Words

qualification, surrogate endpoint, target engagement, validation

Abstract

The strategic, fit-for-purpose use of the combination of robust target engagement and well-qualified disease-related biomarkers enhances understanding of the mechanism of action, ties together preclinical and clinical data, enables the assessment of target engagement, facilitates early proof of concept and dose focusing, and increases the efficiency of early clinical development with improved quality of decision making. Significant progress in biomarker discovery, validation, and qualification has increased drug-development decision making and regulatory applications. Target engagement biomarkers are present early in a pathophysiologic cascade and inform on physical or biological interactions with the molecular target of the drug. Disease-related biomarkers are present late in the pathophysiologic cascade and are linked to clinical benefit; thus, they assess a drug's effect on a particular disease. Together, these concepts lay the groundwork for high-quality drug-development decision making and a framework for the acceptance and qualification of biomarkers for regulatory use.

INTRODUCTION

Drug development remains an uncertain, expensive, time-consuming, and inefficient enterprise. According to some estimates, it costs over 800 million dollars to develop a new drug (1). Biomarkers that enable rational decision making early in development allow the potential for increased efficiency, as well as resource and time savings (2–8). Biomarkers have found utility in many aspects of drug development and medical practice. Investigating biomarkers in the drug-development setting may aid in determining or refining the mechanism of action and assessing target engagement for new or existing therapeutics. Along similar lines, investigating biomarkers may help determine or refine pathophysiology. One of the critical roles for biomarkers, particularly the combination of target engagement and disease-related biomarkers, entails defining and interpreting proof of concept.

If a particular biomarker qualifies for use as a surrogate endpoint, then its use can drive a major improvement in public health by aiding regulatory agencies in their interactions for the review and approval of new therapeutics, and it ultimately can benefit medical practice by allowing the use of new diagnostic tests. Qualified surrogate endpoints enable and accelerate drug development. In fact, for diseases in which surrogate endpoints are qualified, many new drugs are available. Examples include hemoglobin$_{A1c}$ for diabetes and blood pressure for cardiovascular disease. Conversely, for diseases in which no qualified surrogate endpoints are available, there are far fewer new drugs, in part because of the difficult path for drug development; a prime example is Alzheimer's disease.

Many important issues are relevant to a discussion of biomarkers. First, there are many different systems of biomarker nomenclature, based on different types of biomarkers and different approaches to classification. A key biomarker nomenclature distinction for drug development is between target engagement and disease-related biomarkers, which I discuss in greater detail below. Second, there are different uses of biomarkers, which range from exploratory hypothesis generation to definitive go/no-go decision making in late-phase drug development. Third, there are many different technology platforms for biomarker assays, ranging from immunologic assays to imaging. Fourth, there are different strategies for the validation (assay or method validation) and qualification (clinical validation) of biomarkers. Finally, the regulatory needs for scientific consensus and a robust data set highlight the potential for the role of consortia in biomarker discovery and development.

Biomarkers are the subject of an increasing number of important recent initiatives. An important initial effort was the Biomarkers Definitions Working Group (9) with members from the Food and Drug Administration (FDA), the National Institutes of Health (NIH), academia, and industry. I discuss the consensus definitions that arose from this working group below. More recently, the NIH Road Map announced in 2003 (10) and the FDA Critical Path initiative (8) both prominently feature biomarkers to support improvements in the efficiency of pharmaceutical development. In fact, the FDA Critical Path opportunity list highlights prominently developing and qualifying biomarkers. In October 2006, a biomarker consortium with the FDA, the Foundation for the National Institutes of Health, and the Pharmaceutical Research

and Manufacturers of America was launched, which focuses on developing biomarkers for use in regulatory decision making, as well as biomarker discovery (11).

DEFINITIONS

The Biomarkers Definitions Working Group (with members from the FDA, NIH, academia, and industry) arrived at a consensus definition of a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (9). **Table 1** specifies the definitions of biomarker terms, including biomarkers, surrogate endpoints, and clinical endpoints. The term biomarker is the most general case; it refers to any useful characteristic that can be measured and used as an indicator of a normal biologic process, a pathogenic process, or a pharmacologic response to a therapeutic agent (9). A clinical endpoint actually quantifies a characteristic related to how a patient feels, functions, or survives, and a surrogate endpoint is a biomarker that is meant to substitute for a clinical endpoint. There are relatively few biomarkers that qualify for the evidentiary status of surrogate endpoints. Occasionally, surrogate endpoints are also referred to as surrogate markers in the biomarker literature. The Biomarkers Definitions Working Group has pointed out that the term surrogate endpoint is preferred because the use of this term properly connotes that the biomarker is being used to substitute for a clinical endpoint (9).

Validation and qualification are other key terms used for the discussion of biomarkers, and I discuss them in more detail below. Validation is the process of assessing the assay and its measurement performance characteristics and determining the range of

Table 1 Biomarker definitions

Term	Definition
Biomarker	A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic response(s) to a therapeutic intervention
Surrogate endpoint	A biomarker that is intended to substitute for a clinical endpoint and is expected to predict clinical benefit (or harm or lack of benefit or harm) based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence
Clinical endpoint	A characteristic or variable that reflects how a patient feels, functions, or survives
Target engagement biomarker	A biomarker that occurs early in a pathophysiologic cascade and informs on physical or biological interactions with the molecular target of the drug and, thus, assesses how hard a drug hits the target
Disease-related biomarker	A biomarker that occurs late in the pathophysiologic cascade and is linked to clinical benefit and, thus, assesses the effect of a drug on a particular disease
Proof of concept	Proof of concept is achieved when it is established that a drug candidate works to improve a disease condition in a way predicted by the proposed mechanism of action
Validation	The fit-for-purpose process of assessing the assay and its measurement performance characteristics, determining the range of conditions under which the assay will give reproducible and accurate data
Qualification	The fit-for-purpose evidentiary process of linking a biomarker with biological processes and clinical endpoints

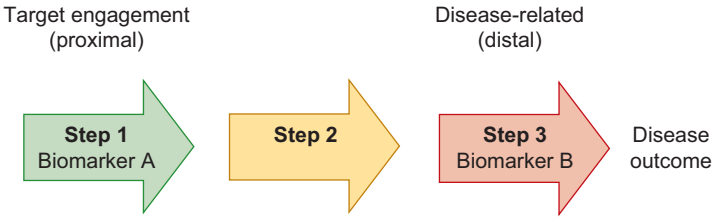

Figure 1

Pathophysiologic processes are generally multistep cascades, as shown in this schematic. If a biomarker is directly involved in the pathophysiology of a biologic process or disease, it may occur early or late in the cascade. Biomarkers that occur early in the pathophysiologic cascade are known as target engagement or proximal biomarkers (biomarker A). Biomarkers that occur late in the pathophysiologic cascade are known as disease-related or distal biomarkers (biomarker B).

conditions under which the assay will provide reproducible data meeting the individual study objectives, including sensitivity, specificity, and reproducibility. Qualification, or evaluation, is the evidentiary process of linking a biomarker with biological processes and clinical endpoints such that it can be used as a surrogate endpoint (11, 12).

Figure 1 shows schematically another useful distinction. Pathophysiology is typically a multistep cascade. If a biomarker is directly involved in the pathophysiology of a disease, it may occur early or late in the cascade. Biomarkers that occur early in the pathophysiologic cascade are known as target engagement or proximal biomarkers. Target engagement biomarkers inform on physical or biological interactions with the molecular target of the drug. Biomarkers that occur late in the pathophysiologic cascade are known as disease-related or distal biomarkers. Qualified disease-related biomarkers are capable of predicting clinical benefit. Thus, in **Figure 1**, putative biomarker A is identical to one of the early pathophysiologic steps leading to the disease outcome and is designated a target engagement biomarker. Putative biomarker B substitutes for one of the late pathophysiologic steps leading to the disease outcome and is designated a disease-related biomarker. The combination of target engagement and disease-related biomarkers is extremely useful in drug development as illustrated below.

VALIDATION AND QUALIFICATION

As defined above, an important distinction should be made between biomarker validation (assay or method validation) and qualification (or clinical validation or evaluation). Method validation is the process of assessing the assay or measurement performance characteristics, whereas qualification is the evidentiary process linking a biomarker with biological processes and clinical endpoints. There are a series of issues relevant to this distinction, including the distinction between these terms; the differences between biomarker and pharmacokinetic assays, and novel biomarker and diagnostic assays; the role for fit-for-purpose biomarker method validation and qualification; and the interaction between validation and qualification.

In the biomarker literature, the terms validation, qualification, and evaluation have been used interchangeably. A key reason to distinguish between validation and qualification is to avoid confusion between method performance and the evidentiary link between a biomarker with biological processes and clinical endpoints. The use of the term qualification emphasizes the relative, fit-for-purpose nature of evidentiary links inherent in qualification. Even biomarkers qualified as surrogate endpoints have varied levels of evidence and confidence in their use.

Biomarker method validation, especially for novel biomarkers, is different from pharmacokinetic assay and diagnostic assay validation. The FDA guidance offers a detailed description on bioanalytical method validation for the industry. However, this guidance is not well-suited for biomarkers, typically endogenous analytes, because it focuses on the validation of assays for small-molecule drugs. Similarly, routine diagnostic laboratory validation is not well-suited for novel biomarker validation. There is an exemption for research laboratory uses such as novel biomarker assays under the Clinical Laboratory Improvement Amendments of 1988, which govern laboratories that perform diagnostic assays. Thus, there is no clear regulatory guidance on requirements for novel biomarker assay validation. Because of the diverse purposes of biomarker research (particularly for biomarker discovery and exploratory biomarker use), the FDA regulations, bioanalytical drug assays guidance, and the Clinical Laboratory Improvement Amendments guidelines all fail to meet adequately the needs of novel biomarker study purposes.

Biomarker method validation assesses the assay or measurement performance characteristics. Method validation should be fit-for-purpose, that is, demonstrate a particular method to be reliable for the intended application; thus, the rigor of method validation depends on the purpose. Typically, the rigor of method validation should increase from the initial validation proposed mainly for exploratory purposes to more advanced validation dependent on the evidentiary status of the biomarker and/or the use of the results. Thus, there are two general categories of method validation in clinical drug development (13): (*a*) exploratory validation with crucial components, including accuracy, recovery, precision, relative selectivity, initial target ranges, analyte integrity in matrix, and dilutional linearity; and (*b*) advanced validation with the graded addition of other necessary components, including additional specificity, sensitivity, parallelism, expanded reference range, extended stability, method robustness, and document control. Different applications of biomarkers require appropriate levels of targeted method validation; i.e., method validation should be considered as an iterative fit-for-purpose and evolving process. For example, a biomarker used for a purely exploratory objective in a Phase I study may be subject to exploratory method validation, whereas a well-qualified biomarker used for a primary objective or decision making in a Phase I study may be subject to advanced method validation.

Although there is currently a lack of a consistent approach for accepting biomarkers for advanced regulatory uses, there is a growing recognition that the primary distinction between less well-qualified biomarkers and surrogate endpoints is based on evidence. Many biomarker nomenclature systems categorize biomarkers according to their evidentiary status. For example, in AIDS research, one early nomenclature scheme includes type 0 markers of natural history, type 1 markers that assess

biologic activity, and type 2 markers that act as surrogate endpoints for the clinical outcome of therapy (14). Regulatory guidances also highlight the role for evidence in defining surrogate endpoint status. For example, the International Conference on Harmonization E9 "Statistical Principles for Clinical Trials" (15) describes three primary criteria for surrogacy: (*a*) the biological plausibility of the relationship, (*b*) the prognostic value of the surrogate for the clinical outcome (linkage to disease), and (*c*) evidence from clinical trials that the treatment effect on the surrogate corresponds to the clinical outcome effect. All three of these criteria relate to the evidentiary status of the biomarker. In addition, selected FDA guidances emphasize the evidentiary status in biomarker classifications. For example, in the exposure-response guidance, the FDA (16) indicates that "[b]iological marker (biomarker) refers to a variety of physiologic, pathologic, or anatomic measurements that are thought to relate to some aspect of normal or pathological biologic processes," and "these biomarkers include measurements that suggest the etiology of, the susceptibility to, or the progress of disease; measurements related to the mechanism of response to treatments; and actual clinical responses to therapeutic interventions." Furthermore, the exposure-response guidance classifies biomarker types by their relationship to the intended therapeutic response or clinical benefit endpoints: (*a*) valid surrogates for clinical benefit (e.g., blood pressure), (*b*) candidate surrogates reflecting the pathologic process (e.g., brain structure in Alzheimer's disease), (*c*) those with measurement of drug action but with uncertain relation to clinical outcome (e.g., inhibition of ADP-dependent platelet aggregation), and (*d*) biomarkers that are remote from the clinical benefit endpoint (e.g., degree of binding to a receptor or inhibition of an agonist-provoked response). Again, all these categories relate to the evidentiary status of the biomarker. The FDA has also provided insight into its view of biomarkers through guidance on pharmacogenomic data submissions that shows it is likely to further distinguish between "probable valid biomarkers" and "known valid biomarkers," depending on the weight of supporting evidence (17).

This evidentiary distinction between biomarkers and surrogate endpoints leads directly to the concept of qualification. Biomarker qualification is a graded, fit-for-purpose evidentiary process linking a biomarker with biological processes and clinical endpoints, dependent on the intended application (11). Biomarkers used in drug development can be categorized into four classes of qualification (**Figure 2**): (*a*) Exploration biomarkers are research and development tools accompanied by in vitro and/or preclinical evidence, but with no consistent information linking the biomarker clinical outcomes in humans; (*b*) demonstration biomarkers are associated with adequate preclinical sensitivity and specificity and are linked with clinical outcomes, but have not been reproducibly demonstrated in clinical studies; (*c*) characterization biomarkers are associated with adequate preclinical sensitivity and specificity and are reproducibly linked to clinical outcomes in more than one prospective clinical study in humans; and (*d*) surrogacy reflects a holistic evaluation of the available data, demonstrating that the biomarker can substitute for a clinical endpoint. This schema captures the increasing decision-making and regulatory utility of biomarkers as qualifying evidence is accrued. The category of demonstration biomarker corresponds to the category of probable valid biomarker in the FDA guidance nomenclature. Moreover,

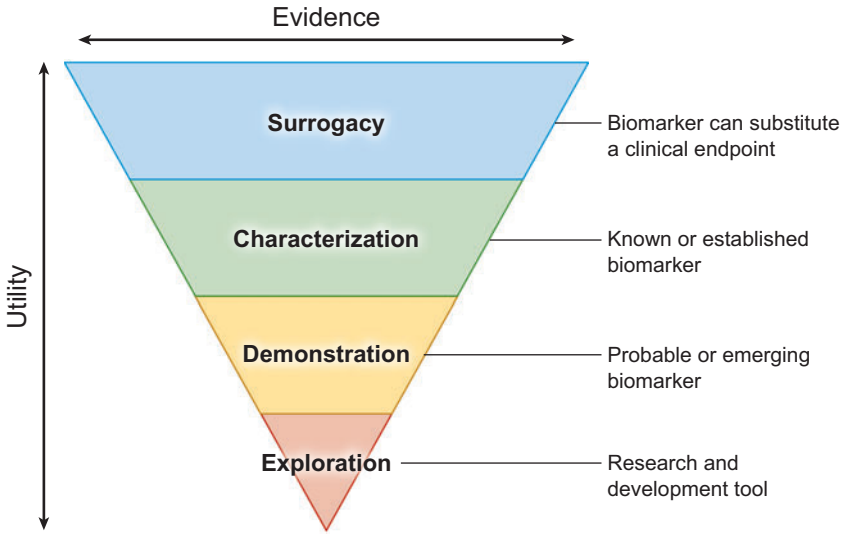

Figure 2

The inverted pyramid of biomarker qualification, demonstrating the relationship between increasing utility and evidence.

the characterization biomarker category corresponds to the known valid biomarkers category in FDA guidance nomenclature. The surrogacy category and designation of a biomarker as surrogate endpoint require agreement with regulatory authorities. Similar graded categories for biomarker qualification have previously been proposed for different purposes (18, 19).

As illustrated in **Figure 2**, the progression of qualification leads to increased utility in decision making and regulatory application. A novel biomarker may be progressively qualified from an exploratory biomarker to a surrogate endpoint for the purpose of drug development. Biomarkers may also enter the qualification pathway from general medical practice or research. Low-density lipoprotein cholesterol is an important example of a biomarker that was in general medical use prior to its role in drug development. Importantly, biomarkers can also be disqualified if evidence no longer supports the intended use. Although the rationale existed for the suppression of intermittent ventricular tachyarrhythmias (i.e., runs of asymptomatic ventricular tachyarrhythmia amid normal sinus rhythm) as a biomarker for the suppression of ventricular arrhythmia and reduction of mortality following myocardial infarction, a well-controlled clinical study disqualified it as a biomarker for this purpose. In fact, the results of the Cardiac Arrhythmia Suppression Trial revealed that mortality was increased by antiarrhythmic therapy following myocardial infarction (20), disqualifying suppression of intermittent ventricular tachyarrhythmias as a biomarker for the suppression of ventricular arrhythmia and reduction of mortality following myocardial infarction.

There is an interaction between method validation and biomarker qualification (**Figure 3**). Different applications of biomarkers require targeted method validation; i.e., method validation should be considered an iterative and evolving process. For example, a biomarker for purely exploratory hypothesis-generation application may

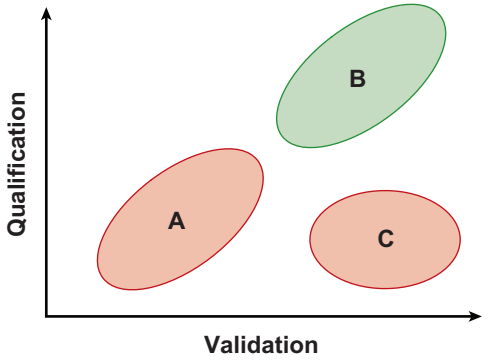

Figure 3

The interaction between method validation and biomarker qualification. The graded nature of the validation and qualification processes allows matching these activities depending on overall purpose. Biomarker A is a biomarker being used for an exploratory purpose. The corresponding method validation can similarly be exploratory. Biomarker B is being used in a more well-qualified fashion. Method validation should likewise be advanced. Biomarker C is a known biomarker used for an exploratory objective and is already associated with a more complete method validation package.

be subject to exploratory method validation, whereas a well-qualified biomarker for definitive decision making may be subject to advanced method validation. This approach also can avoid the investment of larger resources in more extensive method validation when biomarkers fail qualification. The state of the art should not be ignored, however. For example, a known biomarker used for an exploratory hypothesis-generation application may already have a more complete method validation package associated with it. This biomarker may be well qualified in certain settings, but not necessarily for the intended use in the current setting; even though it has a more comprehensive method validation package, the relevance of this must be reviewed for the new intended purpose.

STRATEGIC USE OF TARGET ENGAGEMENT AND DISEASE-RELATED BIOMARKERS IN DRUG DEVELOPMENT

General

The strategic use of biomarkers greatly facilitates drug development. The combination of robust target engagement and well-qualified disease-related biomarkers enables an understanding of the mechanism of action, relates clinical and preclinical experiments, allows the assessment of target engagement, facilitates early proof of concept and dose focusing, increases the efficiency of early clinical development, and improves the quality of decision making. In the following subsections, I discuss four specific examples with and without proof of concept and in the presence and absence of target engagement biomarkers (**Table 2**). These examples are all first-in-class agents at the time of clinical development. This allows a more balanced discussion

Table 2 The role of target engagement and disease-related biomarkers in establishing proof of concept

	Proof of concept?			
	Yes		No	
Biomarker	Target engagement	Disease related	Target engagement	Disease related
Target engagement used	Sitagliptin (DPP-4)		MK-0557 (neuropeptide Y)	
	DPP-4	Glucose	Positron emission tomography	Body weight
Target engagement not used	Troglitazone (PPARγ)		Peptide YY3–36	
	-	Glucose	-	Body weight

of benefits to drug development because subsequent entities may take even greater advantage of biomarkers owing to precedents set by the first-in-class agent.

Sitagliptin

Dipeptidyl peptidase-4 (DPP-4) inhibitors are a new class of oral antihyperglycemic agents for the treatment of patients with type 2 diabetes (21). Sitagliptin is a first-in-class molecule that benefited from robust target engagement and well-qualified disease-related biomarkers and achieved proof of concept. The mechanism of action involves incretin hormones such as glucagon-like peptide-1, which is involved in regulating blood glucose levels (22). Meal ingestion causes the release of incretins, which lowers glucose, at least in part, by stimulating insulin and suppressing glucagon release (21, 23, 24). DPP-4 inhibitors enhance incretin action by blocking their degradation and therefore inactivation, in turn leading to higher levels of active incretins, increased insulin release, lower glucagon levels, and reduced glucose concentrations (**Figure 4**). Several orally active DPP-4 inhibitors have now been developed to treat type 2 diabetes, including sitagliptin, vildagliptin, and saxagliptin (25–27). Sitagliptin was approved in the United States in October 2006 for the treatment of patients with type 2 diabetes (28).

The strategic use of biomarkers greatly facilitated the early clinical development of sitagliptin. Target engagement biomarkers included the inhibition of plasma DPP-4 activity and disease-related biomarkers included plasma glucose in both the preclinical and clinical environments. Preclinical studies demonstrated that oral sitagliptin dose-dependently inhibited plasma DPP-4 activity and reduced blood glucose excursion after an oral glucose load in lean mice (29). In studies in insulin-resistant, diet-induced obese mice, oral sitagliptin produced a glucose excursion after a glucose load to a level comparable to that in the lean control mice (29).

Overall, preclinical experiment studies demonstrated that ~80% inhibition of DPP-4 activity is associated with maximal lowering of glucose levels (29). DPP-4 inhibition measured in vitro is an underestimate of the absolute degree of DPP-4 inhibition in vivo because of dilution; the actual value is estimated to be >90%. Early in clinical development, a Phase Ib placebo-controlled study in patients with type 2 diabetes revealed that single oral doses of sitagliptin dose-dependently inhibited the target

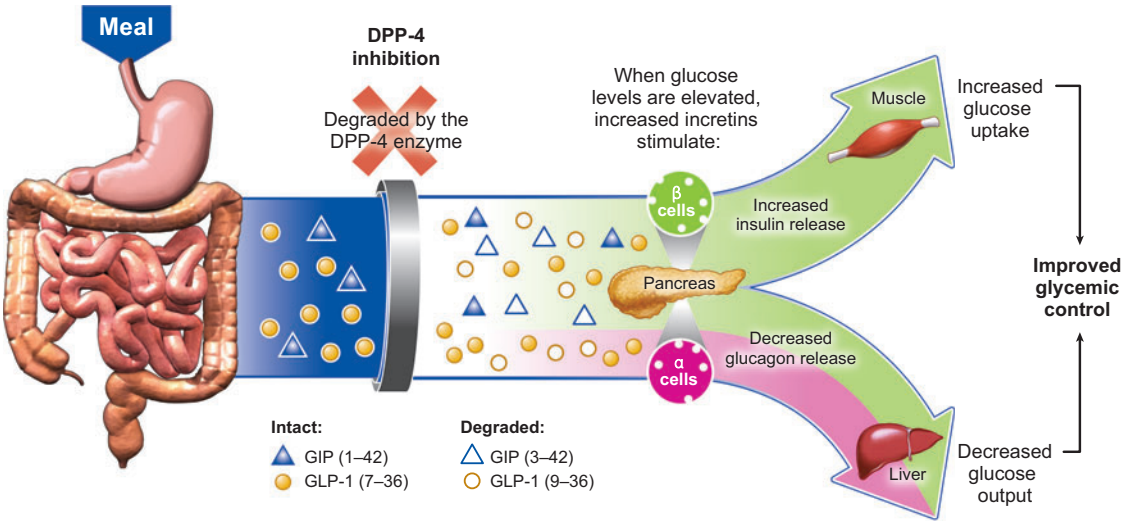

Figure 4

The physiologic role of dipeptidyl peptidase-4 (DPP-4), glucagon-like peptide-1 (GLP-1), and gastric inhibitory polypeptide (GIP) in glucose homeostasis. Following meal ingestion, the incretin hormones, intact (active) GLP-1 and GIP, are released from gut endocrine cells and function to lower blood glucose levels by stimulating glucose-dependent insulin release from pancreatic β-cells (GLP-1 and GIP) and suppressing glucose-dependent glucagon release from pancreatic α-cells (GLP-1). However, once released into the circulation, incretin hormones are rapidly inactivated and degraded by plasma protease enzyme DPP-4. DPP-4 inhibitors such as sitagliptin inhibit the breakdown of incretin hormones, thereby increasing active GLP-1 and GIP levels and promoting fasting and postprandial glycemic control. Adapted with permission from Reference 32.

engagement biomarker DPP-4 activity and decreased the disease-related biomarker of glucose excursion after a glucose load (30). A simple E_{max} model revealed that the EC_{80} of plasma DPP-4 inhibition corresponded to a plasma sitagliptin concentration of approximately 100 nM. These results predicted that a plasma sitagliptin concentration of >100 nM over 24 h would provide sufficient inhibition of plasma DPP-4 activity because ≥80% inhibition produced near-maximal lowering of glucose levels after an oral glucose load. Because a single dose of 100 mg provided maximally effective DPP-4 inhibition (>80%) for 24 h, these studies suggested that a once-daily dosing regimen was appropriate, and a dose of 100 mg per day would be optimal. This dose was later confirmed in a dose-ranging Phase II study in patients with type 2 diabetes (31).

Thus, the strategic use of biomarkers greatly facilitated the early clinical development of sitagliptin (32). The combination of robust target engagement and well-qualified disease-related biomarkers tied the mechanism of action together with the preclinical experiments, allowed an assessment of target engagement, achieved early proof of concept and dose focusing, increased the efficiency of early clinical development, and improved decision-making quality. It should be stressed that none of the

DPP-4 biomarkers discussed here are surrogate endpoints, yet their use was critical from a drug-development perspective. In the case of the sitagliptin clinical development, it provided an opportunity to proceed directly to Phase IIb dose range–finding studies following completion of the Phase I program, bypassing a traditional Phase IIa study. The average time taken for a new molecule to progress from first dose in humans to Phase III is approximately 3.5 years (33). For the sitagliptin program, the biomarker strategy effectively employed in early development enabled the time between first dose in humans and Phase III to be as little as 2.1 years. The reduced time to filing was supported by strategic biomarker use in alignment with a simple pharmacokinetic/pharmacodynamic modeling and increased drug-development efficiency.

Troglitazone

Although troglitazone was ultimately withdrawn from the market because of concerns regarding hepatotoxicity, it serves as a useful example of a first-in-class molecule that benefited from well-qualified disease-related biomarkers without a robust target engagement biomarker, and achieved proof of concept. Troglitazone was the first of the thiazolidinedione insulin-sensitizing agents and was quickly followed by rosiglitazone and pioglitazone. Troglitazone was subsequently withdrawn because of concerns about hepatotoxicity, which appears to be less of a problem with rosiglitazone and pioglitazone. Thiazolidinediones are ligands for the peroxisome proliferator-activated receptor gamma (PPARγ), and the mechanism of action involves PPARγ agonism. This mechanism of action opens the prospect for target engagement biomarkers; however, these were not available for the development of troglitazone.

Preclinical animal models of diabetes suggested that thiazolidinediones would achieve favorable clinical effects in diabetic subjects by reducing insulin resistance. Early clinical findings supported this hypothesis. The disease-related biomarker used was fasting plasma glucose, in some cases supplemented by oral glucose tolerance tests as a measure of insulin sensitivity and glucose handling (34). However, the clinical development timelines required to identify the clinical doses were lengthy owing, in part, to the lack of a target engagement biomarker.

Despite the use of the well-qualified disease-related biomarker of fasting plasma glucose, the lack of a target engagement biomarker hindered clinical development and decision making for troglitazone. Although proof of concept was achieved with disease-related biomarkers, dose selection would have been greatly facilitated by a robust target engagement biomarker. Since the development of troglitazone, rosiglitazone, and pioglitazone, the biomarker adiponectin (a putative target engagement biomarker) has been identified (35). Adiponectin is a 30-kDa protein composed of an N-terminal collagenous domain and a C-terminal globular domain. It exists in the circulation in complex oligomeric forms. Numerous studies have indicated that circulating concentration of adiponectin is closely linked to insulin sensitivity. A gradual decrease in adiponectin concentration is observed from lean to obese to diabetic individuals. Furthermore, PPARγ agonists increase the plasma concentration of adiponectin in rodents and humans. Thus, there is a body of evidence that adiponectin

is linked to glucose and lipid metabolisms in relation to insulin responsiveness and that it acts as a biomarker of PPARγ activation. Although the majority of patients have dose-dependent increases in adiponectin levels in response to PPARγ agonist treatment, only 50%–70% of patients demonstrate clinically improved insulin sensitivity. This suggests that an adiponectin level increase in any particular individual does not correlate with quantitative improvements in insulin sensitivity, but may be indicative of target engagement and dose response.

Neuropeptide Y

A potent orexigenic neuropeptide, neuropeptide Y (NPY), provides the molecular rationale for the antagonism of NPY Y5 receptors as an antiobesity drug target. MK-0557, a potent, selective, orally active NPY5R antagonist, is an example of a potential first-in-class molecule that benefited from a well-qualified disease-related biomarker and a robust target engagement biomarker, which did not ultimately achieve proof of concept. Initial clinical studies including a multiple-dose positron-emission-tomography study and a 12-week weight-loss/dose-ranging study suggested an optimal MK-0557 dose of 1 mg per day. In subsequent clinical development, the NPY5R mechanism was more rigorously tested in a 52-week, multicenter, randomized, double-blind, placebo-controlled trial involving 1661 overweight and obese patients. A statistically significant, but not clinically meaningful, degree of weight loss was observed at 52 weeks, demonstrating a lack of proof of concept for NPY5R antagonism (36).

Thus, the strategic use of biomarkers facilitated clinical development and decision making for MK-0557. The combination of robust target engagement and well-qualified disease-related biomarkers tied the mechanism of action together with the preclinical experiments, allowed the assessment of target engagement, facilitated dose focusing, increased the efficiency of early clinical development, and improved decision-making quality. In the case of the MK-0557 program, the combination of robust target engagement, with positron-emission-tomography imaging, and a well-qualified disease-related biomarker of weight loss provided the definitive assessment that MK-0557 adequately engaged the target NPY5 receptors yet did not achieve sufficient, clinically meaningful weight loss. Thus, with robust target engagement and well-qualified disease-related biomarker, a definitive assessment of this obesity target could be rendered. Without a target engagement biomarker, even in the absence of proof of concept, there would have been doubt about whether the mechanism or the molecule failed to establish proof of concept.

Peptide YY3–36

The gastrointestinal hormone peptide YY3–36 (PYY3–36) has been implicated as a postprandial satiety factor. PYY3–36 is an example of a potential first-in-class molecule that benefited from a well-qualified disease-related biomarker without a robust target engagement biomarker, which did not achieve proof of concept. Although no target engagement biomarker was available, previous Phase I clinical studies

demonstrated that intranasal PYY3–36 at 600 μg three times daily was the maximum tolerated dose. Subsequently, a randomized, double-blind, placebo-controlled 12-week weight-loss study was conducted in 133 obese patients. Placebo or PYY3–36 was administered as a 200- or 600-μg intranasal spray 20 min before breakfast, lunch, and dinner in conjunction with a hypocaloric diet and exercise. In the 600-μg group, 59% patients discontinued use owing to nausea and vomiting. Intranasal PYY3–36 as administered at these doses and preprandial timing was not effective in inducing weight loss in obese patients after 12 weeks of treatment (37).

Despite the use of the well-qualified disease-related biomarker of weight loss, the lack of a target engagement biomarker hinders clinical development and decision making for PYY3–36. To maximize the potential assessment of the mechanism, the researchers chose a high dose that ultimately led to excessive discontinuations. Dose selection would have been facilitated by a robust target engagement biomarker. Furthermore, even though PYY3–36 was not shown to be efficacious in inducing weight loss in obese patients after 12 weeks of treatment in a well-designed clinical study, uncertainty exists about the validity or lack thereof for the mechanism because the lack of target engagement biomarkers makes it difficult to understand if the molecule engaged the target receptor robustly enough. Thus, unlike NPY5R, in which a target engagement biomarker was available, there remains uncertainty surrounding the validity of the PYY3–36 mechanism.

STRATEGIC USE OF SAFETY BIOMARKERS IN DRUG DEVELOPMENT

Safety biomarkers are often used to characterize specific safety issues during drug development. A prominent example is the role of the QT interval as a safety biomarker, which has been largely driven by concern regarding the ability of some nonantiarrhythmic drugs to delay cardiac ventricular repolarization (38). The prolongation of the QT interval and its association with Torsades de Pointes have been important causes of the withdrawal or restriction of the use of marketed drugs (39). Despite great interest in the QT interval, the nature of cardiac repolarization caveats its role for quantifying the effect of a drug on the QT interval. First, cardiac repolarization is plagued by a large amount of intrinsic variability owing to both its effect on heart rate and the role of the autonomic nervous system. Demographic characteristics are also a source of variability (e.g., gender) (40–45). Second, the measurement of the QT interval itself is technically difficult, particularly the end of the T wave because it is difficult to discriminate with precision. Despite these limitations, the recent International Conference on Harmonization E14 clinical guidance document outlines the use of the QT interval in thorough QT studies, and it has become a mainstay for assessing cardiac safety in drug development (46).

Some drug targets and development programs are designed to avoid specific safety and tolerability issues, thus gaining an additional clinical benefit. This is a different strategic focus for safety biomarkers in drug development as opposed to that described above. As a treatment for dyslipidemia, niacin is currently the most effective for raising high-density lipoprotein cholesterol (47–49). However, niacin use is limited

by tolerability issues, including symptoms associated with cutaneous vasodilation, such as flushing (50). Flushing symptoms associated with niacin likely result from the vasodilation of the skin and include symptoms such as skin redness, the sensation of warmth, itching, and tingling. Niacin-induced flushing appears to be primarily mediated by prostaglandin D_2 (PGD_2) released by cells in the skin (51). PGD_2 binds to the G-protein-coupled receptors DP1 and DP2, and stimulation of DP1 by PGD_2 induces vasodilation (52). Laropiprant is a selective DP1 receptor antagonist capable of blocking PGD_2- and niacin-induced vasodilation in a murine model (51). A clinical proof-of-concept study was performed to evaluate the effectiveness of single doses of laropiprant in suppressing safety biomarkers, including niacin-induced flushing symptoms and cutaneous blood flow in the face as measured by malar skin laser Doppler perfusion imaging (53). In this single-dose crossover study with extended-release niacin at doses of 1500 mg, laropiprant at doses of 30 mg through 300 mg reduced peak flushing scores by up to 74%, compared to placebo, in a dose-dependent fashion. The efficacy of laropiprant in reducing flushing symptoms was corroborated via measures of malar skin blood flow (**Figure 5**). Thus, safety biomarkers are useful not only in determining whether a particular safety signal is present, but also in describing the potential therapeutic advantages of new molecules.

ROLE FOR CONSORTIA IN BIOMARKER DISCOVERY AND DEVELOPMENT

Discovery and, in particular, qualification of biomarkers are resource intensive and time consuming. In part, resources are driven by the fact that the advanced qualification of biomarkers, particularly surrogate endpoints, depends on evidence, largely from well-designed clinical trials. Regulatory guidance also highlights the weight of convergent data from several investigative approaches, including biologic and clinical, before a biomarker is likely to be accepted as qualified for regulatory decision making. The role of evidence in qualification highlights the importance of collaboration in biomarker qualification. Collaboration on biomarker qualification is precompetitive in nature because increased regulatory and decision-making utility benefits all biomarker stakeholders. The advantage of the open-source sharing of biomarker data for qualification is a critical point in favor of collaboration. There are also practical arguments in favor of collaboration for the development and qualification of biomarkers. A collaboration model provides a potential forum to bring together different stakeholders who share an interest in developing biomarkers (e.g., regulatory, industrial, academic, and governmental institutions). Such a forum also allows the leverage of subject-matter expertise, often from different perspectives. Collaboration provides a mechanism to identify joint solutions, synergies, gaps, duplicative efforts, and opportunities for cost sharing. In addition, collaboration may augment interaction with regulatory authorities, particularly with regulatory involvement in the collaboration. All these reasons lead to an interest in collaboration between multiple parties involved in the qualification of biomarkers.

One model for collaboration is a consortium among interested stakeholders. The Semiconductor Research Corporation is a long-lived and successful example of a

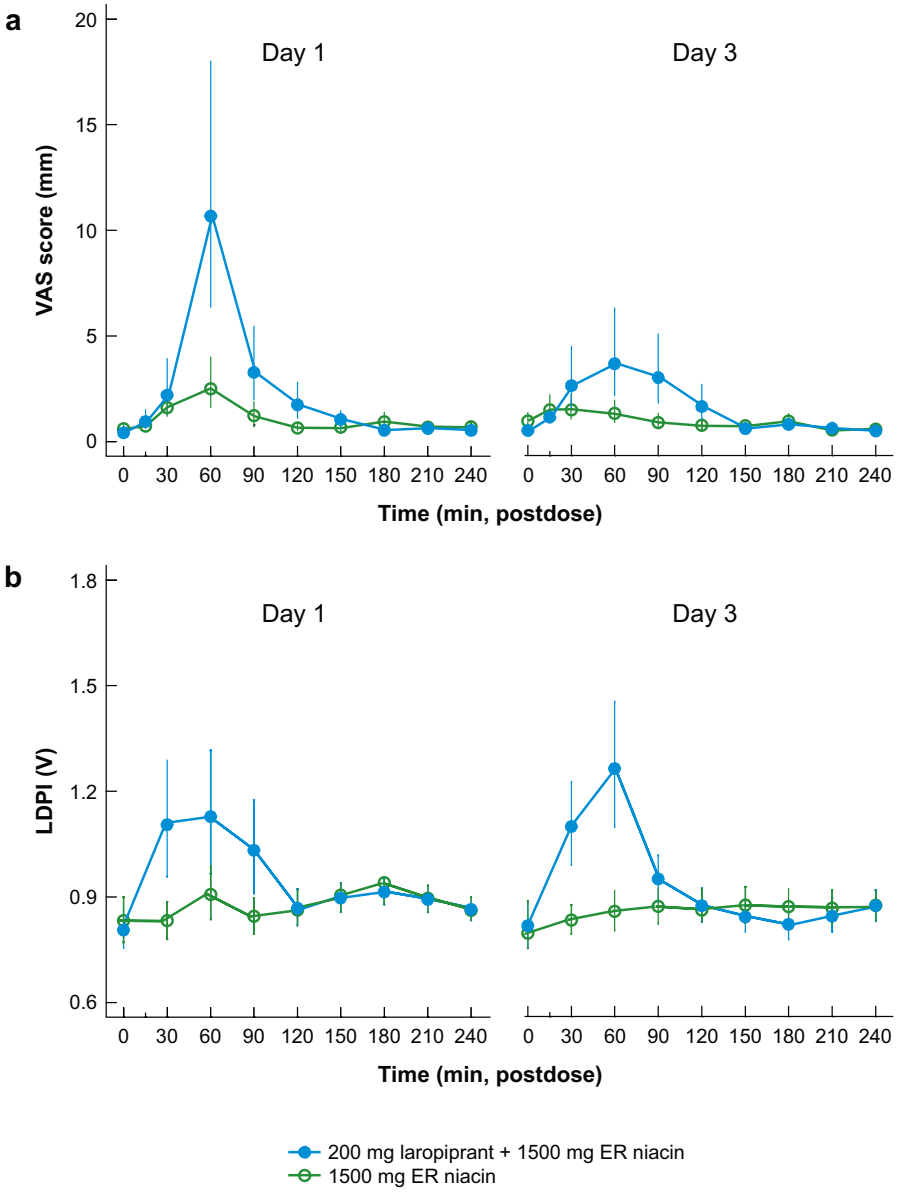

Figure 5

Time profile of niacin-induced flushing and vasodilation over 3 days. (*a*) Time profile of flushing symptoms [mean ± SE (*bars*)] of visual analog scale flushing score (mm) and (*b*) time profile of malar skin blood flow [laser Doppler perfusion imaging (LDPI) measurement in vasodilation] over 6 h postdose on the first and third day during a 3-day multiple dosing of extended-release niacin. Open circles represent laropiprant at 200-mg dose, extended-release niacin at 1500-mg dose; filled circles represent extended-release niacin at 1500-mg dose. Adapted with permission from Reference 53.

notable consortium involving members from industrial, academic, and governmental institutions. Examples of consortia involving the pharmaceutical industry include the SNP Consortium, enabling discovery and open-source publication of single nucleotide polymorphisms, and SEBiX (Secure Electronic Biopharmaceutical Information Exchange), a joint project between pharmaceutical sponsors to create an electronic system to exchange information efficiently with the FDA and other stakeholders. Thus, consortia have been used to provide the framework for precompetitive collaboration and interaction with stakeholders under an accepted governance. To facilitate precompetitive collaboration, the Biomarkers Consortium, a public-private biomedical research partnership of the Foundation for the National Institutes of Health with public and private stakeholders (including the NIH; the FDA; members of the pharmaceutical, biotechnology, diagnostics, and medical device industries; and advocacy groups) was launched in October 2006 (11). The consortium will manage biomarker projects to ensure scientific rigor, appropriate prioritization and funding, and compliance with relevant laws. The involvement of the FDA in the process of biomarker qualification in the United States is crucial. Because of the substantial resources required to qualify biomarkers as new surrogate endpoints or in other well-qualified decision-making uses, prospective regulatory input is crucial for the ultimate acceptance of biomarkers for different purposes. Prospective, nonbinding regulatory input provides the justification for the resource investment necessary to qualify new biomarkers similarly to how the end of a Phase II meeting justifies the resource investment for the Phase III drug development. Because different stakeholders approach biomarker qualification from different perspectives, a unifying framework would greatly facilitate biomarker qualification (54).

SUMMARY POINTS

1. Method validation is the process of assessing the assay and its measurement performance characteristics and determining the range of conditions under which the assay will give reproducible data. Qualification is distinct from method validation, and it is the evidentiary process of linking a biomarker with biological processes and clinical endpoints. These distinct, fit-for-purpose processes are necessary for appropriate biomarker usage.
2. Target engagement biomarkers are present early in a pathophysiologic cascade and inform on physical or biological interactions with the molecular target of the drug. Disease-related biomarkers are present late in the pathophysiologic cascade, are linked to clinical benefit, and, thus, assess the effect of a drug on a particular disease.
3. One of the critical roles for the strategic use of biomarkers, particularly the combination of target engagement and disease-related biomarkers, is the definition and interpretation of proof of concept.
4. Safety biomarkers are increasingly used to characterize and avoid specific safety issues during drug development.

5. To facilitate precompetitive collaboration, the Biomarkers Consortium (a public-private biomedical research partnership) was launched in October 2006 and will have an increasing role in biomarker discovery, qualification, and sharing of open-source biomarker data.

FUTURE ISSUES

1. A consistent, widely accepted framework for the qualification of biomarkers for regulatory use remains lacking. Will increased focus and collaboration on biomarkers facilitate innovative regulatory approaches and the subsequent application of biomarkers in drug development?
2. How will biomarker consortia benefit the discovery and qualification of biomarkers?
3. Will the sharing of placebo data (e.g., ECG warehouse) have a role in the research and qualification of biomarkers?
4. Many of the same questions regarding biomarkers apply to clinical experimental models. How will the role for qualification of clinical experimental models and correlation with preclinical models benefit the exploration of new mechanisms and drug development?
5. Composite and multiplexed biomarkers will have an increasing and evolving role in drug development and medical practice. Will composite biomarkers prove even more useful and predictive than traditional biomarker use?
6. The relationship and regulatory pathway between biomarkers in drug development and diagnostics will be an increasing focus of the industry, particularly for personalized medicine.

DISCLOSURE STATEMENT

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RELATED RESOURCES

Biomarker Consortium. **http://www.fnih.org/Biomarkers%20Consortium/Biomarkers_home.shtml**

Critical Path. **http://www.fda.gov/oc/initiatives/criticalpath/**

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