Worldwide Clinical Trials

Fit-for-Purpose Biomarker Development

A Translational Medicine Perspective

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During a drug discovery/development process, a drug candidate is initially characterized in a preclinical setting to attest to its performance characteristics with anticipation that these characteristics will both confirm the engagement of a molecule with a pathophysiologically relevant molecular target and subsequently inform the potential efficacy and safety of the product once a candidate molecule is introduced into clinical development. Target engagement biomarkers, present early within a pathophysiological cascade, are different than disease-related biomarkers that ultimately might be linked to clinical benefit. Both are essential elements in a discovery algorithm, particularly for targeted base therapeutics, with translatable value into the earliest phases of clinical research development phases, potentially leading to shorter and smaller clinical research designs in earlier phases of research antecedent to a more definitive investigation. The essential framework of a "fit-for-purpose" biomarker development strategy is highlighted, emphasizing the importance of strategic as well as transactional engagements with clinical research organizations.

A final goal of any therapy is a disease cure or its stabilization, attenuation of symptoms, or survival — in other words, a clinical outcome. Such outcomes constitute measurable changes in a patient's health, ability to function, quality of life, or survival, and have been used to characterize the safety and efficacy of a new drug since the early days of clinical research. However, certain clinical outcomes are difficult to measure in the absence of longer duration studies and larger patient sample sizes, and neither may be plausibly entertained at the earliest phases of clinical research.

Fortunately, advancements in life sciences have provided researchers and clinicians with novel tools to evaluate a health state or monitor disease progression or its cure. These tools are biomolecules that can be related to clinical outcomes and thus play a role of biomarkers. A biomarker — defined by the NIH as a characteristic that can be objectively measured and that indicates the state of a biological or pathogenic process[1] — or to be more precise, its concentration, can provide *indirect* insight into the state of a clinical endpoint by detecting changes taking place at the molecular level.

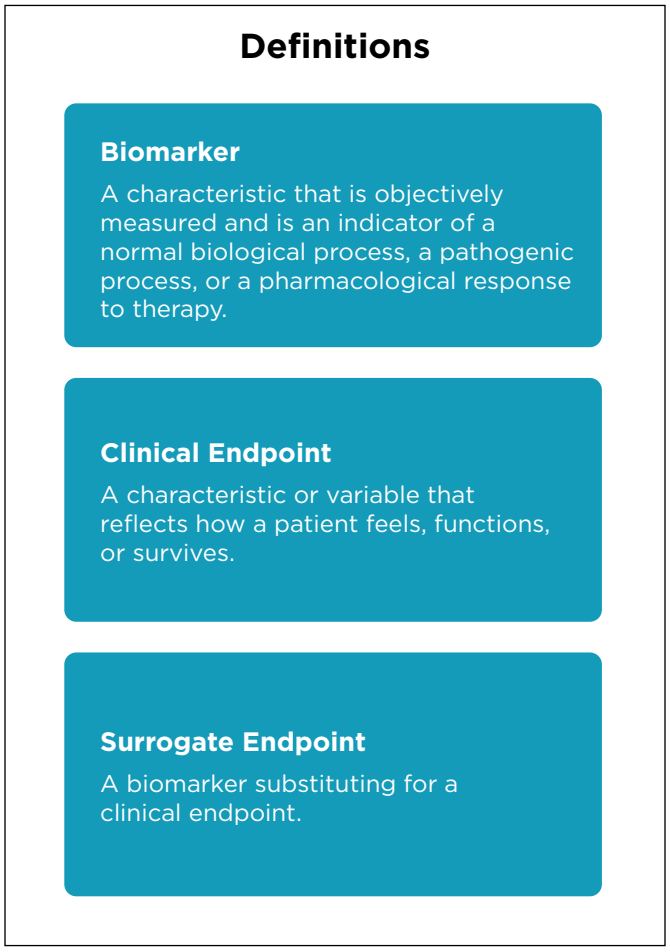

Figure 1: From the Biomarkers Definitions Working Group.[2]

Changes in biomarker concentration or level are plausibly correlated with changes in specific clinical outcome and can often be discerned very quickly, reflecting physical or biological interaction with the molecular target of a potential therapeutic – more proximal to exposure than the analogous clinical outcome might be on its own. Such brisk changes in biomarker levels may enable the identification of an early safety or efficacy signal in studies characterized by short durations of exposure to an investigational product (IP) and limited sample sizes. As such, biomarkers can often provide a clear, precise picture of the status of (or the progression to) a clinical endpoint and enable preliminary dose ranging paradigms that otherwise would not be feasible — while drawing on fewer resources than traditional clinical observation might normally require.

A number of physiology- or pathophysiology-specific biomolecules have been qualified as surrogates for clinical endpoints, although their ubiquitous use as pharmacodynamic (a.k.a. target engagement) biomarkers is far more relevant to early phase clinical research activities. Indeed novel biomarkers are being discovered regularly as a routine byproduct of basic research as a reflection of the unique mechanism of action developed in discovery research and, as such, qualification — which is an evidentiary process linking biomarker with biological processes and clinical endpoints — is uncertain. Although biomarkers are not limited to biomolecules, often equated with digital as well as neurophysiological or neuroimaging modalities, biomolecules represent a majority of biomarkers encountered in the clinic and for that reason are the sole focus of this white paper.

Biomarkers in Clinical Research

The levels of a biomarker and the degree to which that concentration changes can provide insights into fundamental (patho)physiological processes.[2] Depending on their characteristics, biomarkers can be used in many different ways — for therapeutic target identification, disease diagnosis and prognosis, patient stratification, monitoring treatment response, and more. As noted, a key benefit of using a biomarker to

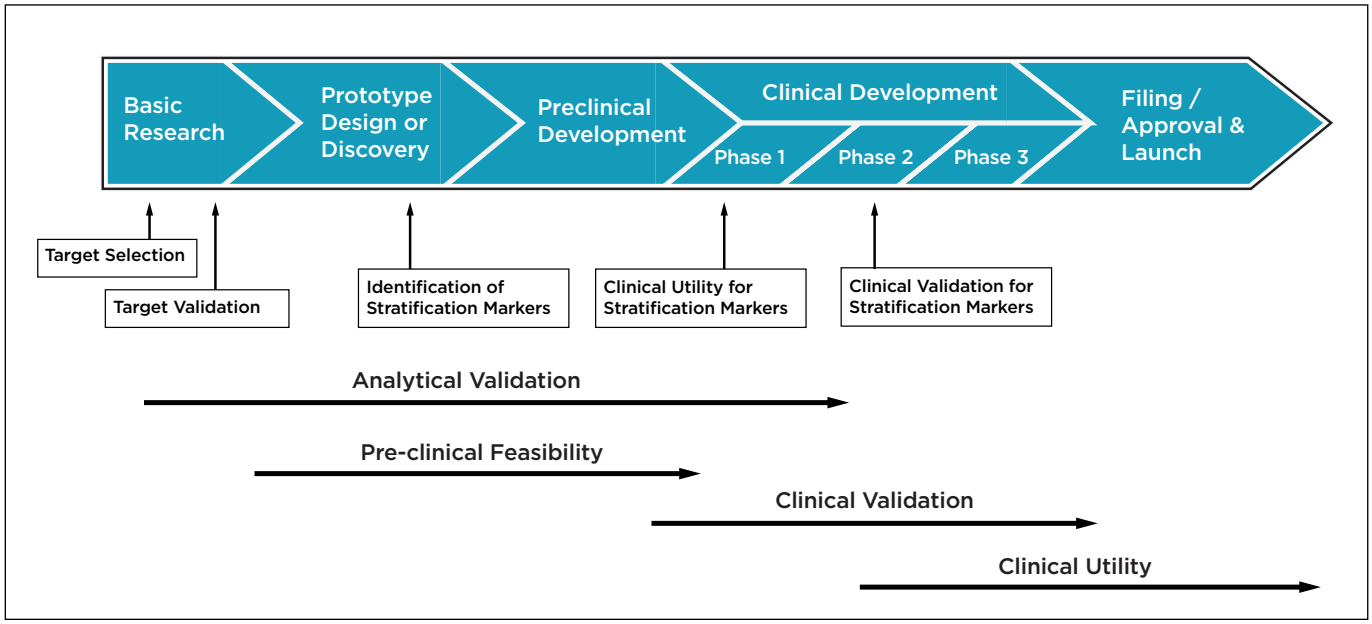

Figure 2: Many possible entry points of biomarkers in clinical research.

gain these insights arises from the fact that changes in biomarker concentrations can often be discerned sooner and with higher sensitivity than changes in clinical observations or symptoms.

Biomarkers can play a role in the drug development process at many different points — from preclinical drug target selection and validation, throughout the optimization process, and through late phase confirmatory clinical trials (see Figure 2). As an example, stratification biomarkers can facilitate the identification of different patient phenotypes and establish appropriate expectations about how a therapy may affect the disease in that particular patient population. This may, in turn, result in phenotypic/genotypic patient segmentation, logically consistent with the putative mechanism of action of the test agent. A novel biomarker identified early in drug discovery and introduced into earlier phase clinical development cycle may provide insights on dose-exposure-response relationships which could be subsequently incorporated into more definitive clinical trials.

Qualification, Evaluation, and Validation

To be useful in a clinical context, though, a biomarker quantification method must be validated (a method validation) before it is ultimately qualified in a regulatory sanctioned analyte which links the biomarker with a biological process and, eventually, a clinical endpoint. Validation involves a process of creating procedures which assess the measurement performance characteristics of an intended assay. Qualification, in contrast, requires a biomarker hypothesis that is grounded in scientific principles explaining the underlying biological processes informing the pathology under investigation, the origin and nature of a dysfunction that leads to the pathology, and, finally, the biological mechanism of the therapy being developed.

Thus there is an iterative process between validation and qualification that occurs along the continuum of the discovery/clinical development process. Clinical utility within this framework depends upon the bioanalytical methods associated with collecting and assaying the biomarkers, expressed as sensitivity, accuracy, precision, range, reliability, and robustness.

Yet the guidelines for biomarker qualification, evaluation, and validation are scant. The FDA released its *Bioanalytical Method Validation* and *Biomarker Qualification: Evidentiary Framework* guidelines in 2018, and the ICH issued its guideline (M10) in 2019 on bioanalytical method validation and study sample analysis, but none of these documents provides much detailed guidance on the qualification, evaluation, and validation of biomarkers.[4-6] The FDA says little more than that "method validation for biomarker assays should address the same questions as method validation for drug assays....The approach used for drug assays should be the starting point for validation of biomarker assays, although the FDA realizes that some characteristics may not apply or that different considerations may need to be addressed."[4]

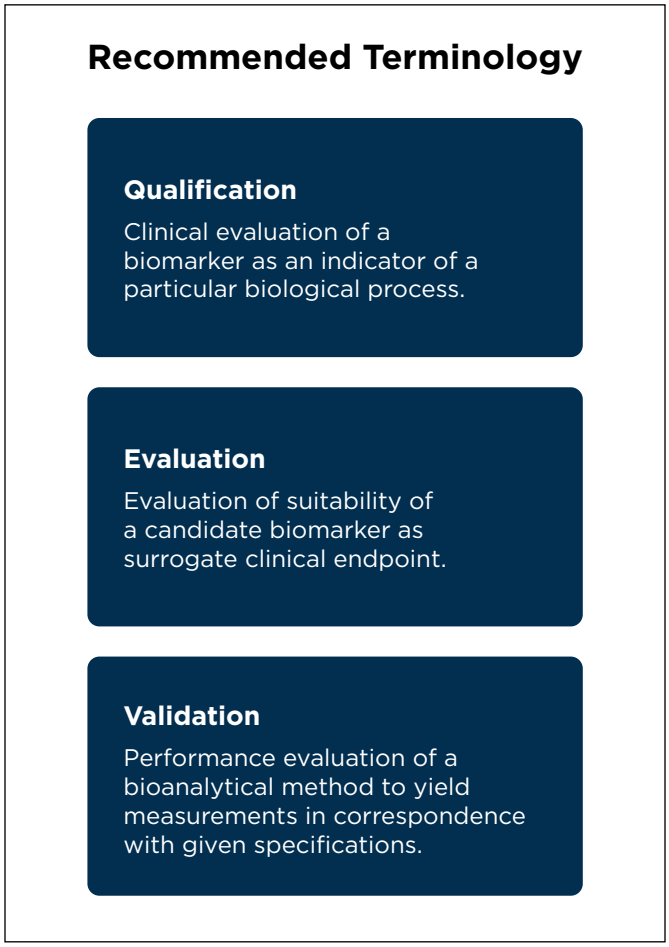

Figure 3: Qualification, evaluation, and validation are not the same.[3]

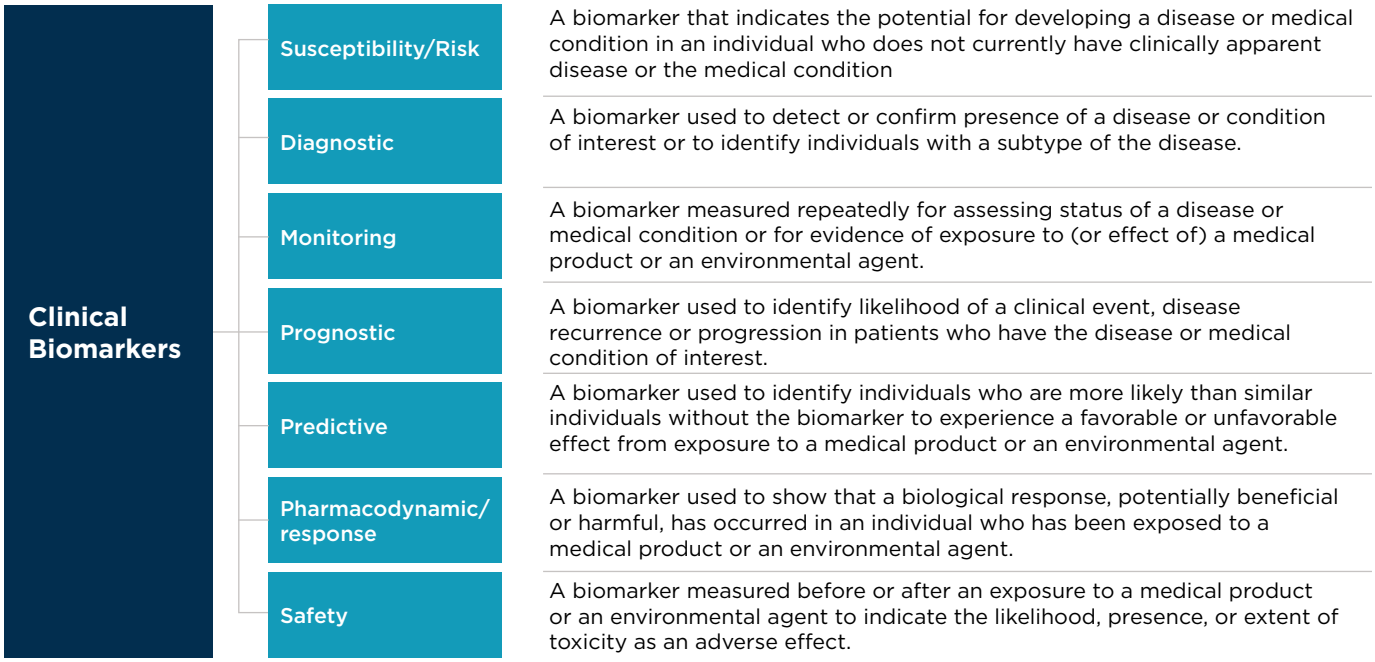

Figure 4: FDA-defined biomarker categories.[7,8]

The FDA defines biomarkers in seven distinct categories: susceptibility/risk, diagnostic, monitoring, prognostic, predictive, pharmacodynamic/response, and safety (see Figure 4).[7] It also distinguishes between biomarkers that a developer might use to validate drug development *methods* from those that a developer might use to support regulatory decision making, "such as the pivotal determination of safety and/or effectiveness or to support dosing instructions in product labeling."[4] These have also been referred to as "category 1" and "category 2" biomarker assays.[9] The central distinction between these categories involves the intent, rather than the nature, of the assay: biomarker assays belonging to category 1 generate data for internal decision making, without any intention to drive regulatory label claims. The quality and robustness of a category 1 assay can vary significantly — from assays that are not approved by the FDA to diagnostic kits that are fully-approved by the FDA. Classically, these are drug discovery tools that confirm that the suspected mechanism of action is indeed one that is deemed to be relevant.

In contrast, biomarker assays belonging to category 2 generate data intended to support a pivotal demonstration of effectiveness or label dosing instructions.[9] For category 2 biomarkers, the assays used to analyze the biomarker samples should be fully validated using the same validation criteria applied to assays associated with pharmacokinetic and toxicokinetic evaluations. However, category 1 biomarkers do not require such extensive validation.[4] Instead, the concept of "fit for purpose" should guide decisions about biomarker qualification, evaluation, and validation. The levels of qualification, evaluation, and validation should be appropriate to the intended purpose of a study and the stage of drug development.[4]

Biomarker Evaluation Throughout the Clinical Development Lifecycle

The concept of "fit-for-purpose" biomarkers and bioanalytical procedures has been discussed in the literature since the early years of the 21st century.[1-3,10,11] Succinctly put, a biomarker assay would be considered fit-for-purpose when the level of validation associated with it is sufficient to support its context of use.[3] For biomarker assays intended to support early drug development activities (e.g., candidate selection, go-no-go decisions, proof-of-concept), the FDA guidelines mirror a sentiment that is long-established within the translational medicine framework: that a developer should "incorporate the extent of method validation they deem appropriate."[4] For many developers, fit-for-purpose provides an ideal conceptual framework for the use of biomarkers because it enables a developer to optimize use of limited resources.[12] A fit-for-purpose biomarker and its assay must still be qualified, evaluated, and validated, but these requirements can be met far more efficiently and at much lower cost than would be required if full validation were undertaken. Thus, the concept of biomarker "qualification" stands apart from "fit for purpose validation" at this phase of clinical development.

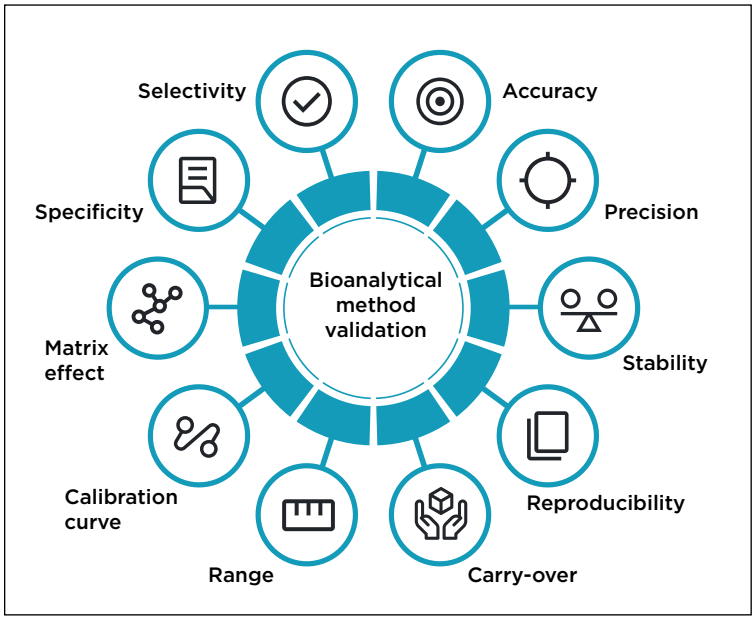

Figure 5: Bioanalytical method validation is carried out across different aspects.

To perform a fit-for-purpose validation, the acceptance criteria must be defined *a priori.*[13] Indeed, the design of a biomarker assay is tailored to its Context of Use (COU).[6] The acceptance criteria will depend on the predefined needs of the study (not simply the limits of the assay methodology), the quality and consistency of the data generated by the assay methodology, and the normal biological variance observed in the population being studied.[3,6] Assay stability, robustness and ruggedness may be evaluated in fewer reagent and assay runs than would be deemed necessary for full validation (three assay runs rather than six, for example).[3] Assay sensitivity may be estimated rather than established.[3] The fit-for-purpose approach to biomarkers and their associated bioanalytical methods is more practical for early development studies and enables faster access to insight regarding the biological confirmation of a presumptive mechanism of action without compromising data quality or incurring the risks of obtaining unreliable data.

This is not to suggest that a biomarker assay initially designed as fit-for purpose has no utility outside of those studies for which it was initially intended. That assay can live through several validation cycles. After development of an initial exploratory method, the validation of that method may be able to support studies probing into the mechanism of action and early toxicity. The subsequent validation of that method at the in-study level will bolster its suitability for early phase or pilot clinical studies in which researchers may shift hypotheses in the study designs toward evidence of early efficacy and toxicity in a clinical setting.[6]

Caveats and Opportunities

When transitioning, first to the exploratory, and later to the confirmatory clinical settings, these assays necessarily will need to be adapted, as there

are notable differences between assays that are suitable for discovery research (preclinical) and assays that have a clinical objective of informing a strategic program decision.

An informative discussion about suitability, as an example, pivots around the concepts of the range and sensitivity of assays. Preclinical experiments in discovery research will typically assess drug effects at higher drug concentrations, and the assays used will be optimized for higher doses. As a consequence, the assays may have lower sensitivity than would be needed in a clinical study, which could distort the potential dose-exposure-response relationship that a subsequent clinical program might examine. Indeed, the dose range selected for evaluation might be precluded clinically, given the enabling safety and toxicology data that informs that discussion.[14]

Another example of where adaptation may be needed involves potential matrix interference. Using a human antibody as a reagent may be acceptable in an assay that is fit-for-purpose designed to analyze samples originating from animals, but such a reagent would clearly be inappropriate if the assay were subsequently used to analyze human samples. Accordingly, the assay would need to be adapted for use in the clinic.[14]

Finally, it should be noted that biomarker research has evolved beyond protein analysis, incorporating cellular and genetic components. This expansion presents both opportunities and challenges in defining assay approaches. For instance, novel biomarker assays related to flow cytometry, enzyme-linked immunosorbent spot (ELISpot), droplet digital polymerase chain reaction (ddPCR), and next generation sequencing (NGS) require thoughtful consideration and strategy of qualification or validation procedures and acceptance criteria.

As these examples illustrate, modification and further validation are inherently necessary in a biomarker development program, but the processes of qualification, evaluation, and validation that begin with the earliest fit-for-purpose biomarker assays is both iterative and cumulative. The more fully and formally a biomarker assay is qualified, evaluated, and validated, the more acceptable that biomarker assay will be when biomarker qualification is proposed as a component of a drug safety or efficacy evaluation within a pivotal phase 3 study, where study design and specific assessments are tailored to an eventual clinical care decision.

Summary

Biomarkers and their associated assays can provide developers with rapid insights into biologically relevant outcomes that can inform clinical trial decisions in a sequence of subsequent studies. An appropriately validated assay can complement an assessment of clinical outcomes that might otherwise require studies of longer duration and sample sizes that extend beyond traditional limits for proof of concept programs. Established, fully validated biomarkers may be well-received by regulators who may be considering the qualification of a biomarker in a decision process involving potentially pivotal trial data.

A fit-for-purpose biomarker assay stratagem need only be qualified, evaluated, and validated to the degree that their intended use determines, and thus is ideally suited to an early phase discovery/ development program within translational research when dose exposure response relationships are poorly defined. Appropriately, the extent of validation efforts is deferred to the developer's assessment of the importance of a given biomarker, or a mosaic of assessments, at a given phase of development, through a series of gated, scientifically sound, and clinically informed decisions.

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