Guidelines for the Development and Incorporation of Biomarker Studies in Early Clinical Trials of Novel Agents


Abstract

The National Cancer Institute (NCI) Investigational Drug Steering Committee (IDSC) charged the Biomarker Task Force to develop recommendations to improve the decisions about incorporation of biomarker studies in early investigational drug trials. The Task Force members reviewed biomarker trials, the peer-reviewed literature, NCI and U.S. Food and Drug Administration (FDA) guidance documents, and conducted a survey of investigators to determine practices and challenges to executing biomarker studies in clinical trials of new drugs in early development. This document provides standard definitions and categories of biomarkers, and lists recommendations to sponsors and investigators for biomarker incorporation into such trials. Our recommendations for sponsors focus on the identification and prioritization of biomarkers and assays, the coordination of activities for the development and use of assays, and for operational activities. We also provide recommendations for investigators developing clinical trials with biomarker studies for scientific rationale, assay criteria, trial design, and analysis. The incorporation of biomarker studies into early drug trials is complex. Thus the decision to proceed with studies of biomarkers should be based on balancing the strength of science, assay robustness, feasibility, and resources with the burden of proper sample collection on the patient and potential impact of the results on drug development. The Task Force provides these guidelines in the hopes that improvements in biomarker studies will enhance the efficiency of investigational drug development. Clin Cancer Res; 16(6); 1745–55. ©2010 AACR.

In an era of increasing number of identified cancer targets and agents affecting those targets, efforts to determine target modulation, and to find subpopulations of patients most likely to benefit or experience harm from therapy, are potentially valuable for rational prioritization of therapies for clinical research evaluation and for individual patient management. Although nearly half of the recently approved oncology therapies have predictive markers, the qualification of putative biomarkers remains limited and the practical realization of successful biomarker use in early clinical drug development remains to be more fully developed. A recent extensive review of the use of biomarker evaluations in early phase I trials of anticancer agents shows the limited contributions resulting from these efforts (1). In addition to the expense and additional time required to incorporate these correlative studies, there are risks to patients who agree to invasive procedures for tissue-based biomarker studies, thus raising ethical issues if assay limitations are poorly understood or the utility of the study poorly justified.

Although the potential for biomarker studies to affect the clinical development strategy may be greatest in appropriately designed early trials by assisting in dose determination or identifying patients likely to benefit or at risk for toxicity, practical measures to achieve the goals are slow to appear, often uncoordinated and, at times, misdirected. Recognizing that biomarker studies have the potential to improve the value of clinical trials and can be integral to the design of a trial, but that this potential has been largely unrealized, the National Cancer Institute (NCI) Investigational Drug Steering Committee (IDSC) created the Biomarker Task Force in
### Table 1. Definitions

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
<th>Example</th>
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<tbody>
<tr>
<td>Biomarker nomenclature</td>
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</tr>
<tr>
<td>Biomarker</td>
<td>A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (25)</td>
<td>Drug-related toxicity, modulation of target protein phosphorylation, alteration of vascular permeability, tumor response</td>
</tr>
<tr>
<td>Pharmacodynamic</td>
<td>Provide evidence that there is a direct pharmacological effect of a drug</td>
<td>Drug-related toxicity, modulation of target protein phosphorylation, alteration of vascular permeability, tumor response</td>
</tr>
<tr>
<td>Prognostic</td>
<td>Provide evidence about the patient’s overall disease outcome independent of any specific intervention.</td>
<td>Performance status, OncotypeDx test</td>
</tr>
<tr>
<td>Predictive</td>
<td>Provide evidence about the probability of benefit or toxicity from a specific intervention.</td>
<td>HER2 amplification and effectiveness of trastuzumab; KRAS mutation and ineffectiveness of cetuximab</td>
</tr>
<tr>
<td>Surrogate</td>
<td>Subsets of biomarkers that are intended to serve as a substitute for a clinically meaningful endpoint (26)</td>
<td>Progression-free survival</td>
</tr>
<tr>
<td>Validation and qualification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assay method validity</td>
<td>Extent of the process by which biomarker assay characteristics are tested</td>
<td>Includes but not limited to analytic sensitivity, analytic specificity, precision, and inter-as well as intrapatient variability under typical clinical scenarios</td>
</tr>
<tr>
<td>Biomarker qualification</td>
<td>The extent of the process by which a biomarker is linked to a clinical significance.</td>
<td></td>
</tr>
<tr>
<td>Known valid biomarker*</td>
<td>A biomarker that is measured in an analytical test system with well-established performance characteristics and for which there is widespread agreement in the medical or scientific community about the physiologic, toxicologic, or clinical significance of the results.</td>
<td>Hercept(TM) test</td>
</tr>
<tr>
<td>Probable valid biomarker</td>
<td>A biomarker that is measured in an analytical test system with well-established performance characteristics and for which there is a scientific framework or body of evidence that appears to elucidate the physiologic, toxicologic, or clinical significance of the results.</td>
<td>Epidermal growth factor receptor mutations in lung carcinoma</td>
</tr>
<tr>
<td>Exploratory biomarker</td>
<td>A biomarker that does not meet the criteria for probable or known valid biomarker</td>
<td>Most biomarkers in early development</td>
</tr>
<tr>
<td>Role within the clinical trial†</td>
<td>Tests that are done for the trial to proceed; Integral studies are inherent in the design of the trial from the onset and are done in real time for the conduct of the trial Of note, if integral markers are to be used to make individual patient decisions, then CLIA regulations apply <a href="http://www.cdc.gov/clia/regs/toc.aspx">http://www.cdc.gov/clia/regs/toc.aspx</a></td>
<td>Biomarker is used to determine eligibility or to stratify to different arms of the trial.</td>
</tr>
<tr>
<td>Integral role</td>
<td></td>
<td>Phase I trial: the biomarker is used to decide whether to dose escalate or expand a dose level.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phase II trial: the biomarker is used to decide whether to continue enrollment or terminate patient enrollment because of lack of activity in biomarker-defined population.</td>
</tr>
</tbody>
</table>
2007 to develop recommendations for the conduct of biomarker studies in early clinical trials. Although initially developed for NCI Cancer Therapy Evaluation Program (CTEP)-sponsored studies, the recommendations are applicable to early investigational drug studies conducted by academic investigators for NCI or other sponsors. Background on the IDSC, the Biomarker Task Force, and the development of the recommendations is provided in Text Box 1.

This document is divided into five sections. It begins with standard definitions and categories of biomarkers based on intended use and level of validation and clinical qualification. Subsequently, recommendations to sponsors and investigators for biomarker incorporation into such trials are provided. Our recommendations for sponsors focus on the identification and prioritization of biomarkers and assays, the coordination of activities for the development and use of assays, and for operational activities. We also provide recommendations for investigators developing clinical trials with biomarker studies for scientific rationale, assay criteria, trial design, and analysis. The final sections provide commentary on the trial design and analytical issues that arise with biomarker studies in early clinical trials.

Definitions

The Biomarker Task Force recommendations use specific nomenclature, on the basis of a number of recent manuscripts and recommendations to define and describe specific types of biomarkers, assay validation and clinical qualification, and the role the biomarker may have within a clinical trial. The terms, definitions, and examples are found in Table 1.

### Recommendations for biomarker studies in support of new therapeutic agents

Biomarker studies in early phase trials require a careful coordination of expertise, infrastructure, and funding resources as well as a careful consideration of scientific rationale, assay characteristics, and trial design Text Box 2. On the basis of the occasional successful integration of biomarker studies in early phase clinical trials and the many examples of less successful ventures in this arena, the following recommendations that are specific for NCI supported trials but modifiable for other applications, are made:

#### Recommendations to the sponsor

**Recommendations for the identification and prioritization of biomarkers and assays**

A review of potential biomarkers by an expert or expert panel should be completed prior to a solicitation for clinical trial proposals of a novel agent. Preferred biomarkers and assays should be prioritized on the basis of the availability of well-characterized assays in humans and/or human specimens as well as on putative mechanism-based considerations. Investigational agents should be more readily and rapidly available to aid assay development.

Biomarker studies conducted in conjunction with pharmacokinetic studies should be encouraged.

Identification and prioritization of biomarkers that might inform clinical evaluations of a novel therapeutic should be based on strength of science supporting the mechanism of action of the agent and the determinants of its activity or toxicity, as well as the robustness of

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integrated role</td>
<td>Tests are intended to identify or validate assays or markers that are planned for use in future trials. Trials are designed to test a hypothesis and include complete plans for specimen collection, laboratory measurements, and analysis. Statistical design and analysis should be prespecified.</td>
<td>Phase I trial: the biomarker is studied at the MTD or at selected doses to confirm effect on target. Phase II trial: predictive marker is measured on all cases but the result is not used for eligibility, treatment assignment, or treatment management in the current trial. Statistical design and sample size are prespecified for the marker analysis.</td>
</tr>
<tr>
<td>Ancillary and/or exploratory role</td>
<td>Trial data are used to develop biomarkers and/or assays or to better understand therapeutic agent potential; biomarker data are not fundamental to the successful completion of the phase I or II trial.</td>
<td>Retrospective biomarker assays, pilot or feasibility biopsies, or methodological assessment; exploratory and/or hypothesis generating analyses.</td>
</tr>
</tbody>
</table>
Table 2. Analytical and performance requirements of laboratory assays of biomarkers with integral or integrated roles in clinical trials

<table>
<thead>
<tr>
<th>Types of laboratory issues</th>
<th>Clinical trial use of the assay</th>
<th>Primary drug effect assay PD assay</th>
<th>Secondary drug effect assay PD, predictive, or response assay</th>
<th>Integral diagnostic assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Role and/or purpose of the assay</td>
<td>Show a direct pharmacological or biological effect of a drug or prove feasibility of a new assay for an established drug effect</td>
<td>Associate a pharmacological or biological effect of a drug to a known therapeutic outcome</td>
<td>Clinical decision-making, e.g., patient eligibility, stratification, or assignment to treatment</td>
<td></td>
</tr>
<tr>
<td>May be exploratory biomarker and serve ancillary role</td>
<td>May be exploratory biomarker and serve ancillary role</td>
<td>May be exploratory biomarker but serves an integral role and needs to be done in a CLIA-certified laboratory</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Assay clinical readiness

Validation of assay analytical performance (using reference and calibration standards)

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Primary drug effect assay PD assay</th>
<th>Secondary drug effect assay PD, predictive, or response assay</th>
<th>Integral diagnostic assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy, precision</td>
<td>Required</td>
<td>Required</td>
<td>Required</td>
</tr>
<tr>
<td>Quantitation of assay endpoint</td>
<td>Required</td>
<td>Required</td>
<td>Required</td>
</tr>
<tr>
<td>Spike-recovery and dilution linearity using intended clinical matrix or linear response with appropriate calibrators for IHC-ISH or nonliquid-based assays</td>
<td>Required</td>
<td>Required</td>
<td>Required</td>
</tr>
<tr>
<td>Specificity</td>
<td>Required</td>
<td>Required</td>
<td>Required</td>
</tr>
<tr>
<td>Assay results parallel modulation of analyte concentration</td>
<td>Required</td>
<td>Required</td>
<td>Required</td>
</tr>
<tr>
<td>Robustness</td>
<td>Required</td>
<td>Not required</td>
<td>Required</td>
</tr>
<tr>
<td>Assay performance is independent of operator and based on a SOP</td>
<td>Assay performance is independent of operator and based on a SOP</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Specimen acquisition, processing, and storage SOP that yields valid assay results

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Primary drug effect assay PD assay</th>
<th>Secondary drug effect assay PD, predictive, or response assay</th>
<th>Integral diagnostic assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of specimen</td>
<td>Using intended specimen types from preclinical models and from relevant clinical donors</td>
<td>Using intended specimen types from preclinical models or from relevant clinical donors</td>
<td>Using intended types of specimens from relevant clinical donors</td>
</tr>
<tr>
<td>Specimen storage procedure</td>
<td>Required</td>
<td>Required</td>
<td>Required</td>
</tr>
<tr>
<td>Quantify how storage time and conditions of intended specimen types affect assay measurements</td>
<td>Quantify how much storage time and conditions of intended specimen types affect assay measurements</td>
<td>Quantify how much storage time and conditions of intended specimen types affect assay measurements.</td>
<td></td>
</tr>
</tbody>
</table>

Clinical readiness of combining specimen and assay SOPS (modeled using intended specimen types from preclinical models and/or intended specimen types from clinical donor protocols)

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Primary drug effect assay PD assay</th>
<th>Secondary drug effect assay PD, predictive, or response assay</th>
<th>Integral diagnostic assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose discrimination</td>
<td>Required</td>
<td>Required, limited to detect changes in assay results from</td>
<td>Required</td>
</tr>
<tr>
<td>Proven ability to detect</td>
<td>Proven ability to detect changes in assay results from</td>
<td>Proven ability to detect changes</td>
<td></td>
</tr>
</tbody>
</table>

(Continued on the following page)
available technologies and assays to measure candidate biomarkers in humans or human tissues. Ideally, proposed biomarkers should have strong preliminary data linking biomarker changes to agent effects, a well-defined assay and interpretation suitable for scientific and clinical inference, and a clearly defined process for specimen collection (including timing and method of sample procurement) and processing with feasibility data. If performance of the assay will be delayed, stability of the samples must be assured. In general, early access to the investigational agent is needed for laboratory-based experiments to accomplish these goals. Such experiments should identify candidate biomarkers that reflect the mechanism of action, toxicity, pharmacokinetics, and activity of the therapeutic agent. The experiments should also analytically validate assays to measure these markers and correlate the marker with dose or exposures as well as the aforementioned details about sample collection and storage (2–4).

**Recommendations for the coordination of activities**

Dialogue between the sponsor and institutional investigators is encouraged to identify the best assays and opportunities for biomarker development and use.

### Table 2. Analytical and performance requirements of laboratory assays of biomarkers with integral or integrated roles in clinical trials (Cont’d)

<table>
<thead>
<tr>
<th>Types of laboratory issues</th>
<th>Clinical trial use of the assay</th>
<th>Primary drug effect assay</th>
<th>Secondary drug effect assay</th>
<th>Integral diagnostic assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PD assay</td>
<td>PD, predictive, or response assay</td>
<td></td>
</tr>
<tr>
<td>LLOQ, ULOQ, and dynamic range</td>
<td>Required</td>
<td>Dynamic range must cover planned dose or exposure levels, and desired drug effect level</td>
<td>Dynamic range must cover planned drug effect level</td>
<td>Dynamic range must cover the clinically expected levels of response or expression</td>
</tr>
<tr>
<td>Absence of pre-analytic variables</td>
<td>Required</td>
<td>Consistency of baseline values; understanding of influence of planned prior sampling on biological variability in the assay</td>
<td>Consistency of baseline values; understanding of influence of planned prior sampling on biological variability in the assay</td>
<td>Consistency of baseline values; understanding of influence of planned prior sampling on biological variability in the assay</td>
</tr>
<tr>
<td>Timing of specimen collection to hit assay dynamic range and sensitivity</td>
<td>Required</td>
<td>Experimental evidence to guide timing of collection</td>
<td>Experimental evidence to guide timing of collection</td>
<td>Experimental (early phase trials) or clinical (late phase trials) evidence to guide timing of collection</td>
</tr>
<tr>
<td>Need for CLIA-certified laboratory</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**NOTE:** This table describes laboratory assays of biomarker issues that determine “clinical readiness.” The concept of “clinical readiness” combines proving analytical performance of a validated assay and fit-for-purpose when replicating the clinical situation in which the assay is intended to be used. It is recommended that the type, purpose, assay analytical performance, and clinical readiness as outlined in this table be described in proposals or protocols to sponsors. Although there are no mandated standards on analytical performance or “clinical readiness” of an assay prior to trial initiation, the greater the rigor of assay optimization and implementation, the greater the potential scientific value of biomarker data derived from the clinical trials. Only “integral” biomarker tests need to be done in a CLIA-certified laboratory; however Good Laboratory Practice (27) standards as well as the performance and reporting standards as outlined in STARD and REMARK (28–31) should be considered for nonintegral biomarkers.
Collaboration between institutions should be encouraged and central reference laboratories should be considered to standardize assay methodologies, reagents, and calibrators. Clinical trial proposals should not be penalized for lack of biomarker studies and instead sponsors should facilitate collaboration with institutions and investigators with applicable biomarker capabilities.

The number of potential biomarkers that might be evaluated for a specific agent and the expertise and resources required for assay development are significant. Such expertise and resources are available within NCI’s Division of Cancer Treatment and Diagnosis (DCTD), industry, and at select institutions. Collaborations between stakeholders for the selection, evaluation, and optimization for biomarkers and assays could facilitate the effective use of the resources and expertise. The use of standardized assays at reference laboratories would assure a greater degree of consistency across studies, and avoid unnecessary duplication of efforts to develop and evaluate assays at individual sites. The centralization of laboratory activities also allows the rapid accrual to well-designed clinical trials at sites that lack in-house resources to run biomarker assays but have the capabilities for sample collection and processing.

**Recommendations for operational activities**

- Funding should be made available for resources that can form the foundation for biomarker-based research with special attention to:
  1. investigational imaging procedure;
  2. collection and storage of biospecimens that can be secured at low cost and low patient risk.

To date, most biomarker studies have assessed pharmacodynamic effects or sought prognostic or predictive markers in tumor specimens even if the state of science, assay methodology, or trial design were insufficient to lead to informative results. Biomarkers that can be measured noninvasively through functional imaging studies or on normal

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**The Development of the Biomarker Recommendations**

- The National Cancer Institute (NCI) Investigational Drug Steering Committee (IDSC) was established in November 2005 in response to NCI Clinical Trials Working Group (CTWG) report to improve the NCI clinical trials enterprise.

- IDSC members include the principal investigators of NCI’s early drug development grants and contracts, representatives from the Cooperative Groups, a patient advocate, biostatisticians, and NCI staff.

- The IDSC provides NCI with broad external scientific and clinical input on the design and prioritization of phase I and phase II trials with agents for which the Cancer Therapy Evaluation Program (CTEP) holds an Investigational New Drug (IND) application.

- The IDSC aims to increase the predictive value of early phase trials, resulting in the design of more successful follow up studies.

- The IDSC created the Biomarker Task Force in 2007 to develop recommendations for the conduct of biomarker studies in early clinical trials.

- Biomarker Task Force members include representatives of the IDSC, Food and Drug Administration (FDA), pathology, imaging, patient advocacy, industry, biostatistics, and NCI staff.

- The Task Force members reviewed past and current biomarker studies, surveyed the peer-reviewed literature, NCI and FDA guidance documents, and conducted a survey of investigators of CTEP sponsored early drug trials to determine practices and challenges to incorporating and executing biomarker studies in early clinical trials.

- Recommendations set out in this document represent the consensus of the Task Force members and were approved by the IDSC in the summer of 2008.
Guidelines for Biomarker Studies in Early Trials

Recommendations for Biomarker Studies in New Therapeutic Studies

To the Sponsor:

- A review of potential biomarkers by an expert or expert panel should be completed prior to a solicitation for clinical trial proposals of a novel agent. Preferred biomarkers and assays should be prioritized based on the availability of well-characterized assays in humans and/or human specimens as well as on putative mechanism-based considerations.

- Investigational agents should be more readily and rapidly available to aid assay development.

- Biomarker studies conducted in conjunction with pharmacokinetic studies should be encouraged.

- Dialogue between the sponsor and institutional investigators is encouraged to identify the best assays and opportunities for biomarker development and use.

- Collaboration between institutions should be encouraged and central reference laboratories should be considered to standardize assay methodologies, reagents and calibrators.

- Clinical trial proposals should not be penalized for lack of biomarker studies and instead sponsors should facilitate collaboration with institutions and investigators with applicable biomarker capabilities.

- Funding should be made available for resources that can form the foundation for biomarker based research with special attention to:
  - Investigational imaging procedures
  - Collection and storage of biospecimens that can be secured at low cost and low patient risk

To the Investigator:

- Provide a hypothesis and rationale for biomarker utility and a description of the impact on therapeutic agent development based on the following considerations:
  - Biological and/or mechanistic rationale with data to support relationship between biomarker and agent effects
  - Intended use within the proposed study
  - Preclinical in vitro, in vivo and clinical results if available

- Describe the assay method’s validity and appropriateness for the study.

- Describe the investigator’s experience and competence with the proposed assays.

- Provide the data supporting the degree of biomarker “fit for purpose” and clinical qualification.

- Justify the number of patients and specimens
  - to demonstrate feasibility
  - to demonstrate that studies are likely to produce interpretable and meaningful results.

- Give thoughtful consideration to the risk to the patient of obtaining samples, specimens or data for biomarker studies in the context of data on biomarker validity and degree of clinical qualification.

Tissues or blood may be preferable to subjecting patients to additional biopsies. Although biomarker studies of readily accessible normal tissue are encouraged, the results of such studies should be used with caution in consideration of decisions about dosing and further development, unless there are convincing preclinical or early phase trial data relating such biomarkers to antitumor activity. Additional resources to support these alternatives are required.
Scientific and design recommendations for the investigator

To comprehensively review proposals for clinical trials with biomarkers, a clear description of the scientific rationale and supporting data for the proposed biomarker, assay as well as the study design, and analysis are needed.

**Scientific recommendations.** Proposals should provide:

- A hypothesis for biomarker utility and a description of the impact on therapeutic agent development based on the following considerations: (1) biological and/or mechanistic rationale with data to support relationship between biomarker and agent effects; (2) intended use within the proposed study (refer to classification, descriptions, and roles as described in Table 1); (3) preclinical *in vitro, in vivo*, and clinical results if available.

- The assay method validity and appropriateness for the study (see Table 2).

- The investigator's experience and competence with the proposed assays.

- The data supporting the degree of biomarker “fit for purpose” and clinical qualification.

To evaluate the clinical trial and prioritize the biomarker studies requires information on the likelihood that the biomarker is (1) relevant to the trial population and intervention; (2) can be measured within the study population and the study samples; and (3) that the assay is reliable and thus the results are likely to be correct and, ideally, contribute meaningful scientific knowledge to the development of the agent. The trial proposal should thus contain the purpose of incorporating a biomarker into a clinical trial and a strong scientific hypothesis that is based on data. The proposed contribution of these measurements to the development of the anticancer agent should also be explained. The expertise and practical experience of the investigator in working with human samples relevant to the proposed assay should be clearly documented. If the “fit-for-purpose” principles outlined by Lee (5), Wagner (6, 7), DeSilva (8), and their colleagues are followed, assay performance within their anticipated clinical trial context will be known (9). Only when the analytical performance of the assay within its clinical study context is characterized will the results of the assay on trial specimens be interpreted with confidence.

**Trial design recommendations**

- Justification of numbers of patients and specimens: (1) to show feasibility; and (2) to show that studies are likely to produce interpretable and meaningful results.

- Consideration about the risk to the patient of obtaining samples, specimens, or data for biomarker studies in the context of data on biomarker validity and degree of clinical qualification.

- Biomarker correlations are frequently done on results obtained from the analysis of samples from a subset of patients. In addition, biomarker results often classify patients into subsets that are then correlated with patient outcomes. In both cases, sample sizes in early clinical trials are generally too small to generate statistically robust conclusions, although results may still be informative. It is important, however, to consider not only how many specimens are likely to be obtained and analyzed but also whether the data will yield a meaningful conclusion that justifies the potential risk to patients, as well as the additional resources and expense required to do the study and/or studies. If the biomarker specimen is to be obtained from patients using invasive procedures, there should be some justification of the potential risks associated with the procedures (10, 11).

**Trial Design and Statistical Considerations**

The incorporation of biomarkers in early clinical trials should be based on specific biomarker hypotheses, availability of an adequate assay, and appropriate design of the study. Although the specifics may vary, the general established objectives for phase I and II trials remain the same. The phase I trial primary goal is to establish safety and to select one or more doses and schedules for further study. The phase II trial explores one or a small number of doses and/or schedules for safety and determines whether the tested agent or regimen has sufficient evidence of clinical activity to warrant further investigation. In these early trials, patient numbers are generally limited, and in phase I studies, patients are generally heterogeneous in terms of tumor histology and prior treatments. Biomarkers tested in early phase trials are often exploratory (Table 1), i.e., to collect preliminary data that may aid in the understanding of the agent and mechanisms, and assays may not be well characterized. These considerations may limit the types of biomarker hypotheses that can be evaluated and the strength of the conclusions that can be drawn.

**Biomarkers in phase I trials**

In phase I studies, pharmacodynamic biomarkers are often of interest based on assumptions that modulation of these markers may provide proof of drug target inhibition and support the selection of drug and dose for further evaluation. These are almost always exploratory biomarkers (Table 1). The design and statistical discussion that follows assumes that (1) the proposed biomarker is relevant for the drug's purported mechanism of action; (2) the assays have been established for the types of samples that will be studied; (3) the correct timing of the sampling has been established; (4) if normal tissues are to be used, that there is a correlation between changes in the normal tissue with changes in the tumor tissue; and (5) that the acquisition of tissue appropriately considers the risks and/or inconvenience to the patient and resources of the institution. In early drug trials, these ideal conditions may not be achieved prior to the initiation of the trial. However, preclinical pharmacokinetic-pharmacodynamic-drug activity modeling and laboratory assay analytical evaluation can

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provide supportive information for the initial selection of biomarker(s), sampling, and analyses for early studies.

The goal in these pharmacodynamic marker studies (e.g., changes in tumors noted during tumor imaging, molecular changes in biospecimen samples) in phase I trials is to provide evidence that the agent reaches or modulates the putative target. These studies can be conducted by analysis of samples and/or images obtained prior to and after treatment, or by comparison to an untreated control. Samples may be collected and analyzed from patients treated at the recommended phase II dose (RP2D) or at several dose levels to construct a preliminary dose-pharmacodynamic response curve.

The required degree of scientific and analytical validation of a biomarker and assay depends on whether the biomarkers are exploratory or to be used to make decisions within the trial. If a biomarker has an integral role (Table 1) to guide the dose escalation decisions for subsequent patients in a phase I trial, key challenges are determining magnitude of biomarker effect related to drug; detecting the effect within patients or samples independent of the variability because of specimen or assay performance; and establishing the methodology for completing the assay within a short turnaround time. In addition, Clinical Laboratory Improvement Amendments (CLIA) regulations imply that if the test results are used for medical decisions for the patient who has the test, the test should be done in a CLIA facility. The rules for deciding whether to dose escalate, expand, or de-escalate trial design will reflect the specifics of the agent tested, the biomarker characteristics, and whether toxicity or other parameters will also be used to guide the decisions. There is no standard or established design, although several approaches have been proposed (12, 13). If a biomarker will play a nonintegral role (i.e., is an exploratory biomarker and has an ancillary role; Table 1) in a phase I trial, the number of patients in the expanded cohort at the MTD or RP2D may be based on estimating the patient-to-patient variability. When the biomarker levels are approximately normally distributed, the number of patients will determine the precision with which the true standard deviation can be estimated (14).

Biomarkers in phase II trials
In phase II trials biomarkers can be used to provide evidence that the agent modulates the putative target or pathway in a pharmacodynamic assessment similar to the phase I setting or to evaluate the association between the biomarker and clinical outcome. Less commonly, biomarkers may be used to determine patient eligibility (for example, HER2 status for trastuzumab trials). Larger patient numbers in phase II allow the determination of the dose-response relationship of a pharmacodynamic marker across a narrow set of dose cohorts (generally one or two) and more homogenous patient population. However, phase II trials to show correlations between clinical outcomes with an investigational agent and biomarkers present at baseline, or changes in markers prior and on treatment, are difficult to design rigorously; there must be a sufficient number of patients with the clinical and biomarker outcomes of interest. When markers are used for medical decision-making such as assignment to therapy or stratification then the assays must be done in a CLIA-certified laboratory. Although a phase II trial is unlikely to definitively establish whether a marker can be used to predict clinical benefit, investigators may identify an association that can then be further explored in definitive phase III trials (15).

Pre-analytical and Analytical Considerations

The precision and accuracy of biomarker measurements, the standardization of specimen or sample collection, storage, and analysis are key to the successful interpretation of clinical trial results. Although exploratory biomarkers may be used in an early clinical trial, it is preferable that both pre-analytical and analytic issues be addressed before clinical trials are initiated to ensure valid scientific inference of trial results.

Analytic measurements can be confounded by biologic and pharmacologic variations among study participants, and by variations in specimen collection and processing. Reliability of biomarker values is dependent on the analytic validation of biomarker assays. Although there are currently no mandated standards for ensuring methodological quality of biomarker measurements for clinical research, there are reference sources that provide specific requirements for quality standards for biomarker tests used for clinical laboratory and imaging diagnostic tests. This is exemplified by the CLIA 1988 standards defined by Clinical and Laboratory Standards Institute\(^8\) and a number of Food and Drug Administration (FDA) and International Committee on Harmonization (ICH) guidance documents (16, 17). Basic analytic validation of biomarkers includes availability of standard reference materials, calibration of equipment, reagent checks, optimization of test protocols, acceptance criteria for standard curves, and assessment of matrix effects and interferences. Quality control measures include determinations of intra-assay and interassay precision and establishment of criteria of acceptability for results obtained with quality control samples at high and low concentrations. Similar principles apply to the evaluation of imaging assessment modalities (18–20). If samples are collected and stored for future use, additional resources and activities are needed such as a sample repository and database with careful definitions of data fields and nomenclature, and quality control procedures to ensure the integrity of the sample repository and data (21, 22).

A number of resources are available to assist investigators in their considerations of imaging- and biospecimen-based biomarkers in early trials of therapeutics. The NCI Translational Research Working Group has outlined

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\(^{8}\) (www.clsi.org)
developmental pathways for both biospecimen and imaging assessment modalities (23, 24). Table 2 outlines the considerations for integral pharmacodynamic marker assays developed by DCTD Clinical Pharmacodynamic Program that have been adapted to describe analytical issues for biomarkers that have integral, integrated, or exploratory roles. The NCI Office of Biorepositories and Biospecimen Research provides guidelines for specimen collection and preservation, which are available through its website (2). Although there are no mandated standards, the greater the rigor of laboratory and imaging practices for biomarker measurements and sample repositories linked to clinical trials, the greater the potential scientific value of biomarker data derived in the course of clinical trials. Support of assay validation, specimen repositories, and accompanying databases (across phase I and II trials) is encouraged.

Concluding Remarks

Biomarker studies have the potential to contribute to our understanding of human cancer biology and optimal patient treatment. Biomarkers in early phase studies can theoretically be used to enhance development of novel therapeutics, and occasionally to prematurely terminate the investigation of a new drug. However, to realize this potential requires careful consideration of study hypothesis, biomarker, technologies, assay development and performance, and patient and sample selection in the design of trials of novel agents that incorporate biomarkers. Prioritization based on solid science and the needs of patients, better coordination of biomarker and assay development evaluation, standardized tools and procedures, and improved operational efficiency and resources are required. In early drug trials, these ideal conditions may not be achievable without considerable coordination of activities for biomarker development prior to initiation of the clinical trials. The Biomarker Task Force submits these recommendations in a genuine effort to enhance the utility of these studies to cancer drug development. Our hope is to encourage the next generation of scientists to fully explore and optimize the use of laboratory and imaging correlates to expedite the identification of exciting novel agents. Perhaps the most important consideration for investigators and reviewers is to clearly understand the goal of incorporating a specific biomarker into a clinical trial and to balance this goal with the risk of sample procurement to the patient. Cost and time implications for developing and including biomarkers within a clinical trial may outweigh gain. Thus, the decision to proceed with biomarker studies within early clinical trials should be based on the strength of the scientific rationale, the feasibility of successfully conducting the studies in the clinical trial, and the potential impact of the results of such studies on drug development and lives of patients.

Disclosure of Potential Conflicts of Interest

M. Koehler, employment, stock holder, Pfizer.

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Guidelines for the Development and Incorporation of Biomarker Studies in Early Clinical Trials of Novel Agents

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