Biomarker Development: Bedside to Bench

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SUMMARY

This commentary complements the report from Nixon and colleagues by addressing the critical definitions, assay and analytical quality control and interpretation, and resources available to advance similar fit-for-purpose biomarker development.

See related articles by Nixon et al., p. 2771 and 2779

In this issue of *Clinical Cancer Research*, Nixon and colleagues report on two correlative translational research projects investigating the possible role of circulating cytokines and growth factors measured prior to patient's initiation of therapy can predict which patients will respond to the addition of bevacizumab to their chemotherapy (1, 2). The collection and careful storage of patient biospecimens as a component of large clinical trials creates an important resource with which to build upon the clinical trial knowledge towards development of fit-for-purpose biomarkers. The most important application of collection of biospecimens from a randomized clinical trial in this setting is for validation of prespecified analytes with locked down numeric cutoffs to apply in a per-patient analysis that examines the biomarker–clinical trial treatment interaction. These investigators examined a series of cytokines and growth factors known to be involved in angiogenesis and vascular remodeling against the clinical value of interferon ± bevacizumab for renal cell carcinoma (3) or combination chemotherapy ± bevacizumab for colorectal cancer (4). Their findings contribute to a growing body of evidence suggesting that patients with renal cancer with high IL6 plasma concentrations prior to initiation of therapy may predict benefit from addition of bevacizumab; similarly, PIGF and VEGF-D may be promising for examination of the role of bevacizumab in colorectal cancer. The different biomarker behavior in different cancers underscores the importance of examining putative biomarkers with rigorous assays and recognizing that there may be disease-specific differences even with the same drug. The clinical implications of these findings are discussed in the companion article by George and colleagues (5). This discussion addresses the critical definitions, assay and analytical quality control (QC) and interpretation, and resources available to advance similar fit-for-purpose biomarker development.

The NCI National Clinical Trials Network (NCTN) supports five network groups in the United States that develop and lead pivotal cancer clinical trials (6). Each NCTN group can collect and store biospecimens in a harmonized network of biobanks. Samples may be collected from consenting patients and biobanked for future unspecified research that further leverages the value of those clinical trials. These irreplaceable biospecimens are a critical resource to allow us to translate forward. The NCTN Navigator is a searchable resource (navigator.ctsu.org) that details biospecimens remaining after NCTN phase II/III and phase III trials are completed and have reported on primary outcome. Navigator includes information on ≥2,000,000 biospecimens (as of Feb2022) from ≥150,000 patients across 240 adult and pediatric NCTN trials in hematologic and solid malignancies. Specimens range from blood and bone marrow to fresh frozen biopsies and tissue recuts and blocks. They are searchable through the clinical trial under which they were collected. Navigator includes links to the clinicaltrials.gov registration page for each trial for interested investigators to learn more about the trial design and results when identifying specimens that may be useful in their research.

NCTN Navigator provides a process for investigators to request access to these valuable biospecimens. Applicants need not be members of an NCTN group, nor are they required to collaborate with the NCTN member group that executed the study from which the samples were collected. Investigators submit letters of intent (LOI; 165 to date) that are reviewed for sample feasibility by the group biobank; requests may include specimens from specific treatment arms or outcome groups, or multiple trials. Scientific proposals may then be submitted via Navigator (88, to date) for feasible LOIs after which they undergo scientific review by NCI's NCTN Core Correlative Science Committee of extramural experts in oncology, biostatistics, laboratory and translational medicine, pathology, and patient advocacy (6). Investigators may elect not submit proposals for several reasons, including lack of sufficient biospecimen number or type. The approval rate of submitted proposals is approximately 65%. Exploratory science may be better executed with samples from earlier phase trials; such requests are proposed directly to the responsible NCTN trial group and undergo scientific review by NCI.

The six most common types of biomarkers are diagnostic, prognostic, predictive, pharmacodynamic, pharmacogenomics, and surrogate (7). Prognosis is a correlation with outcome, independent of therapeutic intervention. Predictive value specifies a statistical treatment-by-biomarker outcome interaction. Such interactions can be negative, such as the effect of *KRAS* mutation on the poor response to EGFR antagonists in colorectal malignancy, or positive, such as the treatment benefit of anti-HER2 therapy for *HER2* amplified tumors. Biomarkers may have different, opposite, or interactive prognostic and predictive effects. The positive predictive value of *HER2* amplification for anti-HER2 treatment has overshadowed the long known negative prognostic effect of *HER2* amplification in breast cancer (8). Pharmacodynamic biomarkers are used to examine modulation of a drug-targeted event, and surrogates are biomarkers whose change is validated to correlate consistently and reliably with a defined outcome.

Biomarkers are also classified for their role in clinical trials (**Fig. 1**; ref. 9). This classification drives the level of required quality-controlled assay stringency and assay analytical outcome. Integral biomarkers are those necessary for execution of a trial, to be used as patient selectors, stratification factors, and/or surrogate endpoints. Integral and surrogate endpoint biomarkers must have validated assays and analytical

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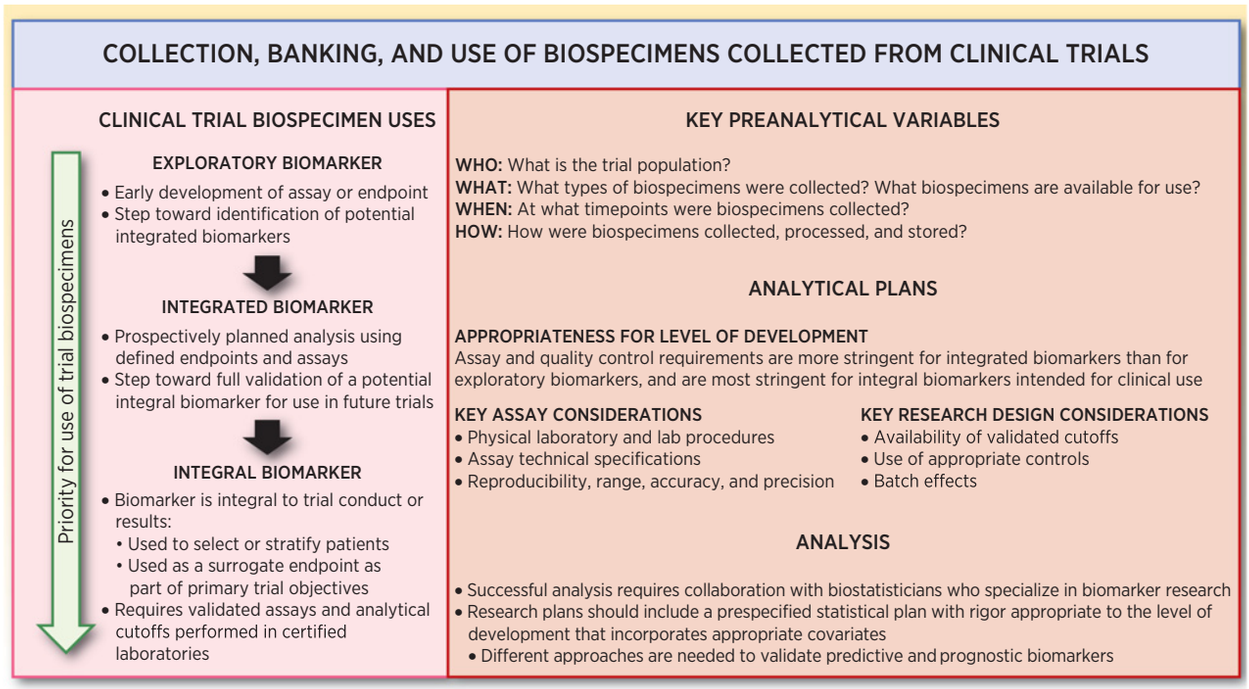

Figure 1.
Collection, banking, and use of biospecimens collected from clinical trials. The flow of decisions related to biomarker inclusion and use in clinical trials are demonstrated. Key considerations required for integrated and integral biomarker analysis are included.

cutoffs that are applied at a per-patient level from a certified laboratory. Median value cutoffs are outcomes of a validated assay. Integral biomarker use is the culmination of discovery and development starting with retrospective biospecimen and clinical data collections, moving in a step-wise fashion to full validation. Integrated biomarkers are those where examination is prospectively planned, samples collected, and analysis is retrospective; this is the penultimate test prior to integral use. These include prospectively planned analyses of defined endpoints with optimized assays examined generally in a blinded fashion with a per-patient predetermined cutoff.

Researchers considering banked biospecimens must consider the context and consent under which those biospecimens were collected for trial patient population(s), type of biospecimens, timing of collection and processing (10). Often, banked biospecimens have been collected years prior, and collection processes may have changed. Outcome reliability also requires knowledge of analyte temperature stability, freeze/thaw stability, and requirement for degradation inhibitors. For instance, cell-free DNA is best collected into specialized clot tubes (Streck tube), or in EDTA-anticoagulant tubes under ice, and doubly spun for plasma collection. EDTA binds divalent cations necessary for many DNAses, thus acting as a DNA protectant. Many pro-angiogenic factors are stored in platelet granules, thus requiring rapid and cold processing of plasma. Careful biospecimen collection, processing, and storage instructions are commonly now found in clinical protocols.

The analytic plan must be appropriate for the level of development (7, 9, 10). Assays need to be fit-for-purpose, which may be different for basic laboratory exploration versus assays for clinical use. Assay and QC requirements are more stringent for integrated than for exploratory biomarkers and are most stringent for integral biomarkers intended for clinical use. Stringency of assay QC increases steeply from exploratory use to patient application. Assays for clinical application have requirements for the physical laboratory, laboratory procedures, and assay technical specifications. Reproducibility over time and biospecimens, range, accuracy, and precision all contribute to assay QC.

Appropriate research designs also vary depending on the development goals. Inclusion of appropriate controls is critical, especially with assays where there needs to be correlation over time and space. Other parameters for analytical consideration include how samples are tested (masked or open-label), number of replicate samples per event or time point, and preplanned batching if possible.

A predefined hypothesis for the translational work will guide the statistical plan, which determines the number of samples necessary (11). The statistical rigor will be determined by the purpose, exploration versus validation. Assays for integral biomarkers and validation of integrated endpoints have a locked-down coordinates against which to make per-patient determinants. Covariates such as clinical characteristics and treatment exposures need to be considered. Specialized biostatisticians are key to application of the correct statistical design, power, and analytical tests.

The collection of clinical trials biospecimens is a unique resource to be leveraged for biomarker discovery, optimization, and validation. Biospecimen use also should be fit-for-purpose. Samples from positive, practice-changing phase III clinical trials are the most valuable as these can be used to validate true predictive treatment-by-biomarker outcome interactions. Whereas, specimens remaining from exploratory trials, trials where the agent or use in question are no longer clinically pertinent, or from negative clinical trials, are logical choices for use for exploration and assay optimization. The quality and optimization of

the assay requires less stringency for exploration than validation. Finally, the statistical design of the experiment(s) is critical to getting an interpretable outcome. Partnering with a biostatistician, even when exploration is planned, will improve the outcome of the biospecimen use.

Authors' Disclosures

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