Predictive Biomarkers in Oncology

Applications in Precision Medicine

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George Louis Kumar
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I would like to thank all the people who have guided, encouraged, and supported me throughout my career. Additionally, acknowledge the contributions of those who did not, but for them, I would not have learnt the value of success and the importance of character. A very humble thank you.

Sunil Badve, MD, FRCPath

"We are like dwarfs on the shoulders of giants, so that we can see more than they, and things at a greater distance, not by virtue of any sharpness on sight on our part, or any physical distinction, but because we are carried high and raised up by their giant size."

- Bernard of Chartres

To my dear father, Joseph, and my late mother, Miriam, for their unconditional love.
To my extraordinarily talented wife, Sujatha, for her continued support of my endeavors.
To my wonderful children, Vikram and Raj, for bringing so much joy to my life.

George Louis Kumar, PhD, MBA

Preface

"Precision/personalized or stratified medicine" refers to the tailoring of medical treatment or drug administration to the individual characteristics of each patient treatment. It does not literally mean that a pharmaceutical company makes a drug for an individual patient for consumption and treatment but rather means the ability to stratify (or classify) individuals into subpopulations that differ in their responsiveness to a specific drug. A marker that provides information on the likely response to therapy, i.e., either in terms of tumor shrinkage or survival of the patient, is termed "predictive biomarker." Examples include HER2 test to predict response to trastuzumab (Herceptin®) in breast cancer, the KRAS test to predict response to EGFR inhibitors like cetuximab (Erbitux®) and panitumumab (Vectibix®) in lung cancer, or the BCR-ABL oncogene detection to predict response to the tyrosine kinase inhibitor imatinib (Gleevec®) in chronic myelogenous leukemia.

Despite their promise in precision medicine and the explosion of knowledge in this area, there is not a single source on this subject that puts all this evidence together in a concise or richly illustrated and easy to understand manner. This book will provide a collection of ingeniously organized, well-illustrated, and up-to-date authoritative chapters divided into five parts that are clear and easy to understand.

Part I will provide an overview of biomarkers and introduce the basic terminologies, definitions, technologies, tools, and concepts associated with this subject in the form of illustrations/graphics, photographs, and concise texts.

Part II describes the signaling pathways controlling cell growth and differentiation altered in cancer. This part will analyze how predictive biomarkers are altered (expressed or amplified) across cancer types.

Part III will explore how predictive biomarkers play a role in patient stratification and tailored treatment in relationship to specific cancers (e.g., breast, gastric, lung, and other tumors).

Part IV will discuss how regulatory processes, quality and policy issues, companion diagnostics, and central laboratories help validate predictive biomarker assays.

Part V will wrap up with a description of precision medicine clinical trials around the world, and its successes and disappointments, challenges, and opportunities. This part will also summarize all FDA-approved drugs in oncology.

We hope that the proposed textbook will serve as a definitive guide for practicing pathologists, pathology residents, and personal in the pharmaceutical

or diagnostic industry interested in learning on how "predictive biomarkers" are used in precision cancer therapy.

We wish to thank Sujatha Kumar, Yesim Gökmen-Polar, Bharat Jasani, Katherina Alexander, and Victoria Alexander for proofreading. Special thanks to Michael D. Sova, Developmental Editor at Deved, Inc., for superb editorial assistance during the production of this book.

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Part I

Basic Principles and Methods

Check for updates

1 Introduction to Predictive Biomarkers: Definitions and Characteristics

Clive R. Taylor

Biomarkers

The concept of "biomarkers" as indicators of health or disease is not new. Under the broadest interpretation, the use of biomarkers extends back to the "ancients," who elicited medical signs, measured the pulse, observed, and even tasted the urine and the like [1]. However, the use of the term biomarker is relatively recent in the field of medicine, where the definition continues to shift with context.

Certainly many clinical laboratory tests fall under a broad definition. Examples include hormone levels for endocrine disease, a succession of enzymes and proteins, up to present day troponin for myocardial infarction, and prostatic acid phosphatase, then PSA (prostate-specific antigen), for prostate cancer. Extending the definition to its limits, the structural changes observed in anatomic pathology, or in radiology, also meet the definitional criteria; a tissue diagnosis of prostate cancer, plus or minus grading (e.g., Gleason), is a biomarker in a very real sense. Other "biomarkers" of diverse variety also have long been applied in unrelated fields, such as archeology, geology, and the petrochemical industry.

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This introductory chapter has a more restricted focus, namely, the utilization of "biomarkers" as identified by laboratory tests in relation to cancer; still more specifically, the focus is upon biomarkers detected directly in tissues from cancer patients (Table 1.1). Within this context of tissue and cancer, biomarkers include proteins and nucleic acids and derivatives and parts thereof. While the focus is narrow, the levels of complexity are manifold and growing day by day.

Biomarkers in Cancer

Tests for biological markers in malignant disease, for diagnosis, prognosis, and monitoring of progression, can be traced back at least a century and a half to the example of Bence-Jones protein in urine (Henry Bence-Jones 1813–1873) [1] for Kahler's disease (Otto Kahler 1849–1893), a surrogate for the detection and measurement of monoclonal (malignant-M) proteins that identify the condition that we now know as multiple myeloma. The modern era of biomarkers with respect to cancer in general may, on the one hand, be traced back to the discovery and use of CEA (carcinoembryonic antigen), a protein biomarker, and, on the other, to the Philadelphia chromosome, a genetic marker of chronic myeloid leukemia [1]. While CEA did not meet initial hopes of diagnostic utility in terms of sensitivity or specificity, measurement of CEA in the serum did find

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Table 1.1 Biomarkers in the context of cancer

Biomarker: general definition	A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to therapeutic intervention
Diagnostic	Design and usage; primarily to assist diagnosis; commonly in IHC on tissue sections, but also sometimes indicative in serum
Prognostic	Design and usage; primarily as a guide to prognosis; the course and progress of disease –therapy unspecified
Predictive	Design and usage; specifically for classification of responders vs. nonresponders for a defined (usually targeted) therapy; assay and threshold developed in conjoint clinical trial with the specified drug
Companion	Predictive; co-developed with a specified therapy and "required" prior to use of said therapy
Complementary	Predictive; co-developed with a specified therapy; accepted as providing guidance for therapy but not required
Pharmacodynamic	Definitional within the pharmaceutical field, such as providing a surrogate marker for disease status, as in remission or progression
Monitoring	Design and usage; for evaluation of status, progression, and/or recurrence of established disease process

a place in monitoring of established disease and as a "biomarker" of recurrence, likewise for CA-125 and arguably PSA. Notably, in a different context that still is within the field of cancer, all three of these biomarkers maintain a (variable) role as diagnostic biomarkers when demonstrated in situ within tissue or cell by immunohistochemistry (IHC). Thus context matters.

The decade of the 1990s saw major developments in the measurement of estrogen (and progesterone) receptors (ER and PR) in breast cancer, with applications that were prognostic and, to a degree, predictive in terms of choice of therapy. Cytosol-based competitive assays, relying upon extracts of purported tumor tissue, gradually gave way to a different methodology based on the detection of ER (and or PR) in situ within tissue sections by labeled antibody methods, with IHC (immunohistochemistry) using FFPE (formalin-fixed paraffin-embedded) sections emerging as the standard.

This transition occurred in spite of the arguments levied against FFPE tissue, because of the unknown effects of protein "masking," and against IHC, because of subjectivity in interpretation and hence variability in scoring, and also because of the nonlinear relationship between signal intensity and target antigen (in this instance the estrogen receptor protein) [2]. The efforts of Craig Allred and others in the development of defined (but semi-quantitative) scoring methods were critical to acceptance of the IHC method for this purpose.

In the presence of proper controls of assay performance [2, 3], IHC brings exquisite specificity, by scoring only recognizable cancer cells, and extraordinary technical sensitivity, with the ability to detect one ER-positive cell among a 100 identifiable cancer cells (1%; the current threshold of a positive ER IHC test) or in fact 1 positive cell among 1000 or 10,000 or more cells. Expressed in these terms, namely, detection of positive cells, this level of sensitivity is far beyond anything that can be achieved by any method using an extract of tissue, which is necessarily an imperfectly known extract of an imperfectly known mixture of normal and cancer cells, themselves imperfectly identified.

In this mode of performance, the IHC ER "test" may be considered to represent the beginning of the current era of employment of biomarkers in cancer, for prognostic and predictive purposes.

The "First" Predictive Biomarker

However, the moment of critical impetus for the current explosion in interest and variety of cancer biomarkers was the day (September 25, 1998) upon which the FDA approved the HercepTest

(Dako, now Agilent, CA, USA) and simultaneously gave approval for the use of the companion drug Herceptin (Genentech, now Roche) for the treatment of patients with Her2-positive breast cancer (as measured by the HercepTest). A vitally important corollary message from the FDA was that drug and test should be developed in concert, during a combined clinical study, hence "companion diagnostic" (Table 1.1) (Fig. 1.1) [4–10].

From the beginning of the millennium to the present time, US and European regulatory and working groups [4–8] offered various definitions of a biomarker, including the following: "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to therapeutic intervention." Subsequently the FDA went further with the definition of a "valid biomarker" – including that it should:

- Be measured in a test system with well-established performance characteristics
- Have a scientific background of evidence including clinical significance
- Be "fit to purpose"

A final consideration extended to a "clinically useful biomarker," which should in addition be reliable and clinically actionable in the specified setting.

The subsequent two decades have seen ongoing evolution of the term, with sub-definitions according to the design and use (Tables 1.1 and 1.2), accompanied by growing emphasis upon objectivity, reproducibility, and elements of true quantification, which reflect back upon methodology and ultimately performance of the "total test" from inception to interpretation, whichever the test modality employed (Table 1.3) [2, 3, 10, 11].

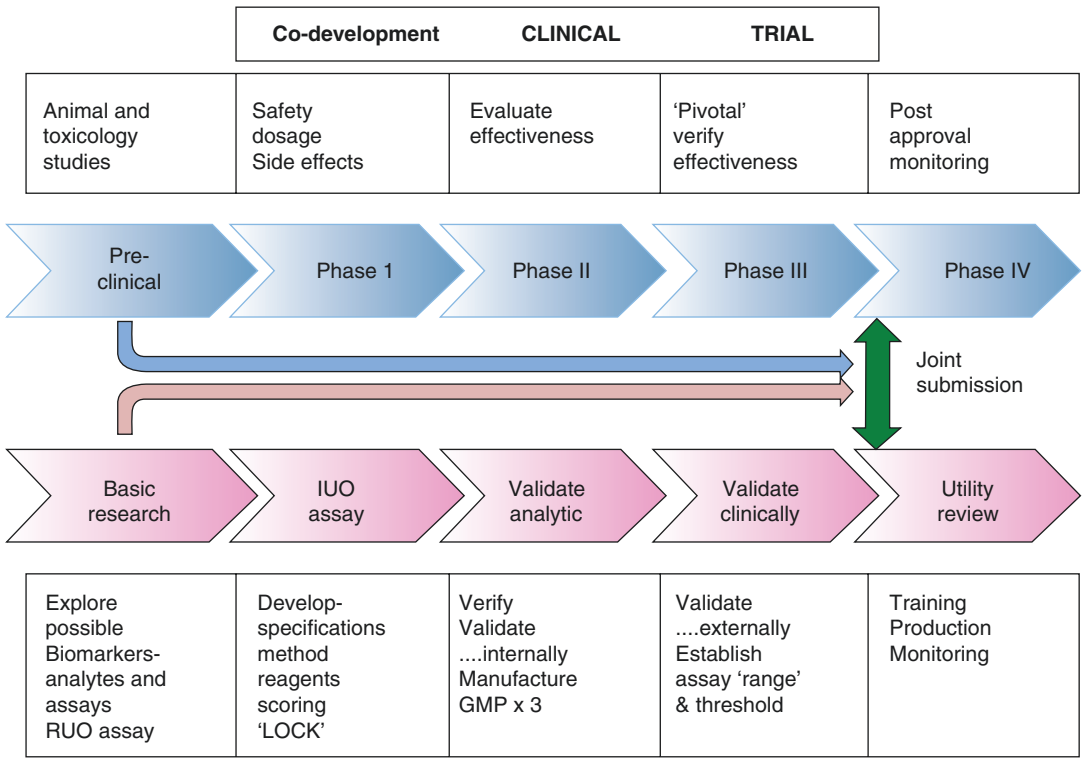

Fig. 1.1 Co-development process for "drug" and companion diagnostic. Time frame, up to 10 years; cost, up to 100 million dollars

Table 1.2 Laboratory reagents and tests; FDA categories

ASR	RUO	IUO	IVD	LDT
Analyte-specific reagent	Research use only	Investigational	In vitro device	Lab developed test
		Use only		
No diagnostic claims	No diagnostic claims	No diagnostic claims	Specified claims	Lab responsible for any claims[a]
			FDA approved	
FDA regulations	FDA regulations	FDA regulations	FDA regulations	CLIA[b] regulations
				FDA discretion
May be used as reagents for RUO, IUO, IVD, and LDT tests	Not for clinical use	Use restricted to specified study	Intended use define by trial	For use only in the lab that developed the test
			Specified in labeling	

https://www.cms.gov/Clia/

[a]LDT may require FDA approval if used as a predictive marker; clinical utility must be validated

[b]*CLIA* Clinical Laboratory Improvement Amendments

Table 1.3 The "total test" approach

Pre-analytical (Sample preparation)	Test selection: indication for the test
	Specimen handling, from operating room to histology laboratory
	Fixation: total fixation time and type of fixative
	Paraffin embedding, storage, and sectioning
	Deparaffinization
Analytical (Reagents and protocol)	Antigen retrieval (exact method)
	Assay (staining) method and protocol
	Reagent validation
	Controls (reference standards)
	Technologist and laboratory certification
	Proficiency testing and quality assurance
Post-analytical (Interpretations and reporting)	Reading of result(s)/scoring/quantification
	Diagnostic, prognostic, or predictive significance
	Report
	Turnaround time
	Outcomes analysis/economics/reimbursement Pre-analytical

Based on data from Taylor [16]

Predictive Biomarkers: Companion Versus Complementary

The distinction of companion versus complementary biomarkers (Table 1.1) emerged from conjoint clinical studies, determined by the level of prediction of clinical response that the test rendered.

With a companion diagnostic, a positive result indicates treatment with the companion drug; a negative result indicates no treatment; and the test is required before the use of the corresponding drug.

With a complementary diagnostic, a positive result usually indicates treatment, but a patient having a negative result may or may not be treated according to an informed clinical decision.

For example, with PD-L1 tests, some "tests" emerged as companion diagnostics, and others as

complementary, varying according to which anti-PD-L1 antibody was employed [8, 12, 13], by which method, and in which specified tumor type.

Intrinsic to the FDA definition of an approved IVD (in vitro diagnostic) companion diagnostic is that it "provides information that is essential for the safe and effective use of a corresponding therapeutic product" and that its use is "stipulated in the instructions for use in the labeling of both the diagnostic device and the corresponding therapeutic agent" (Table 1.2) [6–8]. The current EU definition is less rigorous, but similar in intent, and interestingly admits both "quantitative and qualitative determination of specific markers identifying subjects" [5, 8]. It specifically excludes monitoring.

The FDA definition carries with it an assignment of the IHC IVD to Class III (the highest level) requiring PMA (pre-market approval) in a co-development mode with the drug [4, 6–8, 12], whereas the EU regulations appear to leave companion diagnostics in the current general IVD category [5]; new regulations are afoot that likely will raise the level and may preclude the current self-certification route (for discussion of the subtleties of these definitions, see references 4 and 12 and later chapters in this book). The above statements apply specifically to companion diagnostics; there are as yet no corresponding written rules for complementary diagnostics; the definition of which is at present by precedent and usage, although proposals have been aired.

Method Development

These types of predictive biomarker tests have come to be of critical import in the context of targeted drug therapies, such that the majority of such agents now in clinical studies are following a co-development plan for "test" and "corresponding therapy." Detailed discussion of this co-development process is outside the scope of this chapter but is summarized in Fig. 1.1, examined in detail elsewhere in this book, and well-reviewed in a recent National Policy Workshop [4]. For drug development generally the process includes preclinical (animal) studies: phase 1, toxicity, in which potential biomarkers may also be assessed; phase 2, preliminary efficacy of drug, plus biomarker evaluation; phase 3, definitive efficacy and validation of biomarker; and phase 4, post market surveillance. Total patient accrual will be in the hundreds.

For the biomarker there is a preceding period of basic research and discovery that provides initial evidence of the potential utility of a molecule (biomarker) in the context of diagnosis or prognosis of cancer or a relationship to a potential therapeutic modality (drug – predictive) (Fig. 1.1). This discovery process is followed by evolution of a prototypic test using analyte-specific reagents (ASRs), through an investigational use only (IUO) test, on to an FDA-approved IVD (Table 1.2), which category includes all companion diagnostics. In some instances clinical laboratories may separately develop assays for clinical use, with internal validation under CLIA regulations (LDT, laboratory-developed test) (Table 1.2). The FDA has provided notice that it holds discretionary authority to regulate LDTs and has published guidelines, but not yet enforced them.

The total time span from bench discovery to approval and general clinical application is measured in years, and the total cost is counted in tens of millions of dollars, to be weighed by clinicians, and eventually by society at large, against the undoubted good sense of administering a targeted therapy only to those patients likely to benefit, and the avoidance of side effects and costs of inappropriate treatment of the remainder. This route to approval developed with reference to IHC tests, the most common method adopted for companion diagnostics to date; but other methods as they appear are constrained by similar rules.

As targeted therapies have proliferated, so of course have the corresponding biomarkers, and the methods applied for their detection