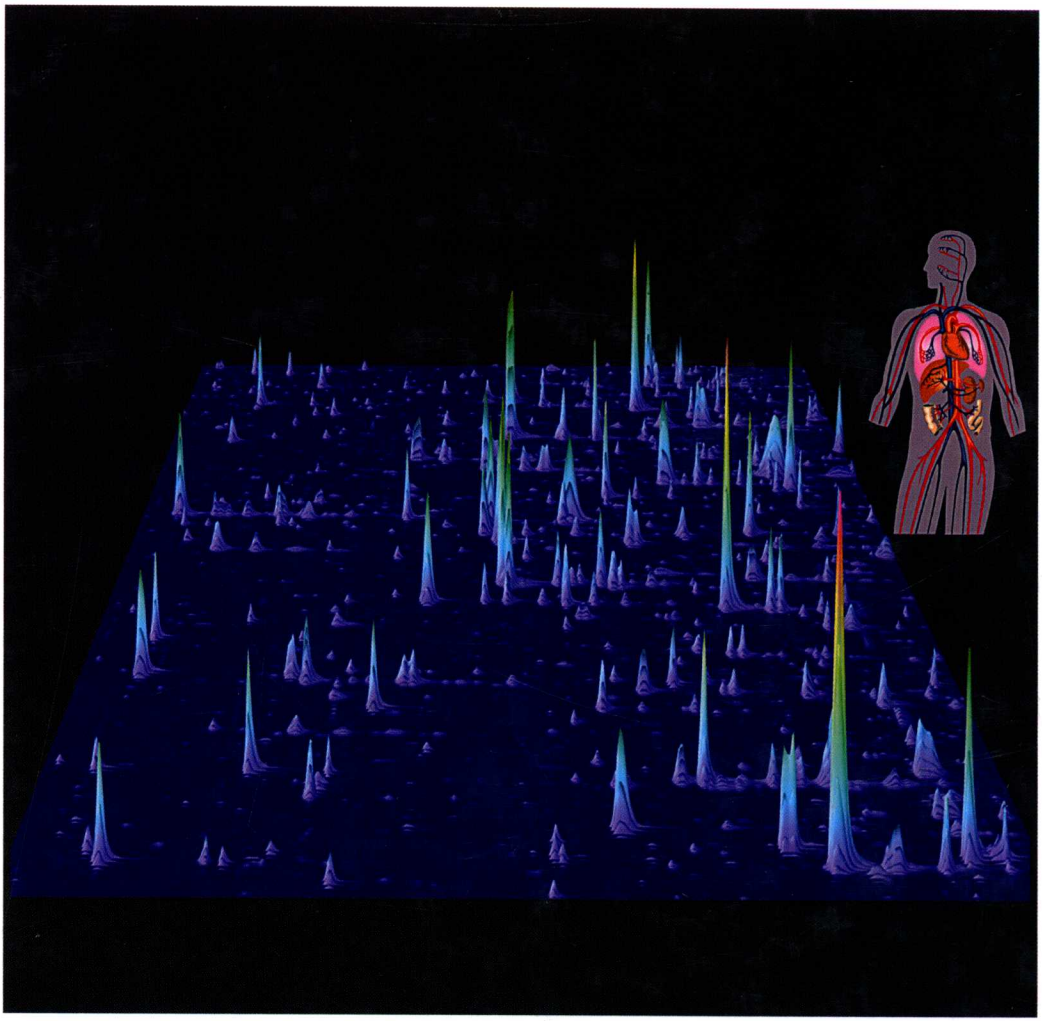
RSC Drug Discovery

Edited by Péter Horvatovich and Rainer Bischoff

Comprehensive Biomarker Discovery and Validation for Clinical Application



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Comprehensive Biomarker Discovery and Validation for Clinical Application

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Comprehensive Biomarker Discovery and Validation for Clinical Application

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PREFACE

Introduction to Biomarker Discovery and Validation

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In 2001, the biomarkers definitions working group of the National Institutes of Health in the United States defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention".[1] A large number of analytical techniques are available to discover and measure biomarkers in complex biological samples such as immunochemical assays, molecular imaging, molecular arrays and mass spectrometry. From these techniques, mass spectrometry excels to be the most versatile analytical instrument able to comprehensively determine the identity and absolute or relative quantity of biomolecules present in complex biological samples. Mass spectrometry, a fairly recent approach to discover and validate biomarkers, requires close collaboration between multiple disciplines such as biology, pathology, analytical chemistry and bioinformatics. This approach is currently gaining momentum in the clinical environment. Different types of biomarkers are needed to support early diagnosis of disease onset, to predict the outcome of therapy, to monitor the efficacy of treatment or to get an objective clinical measure of drug efficiency based on molecular signatures.

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To perform successful biomarker research ending with validated assays that are suitable for clinical application requires collaboration between academic centers and industry with considerable investments of time and money. More importantly it requires meticulous research based on an experimental design, well-characterized biospecimen collections, targeted and comprehensive profiling analytical platforms, and the processing and statistical analysis of enormous sets of data as well as determined leadership to arrive at go/no go decisions throughout the process from discovery to final implementation. Last but not least, biomarker-based assays must provide sufficient benefit for patients and the population at large to be eligible for reimbursement by health-care providers.

This book presents some of the state-of-the-art methodology used by leading academic and industrial research groups worldwide to advance biomarker research from planning sample collection and storage in biobanks, to the initial comprehensive discovery study all the way to the requirements for approval of biomarker assays by regulatory authorities. It provides a comprehensive view of mass-spectrometry-based biomarker discovery in 12 chapters covering all parts of this process.

Introductory Chapters

Chapter 1 covers strategic and practical aspects related to biomarkers for translational and personalized medicine related to pharmaceutical drug development.

Chapter 2 shows the complex and laborious process to take a biomarker product from discovery to commercialization. This chapter will familiarize the reader with regulatory issues and give a general understanding of quality system requirements. The chapter gives an overview over the current regulatory environment at the US Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) for approving a biomarker test for clinical use.

Chapter 3 describes approaches used to design experiments for clinical biomarker discovery, verification and validation, discussing protocols and good practices for sample collection, storage, and quality control. It presents the cardinal role of biobanks and human biospecimen collections in biomarker discovery and validation, discusses the crucial points of patient and control selection to design longitudinal and cross-sectional studies. Finally, this chapter gives guidelines on how to select patient cohorts and to stratify patient populations.

Sample Preparation and Profiling

Chapter 4 deals with approaches of biomarker discovery in easily accessible body fluids such as serum, urine, epithelial lining fluid and cerebrospinal fluid, discussing sample-preparation methods and problems related to sample stability. Sample prefractionation methods that try to cope with the large

dynamic concentration range of proteins in body fluids are described in this chapter.

Chapter 5 describes various profiling platforms that are based on liquid chromatography coupled to mass spectrometry (LC-MS) for proteomic biomarker discovery, verification and validation. Platforms used for biomarker discovery provide qualitative (identity of proteins) and quantitative information about the analyzed proteomics samples. A discussion of targeted proteomics platforms as applied in the postdiscovery verification and validation phases is part of this chapter. Targeted protein analysis by LC-MS/MS allows quantification of peptides directly in clinical samples and contributes to verification and validation of biomarker candidates from biological samples.

Chapter 6 deals with the use of affinity array-based technologies in biomarker discovery and validation. The chapter demonstrates the strength of this technology by the use of peptide arrays to profile kinase activity to characterize phosphorylation-mediated signal transduction. Several applications of peptide arrays for kinome analysis are discussed including specific examples in which arrays have contributed to the identification of disease-associated processes/biomarkers, provided therapeutic targets, and offered novel insight into complex biological processes.

Bioinformatics and Statistics

Chapter 7 describes the structure of data preprocessing pipelines used to extract quantitative information from label-free LC-MS data. The chapter provides an overview of the main steps based on the example of a "Threshold Avoiding Proteomics Pipeline". This chapter further presents data visualization techniques and quality-control methods to assess the accuracy of automated label-free LC-MS data processing.

Chapter 8 describes important aspects of statistical analysis of the extremely complex biomarker discovery data leading to a selected set of highly discriminating peptides and proteins that can be followed-up in a subsequent validation phase. The main concepts of statistical validation to identify potential biomarker candidates are discussed. The presented statistical methods and concepts are described without the use of intricate mathematics, in a way that scientists involved in biomarker research with a general statistical or informatics background can follow. Techniques to avoid false positives are discussed and practical advice is given, including recognition of situations where no reliable biomarker candidate can be identified.

Chapter 9 describes a novel bioinformatics approaches to place biomarker candidates in biological context by exploring the molecular interaction networks and pathways that help in understanding the biological relevance of the discovered proteins. This chapter provides a detailed overview of bioinformatics and statistical approaches based on a case study related to plasma protein markers for breast cancer.

Discovery and Validation Case Studies, Recommendations

Chapter 10 presents a comprehensive study to discover, verify and validate urinary biomarkers for the early detection of bladder cancer. The chapter demonstrates the integration of a discovery study using stable isotope labeling on low numbers of pooled samples followed by the validation of a limited number of candidates with Multiple Reaction Monitoring (MRM) and ELISA approaches using a large set of individual samples. This chapter gives an example of the development of a biomarker panel rather than an individual biomarker.

Chapter 11 demonstrates the power of current mass-spectrometry-based biomarker discovery and validation approaches on the example of developing a biomarker panel to improve the prediction of near-term myocardial infarction. This case shows the power of a pooling strategy in combination with stable isotope labeling and 2-dimensional liquid chromatography coupled to mass spectrometry for biomarker discovery followed by validation with multiplexed immunoassays and MRM.

The last chapter, *Chapter 12*, provides a comprehensive overview of the bottlenecks and challenges of current protein biomarker research and provides guidance and recommendations to avoid common pitfalls. The challenges faced during protein biomarker development are multifactorial from the initial phase of sample collection and study design to final clinical validation comprising financial aspects and regulatory approval. This chapter helps researchers to recognize these potential challenges and help them to plan their study in a way that produces reliable results that can be critically interpreted.

Biomarker research underwent enormous technological and methodological developments during the last decade, a trend that continues today. Biomarker research covers many technological platforms and aspects that are not possible to include in one book. For example, this book does not include discussions on the bioinformatics of mass-spectrometry-based protein identification, analytical methods revealing protein isoforms or the tissue and cellular localization of proteins with optical- or mass-spectrometry-based imaging techniques. This book will aid practitioners ranging from specialists working at biobanks or at regulatory authorities, statisticians, bioinformaticians or researchers working in academic or industrial environments on biomarker discovery and validation projects.

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