PREDICTIVE APPROACHES IN DRUG DISCOVERY AND DEVELOPMENT
Wiley Series on Technologies for the Pharmaceutical Industry

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PREDICTIVE APPROACHES IN DRUG DISCOVERY AND DEVELOPMENT

Biomarkers and In Vitro/In Vivo Correlations

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To Becky and Mary Ann, without whose total support we would never have started and could not have finished this effort. While we like to think we know what we’re getting into, their continuing faith and support of our decisions are amazing.

J. Andrew Williams
David D. Christ

“Essentially all models are wrong, but some are useful”

George E.P. Box

“For nothing ought to be posited without a reason given, unless it is self evident, or known by experience...”

William of Ockham
(known best as Occam’s Razor)
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PREFACE

In the race to discover approvable new drugs faster and with fewer resources, two key elements have emerged that can enhance the drug pipeline and accelerate development, namely, biomarkers and in vitro/in vivo correlations (IVIVCs). At the early stage of the race, identifying the concepts and practices that link in vitro data with projected in vivo performance can lead to the identification of more robust clinical candidates and the more intelligent selection of new leads. Recognizing the limitations of IVIVCs and using IVIVC appropriately are critical to new drug discovery. As clinical trials are conceived, the identification of easily measured, rugged, and reliable markers of disease and the effects drugs have on disease are critical in defining appropriate patients and demonstrating efficacy as early as possible. Biomarkers, defined as an objectively measured indicator of physiological or pathophysiological function, or an indicator of pharmacological response, are important elements in translating basic pharmacology and drug effects into clinical utility and regulatory acceptance. Because of their power, understanding and applying biomarkers is really an expectation for the new drug development paradigm. This book provides a critical compilation of the most important aspects of these two topics from an international perspective.

Everyone involved in the process of new drug discovery, development and regulation should find the concepts and examples described herein useful, both for evaluating the merits of starting programs with these tools and for making decisions based on data from these approaches. Expertise in these two areas is no longer just the province of the pharmaceutical industry and regulatory agencies, but as more academic and government programs become involved in “drug discovery,” more scientists, regardless of location, will need familiarity with these topics. The chapters in this book were written so that all scientific interests could find value; everyone from the technical staff to senior management.
Many of the concepts and strategies behind developing and applying biomarkers and IVIVC are complementary, and much of this book’s value is contained in these reinforcing themes.

The expert authors responsible for each chapter come from a wide background in the pharmaceutical industry, worldwide regulatory agencies, and academia. While each chapter contains a core of basic information, the chapters also contain each author’s perspective and opinion. We hope you will find this important aspect of the book most valuable since it provides the context for much of the science in these rapidly evolving areas.
ACKNOWLEDGMENTS

We are indebted to the chapter authors for their commitment, perseverance, and patience. All are excellent scientists, experts in their field, with overbooked calendars, and we sincerely appreciate the time they dedicated to their chapters. They provided great material to us, and if anything is not clear, we will take editorial responsibility. Thank you.

We would also like to acknowledge the patient guidance and unwavering support of Dr. Sean Ekins, Series Editor for the Wiley Series on Technology for the Pharmaceutical Industry. Sean is a friend and colleague, and his experience and advice throughout our editing efforts have been sustenance. On many levels, this book could not have been completed without Sean.

Jonathan Rose, Amanda Amanullah, and the staff at Wiley have been terrific. We appreciate their expertise, and patience, and the final volume is a product of their support.

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PART ONE

BIOMARKERS IN DRUG DISCOVERY
1

THE IMPORTANCE OF BIOMARKERS IN TRANSLATIONAL MEDICINE

JOSEPH C. FLEISHAKER

1.1 INTRODUCTION

The new millennium was to have ushered in a bright new era of drug discovery. The unraveling of the human genome would provide a host of new therapeutic gene targets to treat debilitating diseases (1). The rest of the “omics” (proteomics, metabonomics, and transcriptomics) would provide additional insights on these targets and methods to assess drug effects early in the development process (2, 3). New therapeutic modalities (sRNAi, therapeutic proteins, and vaccines) would allow us to treat diseases, such as Alzheimer’s disease, that up until now have eluded our best efforts. This was an engaging vision of the future.

What the new millennium has brought so far is steadily decreasing R&D productivity in the pharmaceutical industry. In 2007, only 16 new chemical entities were approved, compared to the 27 approved in 2000 by the U.S. Food and Drug Administration. The success rate for drugs in phase II proof of concept (POC) testing is at 20% or less (4). At the same time, the cost of bringing a new medicine to the market is approaching US$1.7 billion (5). There have also been several high profile withdrawals of products from the market for safety concerns, most notably rofecoxib (VIOXX® Tablets). This is hardly the vision conjured by mapping the human genome.

The key to addressing these issues and realizing the bright future for drug development is to assess, as early as possible, the properties (good and bad) of a potential target for intervention in a disease process and therapeutic modalities against that target. On the basis of these data, one must make a decision...
whether to devote resources (private or public) to the development of that particular agent. The challenge is to do this with limited resources and with less than a 100% certain answer. By making early decisions on compounds and targets, we can then assess more targets/treatments for potential benefit and devote our limited resources to those that show the most promise. Traditional drug development paradigms have relied on large and prolonged studies to make go/no go decisions on new therapeutics. For example, a definitive answer on the utility of a disease-modifying agent for rheumatoid arthritis requires the assessment of the progression of joint narrowing and erosion by radiography (6). For Alzheimer’s disease, long-term studies are necessary to establish a disease-modifying effect (7). How then do we get an answer within 3 months (or less) in 100 patients (or less) that an investigational treatment for these treatments is likely to be of therapeutic benefit and warrant the resources necessary for continued development?

Translational medicine has been proposed as the answer to the above question, and biomarkers are critical to the successful translation of findings in pharmacological studies in animals to therapeutic benefit in humans. The purpose of this chapter is to examine the integral role that biomarkers play in translational medicine and the development of new medicines. We examine successful applications of biomarkers to speed drug development and discuss examples where the lack of biomarkers has led to repeated failure in drug development. Finally, we discuss some future directions in biomarker research that can enhance drug development.

1.2 TRANSLATIONAL MEDICINE AND BIOMARKERS—SOME USEFUL DEFINITIONS

In any discussion on biomarkers, it is important that it is clear exactly what is being discussed. For example, the question, “Is your company working on biomarkers?” can be difficult to answer. Is the questioner referring to biomarkers for use in translational medicine and early decision making during drug development? Or rather, does the question really relate to a company’s development of diagnostic tests to use when a drug is approved? Thus, the various definitions of translational medicine and biomarkers should be clearly understood in order to promote advancement in these areas.

Littman et al. (8) state that “The question of how to define translational research remains unresolved and controversial.” They also provide a table (Box 1.1) that describes the areas that define translational research. The FDA Critical Path Initiative (9) describes translational research as being concerned with “moving basic discoveries from concept to clinical evaluation.” The interesting part of this definition is that it is unidirectional from test tube to animal to human. Equally important is the back translation of clinical observations that may elucidate important insights into human disease, which drive further basic research aimed at new therapies (10).
Box 1.1 GOALS AND AREAS DEFINING TRANSLATION RESEARCH

Goals

The establishment of guidelines for drug development or for the identification and validation of clinically valid biomarkers.
Experimental nonhuman and nonclinical studies conducted with the intent of developing principles for discovery of new therapeutic strategies.
Clinical investigations that provide the biological foundation for the development of improved therapies.
Any clinical trial initiated with the above goals.
Basic science studies that define the biological effects of therapeutics in humans.


The NIH Biomarkers Definition Working Group (11) defined a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.” This is a relatively broad definition of a biomarker, which would include widely disparate methodologies such as FDG-PET, cognitive test batteries, gene expression, protein expression, and biochemical measures under the realm of biomarkers. The same group identified several uses for biomarkers, including diagnosis of disease, a tool for staging disease, and indicator of disease prognosis, or for prediction and monitoring of a clinical response to treatment. Translating these uses to drug development, biomarkers can be used to select which patients should be treated or to monitor beneficial and harmful effects of a medication. Implicit (but often forgotten) in the use of biomarkers in drug development is that they should be decision making; data obtained should affect either the conduct of a protocol or a development program.

In any discussion of biomarkers, one must differentiate between biomarkers and surrogate markers. The NIH group also defined a subset of biomarkers, the surrogate endpoint, as “a biomarker that is intended to substitute for a clinical endpoint. A surrogate endpoint is expected to predict clinical benefit (or harm or lack of benefit or harm) based on epidemiologic, therapeutic, pathophysiologic or other scientific evidence. In this sense, substitute is generally considered to mean substitute in a regulatory sense for a clinical endpoint.” Classical surrogate endpoints are arterial blood pressure reduction as a surrogate for reduced stroke and cardiovascular mortality, LDL-cholesterol reduction for reduced cardiovascular mortality, and prolonged QT interval as a reflection of risk of sudden cardiac
death due to torsades de pointes. In this chapter, we deal with biomarkers in the broadest sense of their use, and do not focus on the development of biomarkers as potential surrogate endpoints.

In developing and using biomarkers, one can use various classification systems. One is related to the type of information that the biomarker provides. According to this, a biomarker can be classified as a target, mechanism, or outcome biomarker (12). A target biomarker measures the interaction of a drug with a target receptor. A common example is measuring the binding of an atypical antipsychotic drug to D2 receptors in the brain using positron emission tomography of a 11C-labeled ligand. A mechanism biomarker measures a physiological, biochemical, genomic, or behavioral change that occurs downstream from the target. Examples would be glucose lowering for a diabetes drug, decreased target phosphorylation after a kinase inhibitor, or sedation after the administration of a benzodiazepine. Outcome biomarkers are those that relate to the efficacy/toxicity of a compound, such as viral load as a function of survival benefit for anti-HIV therapy.

One can also consider the linkage between biomarker effects and clinical outcome (13). For example, mydriasis may be an excellent indication of the activity of a norepinephrine reuptake inhibitor (mechanistic biomarker), but it is not necessarily an indicator of potential efficacy in depression (14). On the other hand, occupancy at the D2 receptor, as measured by PET for an antipsychotic (target biomarker), is very closely related to efficacy for this class of compound (15). Thus, the linkage with outcome, as well as the type of biomarker, should be considered when assessing the ultimate utility of a biomarker.

The terms validation and qualification in relation to biomarker development also cause confusion. Wagner (16) defines validation as “The fit-for-purpose process of assessing the assay and its measurement performance characteristics, determining the range of conditions under which the assay will give reproducible and accurate data.” Qualification is defined by Wagner as “The fit-for-purpose evidentiary process linking a biomarker with biological processes and clinical endpoints.” The key phrase in both definitions is “fit-for-purpose.” The rigor around validation and qualification should be dependent on the use of a biomarker and the decision that it will drive. The rigor around the validation and qualification of a biomarker used to assess whether a compound continues in development may be much less than that for a biomarker used to determine whether a particular patient should be treated with a particular compound. Fit-for-purpose thus means that the assay and its relevance to therapy are sufficient to drive the decision for which they are being developed.

1.3 BIOMARKERS: THE ROSETTA STONE OF TRANSLATIONAL MEDICINE

The term translational medicine suggests that we are translating “something” in animals to “something” in humans. During drug development, this would be
translation of activity in an animal model of disease to activity in the human
disease with great fidelity. Unfortunately, this is not a common occurrence in
drug development. Perel et al. (17) systematically reviewed the concordance
between animal and human data for six disease areas. Table 1.1 describes
the areas reviewed, the number of animal studies reviewed, and the methodologi-
cal aspects of these studies. Three of the interventions showed concordance in
outcomes between animal and human studies (thrombolysis for acute ischemic
stroke, bisphosphonates for osteoporosis, and antenatal corticosteroids), and three did
not. In the case of antifibrinolytics for hemorrhage, animal models yielded no reli-
able data, while clinical trials showed clear benefit. In general, the study designs
of the animal studies were poor, generally lacking in randomized treatment allo-
cation or blinding of the allocation or the assessor. Thus, there is substantial
room for positive bias in the assessment of the results in these animal studies.
There was, however, no correlation between the quality of the experiments and
the concordance between animal and human studies.

Irrespective of methodological considerations, there are often differences
between human disease and disease models in animals (18). If one considers
acute ischemic stroke, many drugs have been studied in animals and humans,
and only one, tissue plasminogen-activating factor, has been found to be
efficacious and is in clinical use. In the neurological trauma arena, many animal
studies are conducted in healthy animals, free of the comorbidities (diabetes,
high blood pressure, etc.) that would be present in an elderly patient with an
acute ischemic stroke. In addition, genetic homogeneity with a rat strain does
not reflect the genetic heterogeneity in the human patient population. Outcome
measures in rodents (infarct size) do not reflect relevant outcome measures in
humans (functional disability). Animal models, where therapeutic interventions

<table>
<thead>
<tr>
<th>Interventions</th>
<th>Random Allocation to Group</th>
<th>Adequate Allocation Concealment</th>
<th>Blinded Assessment of Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroids for traumatic head injury</td>
<td>2 (12)</td>
<td>3 (18)</td>
<td>3 (18)</td>
</tr>
<tr>
<td>(n = 17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antifibrinolytic agents (n = 8)</td>
<td>3 (38)</td>
<td>0</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Thrombolysis for acute ischemic stroke</td>
<td>43 (38)</td>
<td>23 (20)</td>
<td>24 (21)</td>
</tr>
<tr>
<td>(n = 113)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tirilazad for acute ischemic stroke</td>
<td>12 (67)</td>
<td>1 (8)</td>
<td>13 (72)</td>
</tr>
<tr>
<td>(n = 18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antenatal corticosteroids (n = 56)</td>
<td>14 (25)</td>
<td>0</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Bisphosphonates (n = 16)</td>
<td>5 (31)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are number of studies (percentages of total).
Source: Adapted with permission from BMJ Publishing Group Ltd. Comparison of treatment effects
between animal experiments and clinical trials: systematic review. Perel P, Roberts I, Sena E et al.
BMJ, Volume 334, p 197, Copyright 2007 (17).
may be administered before or shortly after a neurological insult, may not be reflective of therapy in humans that will not begin for hours after neurological insult. While these examples are specific to the area of stroke and neurotrauma, similar results are seen across multiple therapeutic areas.

Disease models in animals themselves rarely reflect the human disease in total. Disease models generally reflect some aspect of the human disease. For example, some depression models in animals reflect the learned helplessness typical of human depression, while other models address the cognitive deficits seen in human depression (19). Separate transgenic mouse models in Alzheimer’s disease have been developed to address abnormalities in β-amyloid protein, tau-protein, and pre-senilin (20), which are commonly found together in the human disease. In many cases then, animal models are set up to reflect certain pathways in human disease, rather than the disease per se. Both disease models and pharmacology models in animals may be used in translation to humans, as shown in Figure 1.1, but both pathways require biomarkers for successful translation (21).

![Diagram of linked animal models and clinical biomarkers](Diagram.png)

**FIGURE 1.1** Linked animal models and clinical biomarkers can be used to confirm translation of preclinical efficacy and pharmacology to clinical effects. Clinical measures are used to set dose range and optimize the design of outpatient studies. *Source:* Reprinted from *Drug Discovery Today*, Volume 12, Sultana SR, Roblin D, O’Connell D, pp. 419–425, Copyright 2007, with permission from Elsevier.
The development of translatable biomarkers is still an evolving field, but there are several examples available. Cardiac troponin has been shown to be indicative of cardiac injury in both animals and humans, so that there is high confidence that the increases in cardiac troponin in animals seen in preclinical drug testing would also be seen in humans (22). As such it is a valuable screening tool. Imaging techniques such as PET for receptor occupancy or fMRI have been useful in the development of antischizophrenic compounds (21). Other soluble biomarkers, such as cyclic GMP, can reflect the pharmacology of agents such as the neuroendopeptidase inhibitor and PDE-5 inhibitors in both animals and humans (21).

Target and mechanism biomarkers that would be translatable from animals to humans are absolutely essential to answer key questions during the early development process. These questions are as follows:

1. Does the drug hit the intended target in humans?
2. Does the drug exhibit the intended pharmacology in humans?
3. What is the relationship between pharmacokinetics and pharmacodynamics in humans?
4. What doses/drug concentrations are appropriate for initial studies in patients to more fully explore the efficacy of the compound? Can these be achieved within the tolerable dose range for the compound in humans?

For novel compounds, positive answers to all of these questions are needed to assure that we adequately test the hypothesis that modulating the target mechanism in humans has beneficial effects on a disease process. While this conclusion is intuitive, large scale development programs have been conducted in the absence of this information.

### 1.4 DRUG DEVELOPMENT WITHOUT BIOMARKERS—AN EMPTY EXPERIENCE

Tirilazad mesylate (Tirilazad, Fig. 1.2) is a 21-aminosteroid compound that was developed as a free radical scavenger and antioxidant for the treatment of acute neurological trauma (23). Tirilazad was studied in the treatment of head injury, ischemic stroke, spinal cord injury, and aneurismal subarachnoid hemorrhage and was approved in several countries for the treatment of subarachnoid hemorrhage.

Tirilazad was designed to prevent lipid peroxidation following the generation of free radicals due to the initial tissue damage following a neurological insult. A variety of treatment paradigms in preclinical models were utilized for tirilazad, ranging from single-dose administration following head trauma in mice to administration for 6 days in a canine model of subarachnoid hemorrhage (23). These paradigms were designed to cover the time of penumbral neurological damage that could occur after the initial insult. All of these studies had several characteristics in common. Neurological outcome measures (motor scores,
evoked potentials) or local morphologic/biochemical measures (infarct size, middle cerebral artery vasospasm, lipid peroxide levels, etc.) were the key endpoints for these studies. With the exception of attempts to evaluate the sparing of antioxidant vitamins (vitamins C and E) peripherally by tirilazad (24), neither circulating biomarkers nor circulating or brain levels of tirilazad were measured as part of these studies. Dosing was based on body weight (mg/kg), and exposure was not compared across animal species.

On the basis of the data available in animals and humans, how would we answer the questions outlined in the previous section?

1. Does the drug hit the intended target in humans? We do not know. No assessments of brain uptake of tirilazad were performed in humans.
2. Does the drug exhibit the intended pharmacology in humans? We do not know. Tirilazad elicited no overt pharmacology in early clinical trials.
3. What is the relationship between pharmacokinetics and pharmacodynamics in humans? We do not know. No biomarkers were available to measure tirilazad activity, and there was no correlation between tirilazad dose or exposure and efficacy in humans.
4. What doses/target drug concentrations are appropriate for initial studies in patients to more fully explore the efficacy of the compound? Can these be achieved within the tolerable dose range for the compound in humans? We do not know. The only extrapolation that could be made between animals and humans was based on dose/body weight, not exposure.

Studies of tirilazad in the treatment of head trauma, ischemic stroke, and spinal cord injury failed to show efficacy, and some studies showed worsening of outcome relative to placebo (25–28). Initial studies of tirilazad for the treatment