CONTRIBUTORS

Dmitri Artemov, JHU ICMIC Program, Department of Radiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland, Novel Imaging Agents for Molecular MR Imaging of Cancer

Kadir Aslan, Institute of Fluorescence, University of Maryland Biotechnology Institute, Baltimore, Maryland, Metal-Enhanced Fluorescence: Application to High-Throughput Screening and Drug Discovery

Jürgen Bajorath, Department of Life Science Informatics, B-IT, International Center for Information Technology, Rheinische Friedrich-Wilhelms-University Bonn, Bonn, Germany, Molecular Similarity Methods and QSAR Models as Tools for Virtual Screening


Richard D. Beger, Division of Systems Toxicology, National Center for Toxicological Research, Food and Drug Administration, Jefferson, Arkansas, Combining NMR Spectral Information with Associated Structural Features to Form Computationally Nonintensive, Rugged, and Objective Models of Biological Activity

Oren E. Beske, Vitra Bioscience, Inc., Mountain View, California, Simultaneous Screening of Multiple Cell Lines Using the CellCard System
Zaver M. Bhujwala, JHU ICMIC Program, Department of Radiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland, Novel Imaging Agents for Molecular MR Imaging of Cancer

Sean M. Biggs, Department of Pathology, Department of Cell Biology and Physiology, and Cancer Research and Treatment Center, University of New Mexico School of Medicine, University of New Mexico Health Sciences Center, Albuquerque, New Mexico, High-Throughput Flow Cytometry

George E. Billman, Department of Physiology and Cell Biology, The Ohio State University, Columbus, Ohio, Cardiac Sarcolemmal ATP-Sensitive Potassium Channel Antagonists: Novel Ischemia-Selective Antiarrhythmic Agents

Cristian Bologa, Department of Biochemistry and Molecular Biology and Office of Biocomputing, University of New Mexico School of Medicine, University of New Mexico Health Sciences Center, Albuquerque, New Mexico, High-Throughput Flow Cytometry

Hans Bräuner-Osborne, Department of Medicinal Chemistry, Danish University of Pharmaceutical Sciences, Copenhagen, Denmark, GABA and Glutamate Receptor Ligands and Their Therapeutic Potential in CNS Disorders

Andrej Bugrim, GeneGo, St. Joseph, Michigan, Systems Biology: Applications in Drug Discovery

Dan A. Buzatu, Division of Systems Toxicology, National Center for Toxicological Research, Food and Drug Administration, Jefferson, Arkansas, Combining NMR Spectral Information with Associated Structural Features to Form Computationally Nonintensive, Rugged, and Objective Models of Biological Activity


Zehui Cao, Department of Chemistry, University of Florida, Gainesville, Florida, Cancer Cell Proteomics Using Molecular Aptamers

Hui Chen, Department of Chemistry, University of Florida, Gainesville, Florida, Cancer Cell Proteomics Using Molecular Aptamers
Amy K. Chesterfield, Discovery-Neurosciences Research, Eli Lilly and Company, Indianapolis, Indiana, Methods for the Design and Analysis of Replicate-Experiment Studies to Establish Assay Reproducibility and the Equivalence of Two Potency Assays

C.H. Cho, Department of Pharmacology, Faculty of Medicine, The University of Hong Kong, Hong Kong, China, Herbal Medicines and Animal Models of Gastrointestinal Diseases

Michael J. Corey, Chromos, Seattle, Washington, Coupled Luminescent Methods in Drug Discovery: 3-Min Assays for Cytotoxicity and Phosphatase Activity

Xizhong Cui, Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, Maryland, Factors Influencing the Efficacy of Mediator-Specific Anti-Inflammatory, Glucocorticoid, and Anticoagulant Therapies for Sepsis

Katherine J. Deans, Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, Maryland; Department of Chemistry, Massachusetts General Hospital, Boston, Massachusetts, Factors Influencing the Efficacy of Mediator-Specific Anti-Inflammatory, Glucocorticoid, and Anticoagulant Therapies for Sepsis

Erik de Clercq, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium, Strategies in the Design of Antiviral Drugs

Brian J. Eastwood, Statistics and Information Sciences, Eli Lilly and Company, Indianapolis, Indiana, Methods for the Design and Analysis of Replicate-Experiment Studies to Establish Assay Reproducibility and the Equivalence of Two Potency Assays

Bruce S. Edwards, Department of Pathology and Cancer Research and Treatment Center, University of New Mexico School of Medicine, University of New Mexico Health Sciences Center, Albuquerque, New Mexico, High-Throughput Flow Cytometry

Peter Q. Eichacker, Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, Maryland, Factors Influencing the Efficacy of Mediator-Specific Anti-Inflammatory, Glucocorticoid, and Anticoagulant Therapies for Sepsis

Sean Ekins, GeneGo, St. Joseph, Michigan, Systems Biology: Applications in Drug Discovery
Christian C. Felder, Discovery-Neurosciences Research, Eli Lilly and Company, Indianapolis, Indiana, Methods for the Design and Analysis of Replicate-Experiment Studies to Establish Assay Reproducibility and the Equivalence of Two Potency Assays

Michael D. Feese, deCODE biostructures, Bainbridge Island, Washington, Protein X-Ray Crystallography in Drug Discovery

Bente Frølund, Department of Medicinal Chemistry, Danish University of Pharmaceutical Sciences, Copenhagen, Denmark, GABA and Glutamate Receptor Ligands and Their Therapeutic Potential in CNS Disorders

Brian L. Furman, Department of Physiology and Pharmacology, University of Strathclyde, Strathclyde Institute of Biomedical Sciences, Glasgow, Scotland, Endocrine and Metabolic Agents

Chris D. Geddes, Institute of Fluorescence, University of Maryland Biotechnology Institute, Baltimore, Maryland; Center for Fluorescence Spectroscopy, University of Maryland School of Medicine, Baltimore, Maryland, Metal-Enhanced Fluorescence: Application to High-Throughput Screening and Drug Discovery

Jeremy R. Greenwood, Department of Medicinal Chemistry, Danish University of Pharmaceutical Sciences, Copenhagen, Denmark, GABA and Glutamate Receptor Ligands and Their Therapeutic Potential in CNS Disorders

Ignacy Gryczynski, Center for Fluorescence Spectroscopy, University of Maryland School of Medicine, Baltimore, Maryland, Metal-Enhanced Fluorescence: Application to High-Throughput Screening and Drug Discovery

Michael Haley, Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, Maryland, Factors Influencing the Efficacy of Mediator-Specific Anti-Inflammatory, Glucocorticoid, and Anticoagulant Therapies for Sepsis

Piet Herdewijn, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium, Strategies in the Design of Antiviral Drugs

John G. Houston, Applied Biotechnology Division, Bristol Myers Squibb Co., Pharmaceutical Research Institute, Wallingford, Connecticut, High-Throughput Screening: Evolution of Technology and Methods
Tomi Järvinen, Department of Pharmaceutical Chemistry, University of Kuopio, Kuopio, Finland, *Design and Pharmaceutical Applications of Prodrugs*

Tommy N. Johansen, Department of Medicinal Chemistry, Danish University of Pharmaceutical Sciences, Copenhagen, Denmark, *GABA and Glutamate Receptor Ligands and Their Therapeutic Potential in CNS Disorders*

Hidong Kim, deCODE biostructures, Bainbridge Island, Washington, *Protein X-Ray Crystallography in Drug Discovery*

Robert J. Kinders, *Coupled Luminescent Methods in Drug Discovery: 3-Min Assays for Cytotoxicity and Phosphatase Activity*

J.K.S. Ko, School of Chinese Medicine, The Hong Kong Baptist University, Hong Kong, China, *Herbal Medicines and Animal Models of Gastrointestinal Diseases*

Povl Krogsgaard-Larsen, Department of Medicinal Chemistry, Danish University of Pharmaceutical Sciences, Copenhagen, Denmark, *GABA and Glutamate Receptor Ligands and Their Therapeutic Potential in CNS Disorders*

Duane B. Lakings, Drug Safety Evaluation Consulting, Inc., Elgin, Texas, *Biological and Chemistry Assays Available During Drug Discovery and Developability Assessment*

Joseph R. Lakowicz, Center for Fluorescence Spectroscopy, University of Maryland School of Medicine, Baltimore, Maryland, *Metal-Enhanced Fluorescence: Application to High-Throughput Screening and Drug Discovery*

Ying Li, Department of Chemistry, University of Florida, Gainesville, Florida, *Cancer Cell Proteomics Using Molecular Aptamers*

Tommy Liljefors, Department of Medicinal Chemistry, Danish University of Pharmaceutical Sciences, Copenhagen, Denmark, *GABA and Glutamate Receptor Ligands and Their Therapeutic Potential in CNS Disorders*

Thorsteinn Loftsson, Faculty of Pharmacy, University of Iceland, Reykjavik, Iceland, *Design and Pharmaceutical Applications of Prodrugs*

Arianna Loregian, Department of Histology, Microbiology, and Medical Biotechnologies, University of Padova, Padova, Italy, *Strategies and Methods in Monitoring and Targeting Protein–Protein Interactions*
CONTRIBUTORS

Ulf Madsen, Department of Medicinal Chemistry, Danish University of Pharmaceutical Sciences, Copenhagen, Denmark, *GABA and Glutamate Receptor Ligands and Their Therapeutic Potential in CNS Disorders*

Joanna Malicka, Center for Fluorescence Spectroscopy, University of Maryland School of Medicine, Baltimore, Maryland, *Metal-Enhanced Fluorescence: Application to High-Throughput Screening and Drug Discovery*

Prabodhika Mallikratchy, Department of Chemistry, University of Florida, Gainesville, Florida, *Cancer Cell Proteomics Using Molecular Aptamers*

Mar Masson, Faculty of Pharmacy, University of Iceland, Reykjavik, Iceland, *Design and Pharmaceutical Applications of Prodrugs*

Brian R. McNaughton, Department of Chemistry, University of Rochester, Rochester, New York, *Combinatorial Chemistry in the Drug Discovery Process*

Benjamin L. Miller, Department of Biochemistry and Biophysics, Department of Dermatology, University of Rochester, Rochester, New York, *Combinatorial Chemistry in the Drug Discovery Process*

Peter C. Minneci, Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, Maryland; Department of Chemistry, Massachusetts General Hospital, Boston, Massachusetts, *Factors Influencing the Efficacy of Mediator-Specific Anti-Inflammatory, Glucocorticoid, and Anticoagulant Therapies for Sepsis*

Susan L. Mooberry, Department of Physiology and Medicine, Southwest Foundation for Biomedical Research, San Antonio, Texas, *Targets and Approaches for Cancer Drug Discovery*

Charles Natanson, Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, Maryland, *Factors Influencing the Efficacy of Mediator-Specific Anti-Inflammatory, Glucocorticoid, and Anticoagulant Therapies for Sepsis*

William Neil, Bristol-Myers Squibb, New Brunswick, New Jersey, *Using Microsoft Excel as a Laboratory Data Management Tool*

Mogens Nielsen, Department of Medicinal Chemistry, Danish University of Pharmaceutical Sciences, Copenhagen, Denmark, *GABA and Glutamate Receptor Ligands and Their Therapeutic Potential in CNS Disorders*
Tatiana Nikolskaya, GeneGo, St. Joseph, Michigan, *Systems Biology: Applications in Drug Discovery*

Yuri Nikolsky, GeneGo, St. Joseph, Michigan, *Systems Biology: Applications in Drug Discovery*

Peter Nollert, deCODE biostructures, Bainbridge Island, Washington, *Protein X-Ray Crystallography in Drug Discovery*


Marius Olah, Department of Biochemistry and Molecular Biology and Office of Biocomputing, University of New Mexico School of Medicine, University of New Mexico Health Sciences Center, Albuquerque, New Mexico, *High-Throughput Flow Cytometry*

Paul D. Olivo, Apath, LLC, St. Louis, Missouri, *Respiratory Viruses*

Tudor I. Oprea, Department of Biochemistry and Molecular Biology and Office of Biocomputing, University of New Mexico School of Medicine, University of New Mexico Health Sciences Center, Albuquerque, New Mexico, *High-Throughput Flow Cytometry*

Giorgio Palù, Department of Histology, Microbiology, and Medical Biotechnologies, University of Padova, Padova, Italy, *Strategies and Methods in Monitoring and Targeting Protein–Protein Interactions*

Keykavous Parang, Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, Rhode Island, *Protein Kinase Inhibitors in Drug Discovery*

Steve Pascolo, CureVac, GmbH, Tübingen, Germany, *RNA-Based Therapies*

Eric R. Prossnitz, Department of Cell Biology and Physiology and Cancer Research and Treatment Center, University of New Mexico School of Medicine, University of New Mexico Health Sciences Center, Albuquerque, New Mexico, *High-Throughput Flow Cytometry*

Jarkko Rautio, Department of Pharmaceutical Chemistry, University of Kuopio, Kuopio, Finland, *Design and Pharmaceutical Applications of Prodrugs*
Nathan T. Ross, Department of Biochemistry and Biophysics, University of Rochester, Rochester, New York, Combinatorial Chemistry in the Drug Discovery Process

A. Erik Rubin, Bristol-Myers Squibb, New Brunswick, New Jersey, Using Microsoft Excel as a Laboratory Data Management Tool

Mark E. Russo, Bristol-Myers Squibb, New Brunswick, New Jersey, Using Microsoft Excel as a Laboratory Data Management Tool

Dihua Shangguan, Department of Chemistry, University of Florida, Gainesville, Florida, Cancer Cell Proteomics Using Molecular Aptamers

Peter C. Simons, Department of Pathology and Cancer Research and Treatment Center, University of New Mexico School of Medicine, University of New Mexico Health Sciences Center, Albuquerque, New Mexico, High-Throughput Flow Cytometry

Larry A. Sklar, Department of Pathology and Cancer Research and Treatment Center, University of New Mexico School of Medicine, University of New Mexico Health Sciences Center, Albuquerque, New Mexico, High-Throughput Flow Cytometry

Charles B. Spainhour, Clark’s Summit, Pennsylvania, Natural Products

Bart L. Staker, deCODE biostructures, Bainbridge Island, Washington, Protein X-Ray Crystallography in Drug Discovery

Gongqin Sun, Department of Cell and Molecular Biology, University of Rhode Island, Kingston, Rhode Island, Protein Kinase Inhibitors in Drug Discovery

Weihong Tan, Department of Chemistry, University of Florida, Gainesville, Florida, Cancer Cell Proteomics Using Molecular Aptamers

Zhiwen Tang, Department of Chemistry, University of Florida, Gainesville, Florida, Cancer Cell Proteomics Using Molecular Aptamers

Anna Waller, Department of Pathology and Cancer Research and Treatment Center, University of New Mexico School of Medicine, University of New Mexico Health Sciences Center, Albuquerque, New Mexico, High-Throughput Flow Cytometry
Sandy Weinberg, Fast Trak BioDefense, GE Healthcare, Atlanta, Georgia, *Age of Regulation*


Jon G. Wilkes, Division of Systems Toxicology, National Center for Toxicological Research, Food and Drug Administration, Jefferson, Arkansas, *Combining NMR Spectral Information with Associated Structural Features to Form Computationally Nonintensive, Rugged, and Objective Models of Biological Activity*

Mary C. Wolff, Discovery-Neurosciences Research, Eli Lilly and Company, Indianapolis, Indiana, *Methods for the Design and Analysis of Replicate-Experiment Studies to Establish Assay Reproducibility and the Equivalence of Two Potency Assays*

Susan M. Young, Department of Pathology and Cancer Research and Treatment Center, University of New Mexico School of Medicine, University of New Mexico Health Sciences Center, Albuquerque, New Mexico, *High-Throughput Flow Cytometry*
## CONTENTS

<table>
<thead>
<tr>
<th>Preface</th>
<th>xix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction: Drug Discovery in the 21st Century</td>
<td>1</td>
</tr>
<tr>
<td>1 Natural Products</td>
<td>11</td>
</tr>
<tr>
<td>Charles B. Spainhour</td>
<td></td>
</tr>
<tr>
<td>2 Cancer Cell Proteomics Using Molecular Aptamers</td>
<td>73</td>
</tr>
<tr>
<td>Weihong Tan, Zehui Cao, Dihua Shangguan, Ying Li, Zhiwen Tang,</td>
<td></td>
</tr>
<tr>
<td>Prabodhika Mallikratchy, and Hui Chen</td>
<td></td>
</tr>
<tr>
<td>3 Molecular Similarity Methods and QSAR Models as Tools for</td>
<td>87</td>
</tr>
<tr>
<td>Virtual Screening</td>
<td></td>
</tr>
<tr>
<td>Jürgen Bajorath</td>
<td></td>
</tr>
<tr>
<td>4 Systems Biology: Applications in Drug Discovery</td>
<td>123</td>
</tr>
<tr>
<td>Sean Ekins, Andrej Bugrim, Yuri Nikolsky, and Tatiana Nikolskaya</td>
<td></td>
</tr>
<tr>
<td>5 High-Throughput Flow Cytometry</td>
<td>185</td>
</tr>
<tr>
<td>Larry A. Sklar, Peter C. Simons, Anna Waller, Sean M. Biggs,</td>
<td></td>
</tr>
<tr>
<td>Susan M. Young, Marius Olah, Cristian Bologa, Tudor I. Oprea,</td>
<td></td>
</tr>
<tr>
<td>Eric R. Prossnitz, and Bruce S. Edwards</td>
<td></td>
</tr>
</tbody>
</table>
6 Combining NMR Spectral Information with Associated Structural Features to Form Computationally Nonintensive, Rugged, and Objective Models of Biological Activity 227
Richard D. Beger, Dan A. Buzatu, and Jon G. Wilkes

7 Using Microsoft Excel® as a Laboratory Data Management Tool 287
A. Erik Rubin, Mark F. Russo, and William Neil

8 Age of Regulation 337
Sandy Weinberg and Gerald J. Whartenby

9 Simultaneous Screening of Multiple Cell Lines Using the CellCard System 353
Oren E. Beske

10 Protein X-ray Crystallography in Drug Discovery 373
Peter Nollert, Michael D. Feese, Bart L. Staker, and Hidong Kim

11 Biological and Chemistry Assays Available During Drug Discovery and Developability Assessment 457
Duane B. Lakings

12 Strategies and Methods in Monitoring and Targeting Protein–Protein Interactions 483
Arianna Loregian and Giorgio Palù

13 High-Throughput Screening: Evolution of Technology and Methods 559
Martyn N. Banks, Angela M. Cacace, Jonathan O’Connell, and John G. Houston

14 Metal-Enhanced Fluorescence: Application to High-Throughput Screening and Drug Discovery 603
Kadir Aslan, Ignacy Gryczynski, Joanna Malicka, Joseph R. Lakowicz, and Chris D. Geddes

15 Methods for the Design and Analysis of Replicate-Experiment Studies to Establish Assay Reproducibility and the Equivalence of Two Potency Assays 667
Brian J. Eastwood, Amy K. Chesterfield, Mary C. Wolff, and Christian C. Felder
<table>
<thead>
<tr>
<th>Section Number</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>Coupled Luminescent Methods in Drug Discovery: 3-Min Assays for Cytotoxicity and Phosphatase Activity</td>
<td>Michael J. Corey and Robert J. Kinders</td>
</tr>
<tr>
<td>17</td>
<td>Design and Pharmaceutical Applications of Prodrugs</td>
<td>Tomi Järvinen, Jarkko Rautio, Mar Masson, and Thorsteinn Loftsson</td>
</tr>
<tr>
<td>18</td>
<td>GABA and Glutamate Receptor Ligands and Their Therapeutic Potential in CNS Disorders</td>
<td>Ulf Madsen, Hans Bräuner-Osborne, Jeremy R. Greenwood, Tommy N. Johansen, Povl Krogsgaard-Larsen, Tommy Liljefors, Mogens Nielsen, and Bente Frølund</td>
</tr>
<tr>
<td>19</td>
<td>Cardiac Sarcolemmal ATP-Sensitive Potassium Channel Antagonists: Novel Ischemia-Selective Antiarrhythmic Agents</td>
<td>George E. Billman</td>
</tr>
<tr>
<td>20</td>
<td>Factors Influencing the Efficacy of Mediator-Specific Anti-Inflammatory, Glucocorticoid, and Anticoagulant Therapies for Sepsis</td>
<td>Peter C. Minneci, Katherine J. Deans, Michael Haley, Xizhong Cui, Charles Natanson, and Peter Q. Eichacker</td>
</tr>
<tr>
<td>21</td>
<td>Combinatorial Chemistry in the Drug Discovery Process</td>
<td>Nathan T. Ross, Brian R. McNaughton, and Benjamin L. Miller</td>
</tr>
<tr>
<td>23</td>
<td>Endocrine and Metabolic Agents</td>
<td>Brian L. Furman</td>
</tr>
<tr>
<td>24</td>
<td>Respiratory Viruses</td>
<td>Paul D. Olivo</td>
</tr>
<tr>
<td>25</td>
<td>Strategies in the Design of Antiviral Drugs</td>
<td>Erik De Clercq and Piet Herdewijn</td>
</tr>
<tr>
<td>26</td>
<td>Protein Kinase Inhibitors in Drug Discovery</td>
<td>Keykavous Parang and Gongqin Sun</td>
</tr>
<tr>
<td>27</td>
<td>RNA-Based Therapies</td>
<td>Steve Pascolo</td>
</tr>
</tbody>
</table>
28  Novel Imaging Agents for Molecular MR Imaging of Cancer  1309
    Dmitri Artemov and Zaver M. Bhujwalla

29  Targets and Approaches for Cancer Drug Discovery  1343
    Susan L. Mooberry

Index  1375
This Drug Discovery Handbook represents a unique attempt to survey the different approaches to discovering potential new therapeutic moieties. Such moieties are the backbone of both the pharmaceutical industry and the prime axis for the advancement of medical science.

The volume is unique in that it seeks to cover possible approaches to drug discovery as broadly as possible while not just doing so in a superficial manner. The 29 chapters cover all the major approaches to the problem of identifying potential drugs and were written by leading representatives from each of these approaches.

I hope that this banquet is satisfying and useful to my colleagues in the field.


S.C. Gad
I.1 INTRODUCTION

The discovery, development, and registration of a pharmaceutical is an immensely expensive operation and represents a rather unique challenge. For every 9000 to 10,000 compounds specifically synthesized or isolated as potential therapeutics, one (on average) will actually reach the market. This process is illustrated diagrammatically in Figure 1. Each successive stage in the process is more expensive, making it of great interest to identify as early as possible those agents that are likely not to go the entire distance, allowing a concentration of effort on the compounds that have the highest probability of reaching the market. Compounds “drop out” of the process primarily for three reasons:

1. Toxicity or (lack of) tolerance
2. (Lack of) efficacy
3. (Lack of) bioavailability of the active moiety in humans

Early identification of poor or noncompetitive candidates in each of these three categories is thus extremely important [1], forming the basis for the use of screening in pharmaceutical discovery and development. How much and which resources to invest in screening and each successive step in support of the development of a potential drug are matters of strategy and phasing that are detailed later in this Introduction. In vitro methods are increasingly
providing new tools for use in both early screening and the understanding of mechanisms of observed toxicity in preclinical and clinical studies [2, 3], particularly with the growing capabilities and influence of genomic and proteomic technologies. This is increasingly important as the societal concern over drug prices has grown [4]. Additionally, the marketplace for new drugs is exceedingly competitive. The rewards for being either early (first or second) into the marketplace or achieving a significant therapeutic advantage are enormous in terms of eventual market share. Additionally, the first drug approved sets agency expectations for those drugs that follow. In mid-2004, there were 263 pharmaceutical products awaiting approval (93 of these biotech products)—the “oldest” having been in review 10 years) and some 2300 additional agents in the IND stage. Not all of these (particularly the oldest) will be economically successful).

The usual way in which transition (or “flow”) of new molecules between the different phases is handled in drug discovery/development is to use a tiered screening or testing approach. Each tier generates more specific data (and costs more to do so) and draws on the information generated in earlier tiers to refine the design of new studies. Different tiers are keyed to the support of successive decision points (go/no-go points) in the development process, with the intent of reducing risks (as to efficacy bioavailability and safety) as early as possible.

The first real critical decisions concerning the potential advancement of a compound to evaluation in clinical trials are the most difficult. They require an understanding of how well particular in vitro or in vivo work in predicting
adverse effects in humans (usually very well, but there are notable lapses; for example, giving false positives and false negatives) and an understanding of what initial clinical trials are intended to do. Though an “approved” IND grants one entry into limited evaluations of drug effects in humans, flexibility in the execution and analysis of these studies offers a significant opportunity to also investigate efficacy [5].

Once past the discovery and initial lead or candidate selection stages, each aspect of development becomes more tightly connected with the other aspects of the development of a compound, particularly the potential clinical aspects. These interconnections are coordinated by project management systems. Many times during the early years of the development process, biological evaluation of efficacy and safety constitutes the rate-limiting step—it is, in the language of project management, on the critical path.

Another way in which pharmaceutical development varies from toxicology as practiced in other industries is that it is a much more multidisciplinary and integrated process. This particularly stands out in the incorporation of the evaluation of ADME (absorption, distribution, metabolism, and excretion) aspects in the safety evaluation process. These pharmacokinetic/metabolism (PKM) aspects are evaluated for each of the animal model species utilized to predict the safety of a potential drug prior to evaluation in humans. Frequently, in vitro characterizations of metabolism for model (or potential model) species and humans are performed to allow optimal model selection and understanding of findings. This allows for an early appreciation of both the potential bioavailability of active drug moieties and the relative predictive values of the various biological models. Such data early on are also very useful (in fact, sometimes essential) in setting does levels for later animal studies and in projecting safe dose levels for clinical use. Unlike the case in most other realms of development of biologically active molecules, one is not limited to extrapolating the relationships between administered dose and systemic effects. Rather, one has significant information on systemic levels of the therapeutic moiety—typically, total area under the curve (AUC), peak plasma levels ($C_{max}$), and plasma half-lives, at a minimum.

I.2 SCREENS: THEIR USE AND INTERPRETATION IN DRUG DISCOVERY

Much (perhaps even most) of what is performed in safety assessment can be considered screening—trying to determine if some effect is or is not (to an acceptable level of confidence) present [6]. The general concepts of such screens are familiar to toxicologists in the pharmaceutical industry because the approach is a major part of the activities of the pharmacologists involved in the discovery of new compounds. But the principles underlying screening are not generally well recognized or understood. And such understanding is essential to the proper use, design, and analysis of screens [7, 8]. Screens are
the biological equivalent of exploratory data analysis, or EDA [9]. Each test or assay has an associated activity criterion, that is, a level above which the activity of interest is judged to be present. If the result for a particular test compound meets this criterion, the compound may pass to the next stage. This criterion could be based on statistical significance (e.g., all compounds with observed activities significantly greater than the control at the 5 percent level could be tagged). However, for early screens, such a formal criterion may be too strict, resulting in few compounds begin identified as “active.”

A useful indicator of the efficacy of an assay series is the frequency of discovery of truly active compounds. The frequency is related to the probability of discovery and to the degree of risk (hazard to health) associated with an active compound passing a screen undetected. These two factors in turn depend on the distribution of activities in the series of compounds being tested and the chances of rejecting or accepting compounds with given activities at each stage.

Statistical modeling of the assay system may lead to the improvement of the design of the system by reducing the interval between discoveries of active compounds. The objectives behind a screen and considerations of (1) costs for producing compounds and testing and (2) the degree of uncertainly about test performance will determine desired performance characteristics of specific cases. In the most common case of early toxicity screens performed to remove possible problem compounds, preliminary results suggest that it may be beneficial to increase the number of compounds tested, decrease the numbers of animals per group, and increase the range and number of doses. The result will be less information on more structure, but there will be an overall increase in the frequency of discovery of active compounds (assuming that truly active compounds are entering the system at a steady rate).

The methods described here are well suited to analyzing screening data when the interest is truly in detecting the absence of an effect with little chance of false negatives. There are many forms of graphical analysis methods available, including some newer forms that are particularly well suited to multivariate data (the types that are common in more complicated screening test designs). It is intended that these aspects of analysis will be focused on in a later publication.

The design of each assay and the choice of the activity criterion should, therefore, be adjusted, bearing in mind the relative costs of retaining false positives and rejecting false negatives. Decreasing the group sizes in the early assays reduces the chance of obtaining significance at any particular level (such as 5 percent), so that the activity criterion must be relaxed, in a statistical sense, to allow more compounds through. At some stage, however, it becomes too expensive to continue screening many false positives, and the criteria must be tightened accordingly. Where the criteria are set depends on what acceptable noise levels are in a screening system.
characteristics of screens

an excellent introduction to the characteristics of screens in redman’s [10] interesting approach, which identifies four characteristics of an assay. redman assumes that a compound is either active or inactive and that the proportion of activities in a compound can be estimated from past experience. after testing, a compound will be classified as positive or negative (i.e., possessing or lacking activity). it is then possible to design the assay so as to optimize the following characteristics:

1. sensitivity: the ratio of true positives to total activities
2. specificity: the ratio of true negatives to total inactives
3. positive accuracy: the ratio of true to observed positives
4. negative accuracy: the ratio of true to observed negatives
5. capacity: the number of compounds that can be evaluated
6. reproducibility: the probability that a screen will produce the same result at another time (and, perhaps, in some other lab)

an advantage of testing many compounds is that it gives the opportunity to average activity evidence over structural classes or to study quantitative structure–activity relationships (qsars). quantitative structure–activity relationships can be used to predict the activity of new compounds and thus reduce the chance of in vivo testing on negative compounds. the use of qsars can increase the proportion of truly active compounds passing through the system.

to simplify this presentation, data sets drawn only from neuromuscular screening activity were used. however, the evaluation and approaches should be valid for all similar screening data sets, regardless of source. the methods are not sensitive to the biases introduced by the degree of interdependence found in many screening batteries that use multiple measures (such as the neurobehavioral screen).

1. screens almost always focus on detecting a single end point of effect (such as mutagenicity, lethality, neurotoxicity, or development toxicity) and have a particular set of operating characteristics in common.
2. a large number of compounds are evaluated, so ease and speed of performance (which may also be considered efficiency) are very desirable characteristics.
3. the screen must be very sensitive in its detection of potential effective agents. an absolute minimum of active agents should escape detection; that is, there should be very few false negatives (in other words, the type ii error rate or β level should be low). stated yet another way, the signal gain should be way up.
4. It is desirable that the number of false positives be small (i.e., there should be a low type I error rate of $\alpha$ level).

5. Items 2 to 4, which are all to some degree contradictory, require the involved researchers to agree on a set of compromises, starting with the acceptance of a relatively high $\alpha$ level (0.10 or more), that is, an increased noise level.

6. In an effort to better serve item 2, safety assessment screens are frequently performed in batteries so that multiple end points are measured in the same operation. Additionally, such measurements may be repeated over a period of time in each model as a means of supporting item 3.

7. This screen should use small amounts of compound to make item 1 possible and should allow evaluation of materials that have limited availability (such as novel compounds) early on in development.

8. Any screening system should be validated initially using a set of blind (positive and negative) controls. These blind controls should also be evaluated in the screening system on a regular basis to ensure continuing proper operation of the screen. As such, the analysis techniques used here can then be used to ensure the quality or modify the performance of a screening system.

9. The more that is known about the activity of interest, the more specific the form of screen that can be employed. As specificity increases, so should sensitivity.

10. Sample (group) sizes are generally small.

11. The data tend to be imprecisely gathered (often because researchers are unsure of what they are looking for) and therefore possess extreme within-group variability. Control and historical data are not used to adjust for variability or modify test performance.

12. Proper dose selection is essential for effective and efficient screen design and conduct. If insufficient data are available, a suitably broad range of doses must be evaluated (however, this technique is undesirable on multiple grounds, as has already been pointed out).

It should be kept in mind that there are a number of common mistakes (in both the design and conduct of studies and in how information from studies is used) that have led to unfortunate results, ranging from losses in time and money to the discarding of perfectly good potential drugs. Such outcomes are indeed the great disasters in drug discovery—especially since many of them are avoidable if attention is paid to a few basic principles.

It is quite possible to design a study for failure. Common shortfalls include:

1. Using the wrong animal model.
2. Using the wrong route or dosing regimen.
3. Using the wrong vehicle or formulation of test material.
4. Using the wrong dose level. In studies where several dose levels are studied, the worst outcome is to have an effect at the lowest dose level tested (i.e., the safe dosage in animals remains unknown). The next worst outcome is to have no effect at the highest dose tested (generally meaning that the signs of toxicity remain unknown, invalidating the study in the eyes of many regulatory agencies).
5. Making leaps of faith. An example is to set dosage levels based on others’ data and to then dose all test animals. At the end of the day, all animals in all dose levels are dead. The study is over; the problem remains.
6. Using the wrong concentration of test materials in a screen. Many effects are very concentration dependent.

The design and conduct of discovery screens and programs also require an understanding of some basic concepts:

1. The studies are performed to establish or deny a specific activity of a compound, rather than to characterize the toxicity of a compound.
2. Because pharmaceuticals are intended to affect the functioning of biological systems and safety assessment characterizes the effects of higher-than-therapeutic doses of compounds, it is essential that one be able to differentiate between hyperpharmacology and true (undesirable) adverse effects.
3. Focus of the development process for a new pharmaceutical is an essential aspect of success but is also difficult to maintain. Clinical research units generally desire to pursue as many or as broad claims as possible for a new agent and frequently also apply pressure for the development of multiple forms for administration by different routes.

This volume will present a wide variety of approaches to the discovery and identification of potential new drugs. In assembling this volume, these approaches were derived thinking of four large categories. This approach is a traditional one, focusing on using some accepted (at least to the researcher) screens for a specific therapeutic activity to identify active or promising structures.

**Therapeutic Area Approach: Diseases Seeking Drugs**

This approach is perhaps the most traditional one, focusing on using some accepted (at least to the researcher) screen for a specific therapeutic activity to identify active or promising structure. Most drug discovery in the past started with such mass screening of selected molecules.
Mechanism Approach: Drugs Seeking Diseases

This approach is still currently popular. One or more compounds possessing a mechanism of activity are evaluated for activity in a specific disease. Or several: The challenge is the plausibility of suitable therapeutic activity and the degree of validity of one by hypothesis for a disease process.

Medicinal Chemistry Approach

This is also a traditional approach starting with molecular structures of known properties and seeking to modify structure to improve and optimize desirable features. The medicinal chemist utilizes knowledge of what past structural modifications have meant in terms of functional differences.

Technique-Based Approaches

Since the early 1990s, the most popular approaches have utilized new technologies based on expanded knowledge of how to characterize genetic and molecular level processes in humans and other species. Regrettably, as of late 2004, these methods have not yet borne the expected fruit. But perhaps the necessary period of learning how to use such tools is almost over.

Genomics

The field of genomics, particularly high-throughput sequencing and characterization of expressed human genes, has created new opportunities for drug discovery. Knowledge of all the human genes and their functions may allow effective preventive measures and change drug research strategy and drug discovery development processes. Pharmacogenomics (dating back to the 1970s) is the application of genomic technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. It applies the large-scale systematic approaches of genomics to speed the discovery of drug response markers, whether they act at the level of the drug target, drug metabolism, or disease pathways. The potential implication of genomics and pharmacogenomics in drug discovery and development is that treatments for diseases could be identified according to genetic and specific individual markers, selecting medications and dosages that are optimized for individual patients.

Combinatorial Chemistry

By reacting a set of starting chemicals in every possible combination, combinatorial chemistry gives life scientists the ability to create molecules in huge numbers and to test them for sought-after properties. Those abilities have attracted the attention of pharmaceutical companies. In recent years, virtually every large drug company has set up a combinatorial chemistry group.

The groups have revolutionized drug search programs. Before the mid-1990s, a single chemist could make perhaps 5 compounds per week. Now, he