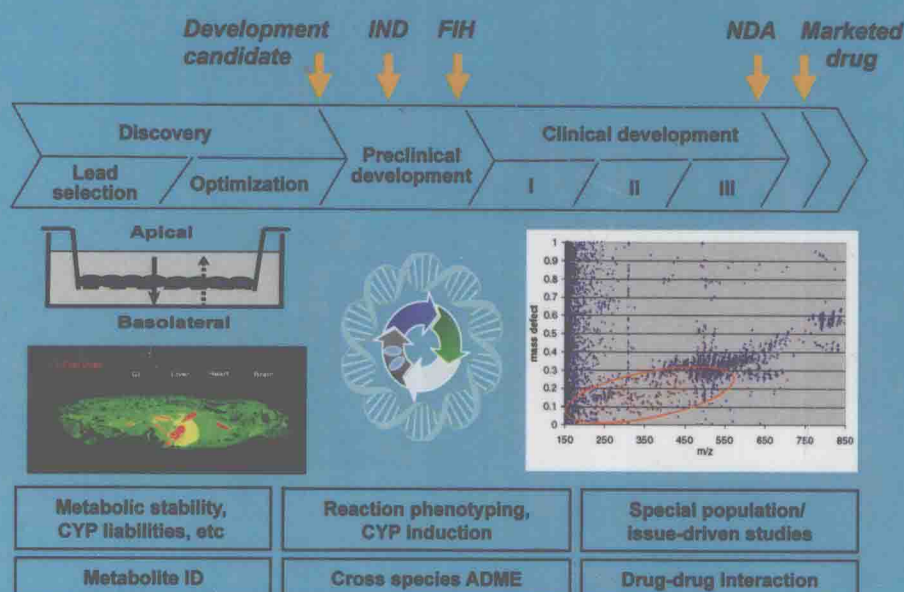


# ADME-Enabling Technologies in Drug Design and Development



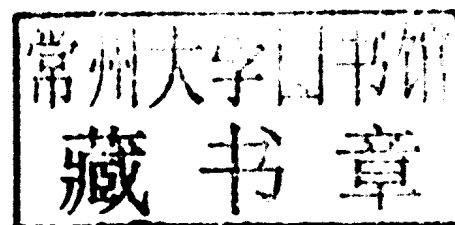
Edited by  
**DONGLU ZHANG**  
**SEKHAR SURAPANENI**

# ADME-ENABLING TECHNOLOGIES IN DRUG DESIGN AND DEVELOPMENT

---

EDITED BY

DONGLU ZHANG  
SEKHAR SURAPANENI



 **WILEY**

A JOHN WILEY & SONS, INC., PUBLICATION

Copyright © 2012 by John Wiley & Sons, Inc. All rights reserved.

Published by John Wiley & Sons, Inc., Hoboken, New Jersey.

Published simultaneously in Canada.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, scanning, or otherwise, except as permitted under Section 107 or 108 of the 1976 United States Copyright Act, without either the prior written permission of the Publisher, or authorization through payment of the appropriate per-copy fee to the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, (978) 750-8400, fax (978) 750-4470, or on the web at [www.copyright.com](http://www.copyright.com).

Requests to the Publisher for permission should be addressed to the Permissions Department, John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, (201) 748-6011, fax (201) 748-6008, or online at <http://www.wiley.com/go/permissions>.

**Limit of Liability/Disclaimer of Warranty:** While the publisher and author have used their best efforts in preparing this book, they make no representations or warranties with respect to the accuracy or completeness of the contents of this book and specifically disclaim any implied warranties of merchantability or fitness for a particular purpose. No warranty may be created or extended by sales representatives or written sales materials. The advice and strategies contained herein may not be suitable for your situation. You should consult with a professional where appropriate. Neither the publisher nor author shall be liable for any loss of profit or any other commercial damages, including but not limited to special, incidental, consequential, or other damages.

For general information on our other products and services or for technical support, please contact our Customer Care Department within the United States at (800) 762-2974, outside the United States at (317) 572-3993 or fax (317) 572-4002.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic formats. For more information about Wiley products, visit our web site at [www.wiley.com](http://www.wiley.com).

***Library of Congress Cataloging-in-Publication Data:***

ADME-enabling technologies in drug design and development / edited by Donglu

Zhang, Sekhar Surapaneni.

p. ; cm.

Includes bibliographical references and index.

ISBN 978-0-470-54278-1 (cloth)

I. Zhang, Donglu. II. Surapaneni, Sekhar.

[DNLM: 1. Drug Design. 2. Drug Evaluation, Preclinical. 3. Pharmaceutical Preparations--metabolism. 4. Pharmacokinetics. 5. Technology, Pharmaceutical--methods. QV 744]

LC-classification not assigned

615.1'9--dc23

2011030352

Printed in the United States of America.

ISBN: 9780470542781

10 9 8 7 6 5 4 3 2 1

## FOREWORD

The discovery, design, and development of drugs is a complex endeavor of optimizing on three axes: efficacy, safety, and druggability or drug-likeness. Each of these axes is a potential cause of attrition as a new molecular entity progresses through the many phases of drug development. Out of the 5000–10,000 compounds evaluated in discovery efforts, only 250 enter preclinical testing, 5 enter clinical trials, and only 1 is granted approval by the Food and Drug Administration at a cost that is estimated between US\$1.3–1.6 billion [1]. Efforts to increase innovation, decrease attrition, and lower the cost of drug development are the focus of the pharmaceutical industry and regulatory agencies alike. Advances have been made in some disciplines such as drug metabolism and pharmacokinetics (PK), particularly in the area of absorption, distribution, metabolism, and excretion (ADME) studies. For example, a root cause analysis of clinical attrition [2] showed that unacceptable PK or bioavailability accounted for 40% of clinical attrition in the 1990s but within a decade had been reduced to less than 10%, in large part by the identification and mitigation of risks associated with ADME/PK properties earlier in the drug discovery process. This was enabled by the introduction of automated high- and medium-throughput screening of lead optimization candidates in the discovery space. While impressive, this improvement alone is not sufficient to reverse the rising costs and long development cycle times. It is, however, a step in the right direction. As the pharmaceutical industry has evolved, the focus of ADME studies has shifted from studies conducted primarily in support of regulatory submissions to playing a significant role in the earliest stages of the discovery phase of drug development. The engagement of ADME scientists in the

discovery space has allowed drug candidates to progress in the development pipeline to the next milestone with greater probability of success because desirable characteristics, such as good aqueous solubility for absorption, high bioavailability, and balanced clearance, have been engineered into the molecules, and liabilities such as high first-pass metabolism and unacceptable drug–drug interactions potential have been engineered out.

The history of the discipline of drug metabolism and PK and ADME studies, with its roots in organic chemistry and pharmacology, has been well chronicled [3–8]. The rapid advancement of the discipline over the past 50 years is clearly linked to the development of ever-increasingly sophisticated analytical tools and the growth of the pharmaceutical industry. The vast number of tools at the disposal of drug metabolism scientists has transformed the study of xenobiotics from descriptive to quantitative, *in vivo* to the molecular levels, and from simply characterizing to predicting ADME properties.

It would be beyond the scope of this introduction to provide a historical accounting of the numerous advances of technology that have shaped the field. There are, however, three noteworthy milestones in the evolution of the discipline that merit mention: the use of radioisotopes in metabolism and distribution studies; the discovery of the superfamily of drug metabolizing enzymes, the cytochrome P450s; and the revolutionizing impact of mass spectrometry as both a qualitative and quantitative tool.

With the discovery of a new radioisotope of carbon,  $^{14}\text{C}$ , by Martin and Ruben [9], this powerful analytical tool enabled the first radiolabeled studies that elucidated the metabolic pathways and the disposition of xenobiotics in rats [10, 11]. The use of radiotracers went

on to become an indispensable tool in biochemical pathway elucidation and in drug disposition studies. While  $^{14}\text{C}$ -labeled compounds are predominantly used in *in vivo* studies to fulfill regulatory requirement, the development of new reagents and techniques in tritium labeling now have allowed stereo- and site-selective synthesis with high specific activity, making these labeled molecule readily available for use in the earliest phases of drug discovery [12, 13].

The discovery of the cytochrome P450s and their role in the metabolism of endo- and xenobiotics opened a field of science that continues to grow and have a tremendous impact on the development of drugs and the practice of medicine. The pioneering research in this field has been well documented by Estabrook, a key contributor to our current understanding of this superfamily of enzymes [14]. The magnitude of research on the cytochrome P450s has exploded since 2003 (from greater than 2000 literature references to over 67,000 citations, as reflected by searching the PubMed database in 2011). The expanding knowledge of the cytochrome P450s has impacted early discovery efforts via assays for metabolic stability, species comparison in the selection of the most relevant species for toxicology studies, identification of the primary enzymes involved in the metabolism of a candidate drug, and potential polymorphic or drug–drug interaction liabilities of a candidate drug. The influence of the research on the cytochrome P450s also reaches into the clinical realm of drug development in the need for and design of clinical drug–drug interaction trials as well as in the regulatory guidance on drug interactions [15, 16].

No single analytical technique has had a more powerful effect on drug development than mass spectrometry, with an impact on multiple disciplines, such as chemistry, biology, and ADME [17]. An excellent review of mass spectrometry and its applications in drug metabolism and PK has recently been published [18]. Mass spectrometry moved from the being a specialized tool largely used in structure identification to a “routine,” but albeit powerful, analytical technology used across the pharmaceutical industry and academia alike. The selectivity, sensitivity, and speed of mass spectrometry enabled much of the success seen with high-throughput screening and advances in bioanalytical analysis in a multitude of biological matrices in both PK and biotransformation studies.

The ADME scientist of today is fortunate to have an arsenal of tools at his or her disposal, many of which will be expanded upon in this book. The advances in technologies often have implications in adjacent technologies that further the discipline of drug metabolism and PK and allow an integrated approach to solving

problems and advancing drug candidates through the phases of drug development.

LISA A. SHIPLEY

## REFERENCES

1. Burrill & Company. *Analysis for Pharmaceutical Research and Manufacturers of America; and Pharmaceutical Research and Manufacturers of America, PhRMA Annual Member Survey* (Washington, DC: PhRMA, 2010). Citations at <http://www.phrma.org/research/infographics>, 2010.
2. Kola I, Landis J (2004) Can the pharmaceutical industry reduce attrition rates? *Nature Reviews. Drug Discovery* 3:711–715.
3. Conti A, Bickel MH (1977) History of drug metabolism: Discoveries of the major pathways in the 19th century. *Drug Metabolism Reviews* 6(1):1–50.
4. Bachmann C, Bickel MH (1985) History of drug metabolism: The first half of the 20th century. *Drug Metabolism Reviews* 16(3):185–253.
5. Murphy PJ (2001) Xenobiotic metabolism: A look from the past to the future. *Drug Metabolism and Disposition* 29:779–780.
6. Murphy PJ (2008) The development of drug metabolism research as expressed in the publications of ASPET: Part 1, 1909–1958. *Drug Metabolism and Disposition* 36:1–5.
7. Murphy PJ (2008) The development of drug metabolism research as expressed in the publications of ASPET: Part 2, 1959–1983. *Drug Metabolism and Disposition* 36:981–985.
8. Murphy PJ (2008) The development of drug metabolism research as expressed in the publications of ASPET: Part 3, 1984–2008. *Drug Metabolism and Disposition* 36:1977–1982.
9. Ruben S, Kamen MD (1941) Long-lived radioactive carbon: C14. *Physical Review* 59:349–354.
10. Elliott HW, Chang FNH, Abdou IA, Anderson HH (1949) The distribution of radioactivity in rats after administration of C14 labeled methadone. *The Journal of Pharmacology and Experimental Therapeutics* 95:494–501.
11. Morris HP, Weisburger JH, Weisburger EK (1950) The distribution of radioactivity following the feeding of carbon 14-labeled 2-acetylaminofluorene in rats. *Cancer Research* 10:620–634.
12. Saljoughian M (2002) Synthetic tritium labeling: Reagents and methodologies. *Synthesis* 13:1781–1801.
13. Voges R, Heys JR, Moenius T *Preparation of Compounds Labeled with Tritium and Carbon -14*, Chichester, U.K.: John Wiley and Sons, 2009.
14. Estabrook RW (2003) A passion for P450's (remembrances of the early history of research on cytochrome P450). *Drug Metabolism and Disposition* 31:1461–1473.

15. *Guideline on the Investigation of Drug Interactions (EMA/CHMP/EWP/125211/2010)*. (2010) [http://www.ema.europa.eu/ema/pages/includes/document/open\\_document.jsp?webContentId=WC500090112](http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500090112).
16. *Guidance for Industry: In Vivo Drug Metabolism/Drug Interaction Studies-Study Design, Data Analysis, and Recommendations for Dosing and Labeling*. (1999) <http://www.fda.gov/cder/guidance/index.htm>.
17. Ackermann BL, Berna MJ, Eckstein JA, Ott LW, Chadhary AK (2008) Current applications of liquid chromatography/mass spectrometry in pharmaceutical discovery after a decade of innovation. *Annual Review Of Analytical Chemistry* 1:357–396.
18. Ramanathan R, ed. *Mass Spectrometry in Drug Metabolism and Pharmacokinetics*. Hoboken, NJ: John Wiley and Sons, 2009.



## PREFACE

Understanding and characterizing absorption, metabolism, distribution, and excretion (ADME) properties of new chemical entities and drug candidates is an integral part of drug design and development. ADME is the discipline that is involved in the entire process of drug development, right from discovery, lead optimization, and clinical drug candidate selection through drug development and regulatory process. The complexity of ADME studies in drug discovery and development requires a drug metabolism scientist to know all available technologies in order to choose the right experimental approach and technology for solving the problems in a timely manner. During the last decade, tremendous progress has been made in wide array of technologies including mass spectrometry and molecular biology tools, and these enabling technologies are widely employed by ADME scientists. The generation of ADME data to support discovery and development teams is a gated process and timely generation of data to make right decisions is of paramount importance. Given the complexity of the drug discovery and development process, right techniques and tools should be used to generate timely data that is useful for decision making and regulatory filing. This requires an understanding of not only the breadth and depth of ADME technologies but also their limitation and pitfalls so scientists can make appropriate choices in employing these tools. A book on integrated enabling technologies will not only be useful to drug metabolism scientists but also could be a very helpful reference for scientists from the fields of pharmacology, medicinal chemistry, pharmaceuticals, toxicology, and bioanalytical sciences in academia and industry.

This book is divided into four main sections. Part A provides the reader with an overview of ADME con-

cepts and current topics including ADME and transporter studies in drug discovery and development, active and toxic metabolites, modeling and simulation, and developing biologics and individual medicines. Part B describes the ADME systems and methods; these include ADME screening technologies, permeability and transporter studies, distribution across specialized barriers such as blood–brain barrier (BBB) or placenta, cytochrome P450 (CYP) inhibition, induction, phenotyping, animal models for studying metabolism and transporters, and bile collection. Part C of the book discusses analytical tools including liquid chromatography-mass spectrometry (LC-MS) technologies for quantitation, metabolite identification and profiling, accelerator mass spectrometry (AMS) and radioprofiling, nuclear magnetic resonance (NMR), supercritical fluid chromatography (SFC) and other separation techniques, mass spectrometric imaging, and quantitative whole-body autoradiography (QWBA) tissue distribution techniques. Part D presents new and evolving technologies such as stem cells, genetically modified animal models, and siRNA techniques in ADME studies. Other techniques included in this section are target imaging technologies, radiosynthesis, formulation, and testing of cardiovascular toxicity potential.

We would like to thank our colleagues who are the experts and leading practitioners of the techniques described in the book for their contributions. We hope that this book is useful and serves as a quick reference to all drug hunters and to all those who are new to the discipline of ADME.

DONGLU ZHANG  
SEKHAR SURAPANENI

## CONTRIBUTORS

- Suresh K. Balani**, DMPK/NCDS, Millennium: The Takeda Oncology Company, Cambridge, MA, USA
- Praveen V. Balimane**, Bristol-Myers Squibb, Princeton, NJ, USA
- Vanessa N. Barth**, Translational Sciences, Eli Lilly and Company, Indianapolis, IN, USA
- Leslie Bell**, Novartis Institutes for BioMedical Research, Cambridge, MA, USA
- Rajinder Bhardwaj**, DMPK, Chemical Sciences and Pharmacokinetics, Lundbeck Research USA, Paramus, NJ, USA
- Catherine L. Booth-Genthe**, Respiratory Therapeutic Area Unit, GlaxoSmithKline, King of Prussia, PA, USA
- Hong Cai**, Bristol-Myers Squibb, Pennington, NJ, USA
- Gamini Chandrasena**, DMPK, Chemical Sciences and Pharmacokinetics, Lundbeck Research USA, Paramus, NJ, USA
- Jiwen Chen**, Bristol-Myers Squibb, Pennington, NJ, USA
- Saeho Chong**, College of Pharmacy, Seoul National University, Seoul, Korea
- Lisa J. Christopher**, Bristol-Myers Squibb, Princeton, NJ, USA
- Jun Dai**, Bristol-Myers Squibb, Princeton, NJ, USA
- Li Di**, Pfizer Global Research and Development, Groton, CT, USA
- Ashok Dongre**, Bristol-Myers Squibb, Pennington, NJ, USA
- Dieter M. Drexler**, Bristol-Myers Squibb, Wallingford, CT, USA
- Richard W. Edom**, Janssen Pharmaceutical Companies of Johnson & Johnson, Raritan, NJ, USA
- Charles S. Elmore**, Radiochemistry, AstraZeneca, Mölndal, Sweden
- Adrian J. Fretland**, Nonclinical Safety, Early ADME Department, Roche, Nutley, NJ, USA
- Timothy J. Garrett**, Clinical and Translational Science Institute, University of Florida, Gainesville, FL, USA
- Lingling Guan**, Ricerca Biosciences, Concord, OH, USA
- Anshul Gupta**, Drug Metabolism and Pharmacokinetics, AstraZeneca, Waltham, MA, USA
- Yong-Hae Han**, Bristol-Myers Squibb, Princeton, NJ, USA
- Imad Hanna**, Drug Metabolism and Pharmacokinetics, Novartis Institutes for BioMedical Research, East Hanover, NJ, USA
- David C. Hay**, MRC Centre for Regenerative Medicine, Edinburgh, UK
- Haizheng Hong**, College of Oceanography and Environmental Sciences, Xiamen University, Fujian, China
- Cornelis E.C.A. Hop**, Department of Drug Metabolism and Pharmacokinetics, Genentech, South San Francisco, CA, USA



**Matthew Hoffmann**, Celgene Corporation, Summit, NJ, USA

**Stella Huang**, Bristol-Myers Squibb, Wallingford, CT, USA

**W. Griffith Humphreys**, Bristol-Myers Squibb, Princeton, NJ, USA

**Wenying Jian**, Johnson & Johnson Pharmaceutical Research & Development, Raritan, NJ, USA

**Xi-Ling Jiang**, Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, The State University of New York, Buffalo, NY, USA

**Kim A. Johnson**, Bristol-Myers Squibb, Wallingford, CT, USA

**Janan Jona**, Small Molecule Process and Product Development/Preformulation, Amgen Inc., Thousand Oaks, CA, USA

**Elizabeth M. Joshi**, Department of Drug Disposition, Lilly Research Laboratories, Indianapolis, IN, USA

**Nataraj Kalyanaraman**, Pharmacokinetics and Drug Metabolism, Amgen Inc., Thousand Oaks, CA, USA

**Jiesheng Kang**, Sanofi-Aventis U.S. Inc., Bridgewater, NJ, USA

**Edward H. Kerns**, Therapeutics for Rare and Neglected Diseases, NIH Center for Translational Therapeutics, Rockville, MD, USA

**Yuan-Hon Kiang**, Small Molecular Process and Product Development/Preformulation, Amgen Inc., Thousand Oaks, CA, USA

**Wing Wah Lam**, Janssen Pharmaceutical Companies of Johnson & Johnson, Raritan, NJ, USA

**Chun Li**, Metabolism and Pharmacokinetics, Genomics Institute of the Novartis Research Foundation, San Diego, CA, USA

**Mingxiang Liao**, DMPK/NCDS, Millennium: The Takeda Oncology Company, Cambridge, MA, USA

**Heng-Keang Lim**, Janssen Pharmaceutical Companies of Johnson & Johnson, Raritan, NJ, USA

**Zhongping (John) Lin**, Frontage Laboratories, Inc. Malvern, PA, USA

**Chang-Xiao Liu**, State Key Laboratory of Drug Technology and Pharmacokinetics, Tianjin Institute of Pharmaceutical Research, Tianjin, China

**Tom Lloyd**, Worldwide Clinical Trials Drug Development Solutions Bioanalytical Sciences, Austin, TX, USA

**Anthony Y.H. Lu**, Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ, USA

**Qiang Ma**, Receptor Biology Laboratory, Toxicology and Molecular Biology Branch, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, WV, USA

**Daniel P. Magparangalan**, Covidien, St. Louis, MO, USA

**Brad D. Maxwell**, Bristol-Myers Squibb, Princeton, NJ, USA

**Kaushik Mitra**, Merck & Co. Inc., Rahway, NJ, USA

**Voon Ong**, San Diego, CA, USA

**Ryan M. Pelis**, Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada

**Natalia Penner**, Department of Drug Metabolism and Pharmacokinetics, Biogen Idec, Cambridge, MA, USA

**Chandra Prakash**, Department of Drug Metabolism and Pharmacokinetics, Biogen Idec, Cambridge, MA, USA

**Darren L. Reid**, Small Molecular Process and Product Development/Preformulation, Amgen Inc., Thousand Oaks, CA, USA

**Kevin L. Salyers**, Pharmacokinetics and Drug Metabolism, Amgen Inc., Thousand Oaks, CA, USA

**Mark Seymour**, Xceleron, Heslington, York, UK

**Adam Shilling**, Incyte Corp, Wilmington, DE, USA

**Lisa A. Shipley**, Drug Metabolism and Pharmacokinetics, Merck & Co., Inc., West Point, PA, USA

**Yue-Zhong Shu**, Bristol-Myers Squibb, Princeton, NJ, USA

**Jose Silva**, Janssen Pharmaceutical Companies of Johnson & Johnson, Raritan, NJ, USA

**Matthew D. Silva**, Amgen Inc., Thousand Oaks, CA, USA

**Sekhar Surapaneni**, Drug Metabolism and Pharmacokinetics, Celgene Corporation, Summit, NJ, USA

**Adrienne A. Tymiak**, Bristol-Myers Squibb, Princeton, NJ, USA

**Jianling Wang**, Novartis Institutes for BioMedical Research, Cambridge, MA, USA

**Lifei Wang**, Bristol-Myers Squibb, Princeton, NJ, USA

**Xiaomin Wang**, Celgene Corporation, Summit, NJ, USA

**David B. Wang-Iverson**, Bristol-Myers Squibb, Princeton, NJ, USA

**Naidong Weng**, Janssen Pharmaceutical Companies of Johnson & Johnson Raritan, NJ, USA

**Caroline Woodward**, Department of Drug Metabolism and Pharmacokinetics, Biogen Idec, Cambridge, MA, USA

**Cindy Q. Xia**, Biotransformation/DMPK, Drug Safety and Disposition, Millennium: The Takeda Oncology Company, Cambridge, MA, USA

**Yang Xu**, Pharmacokinetics and Drug Metabolism, Amgen Inc., Thousand Oaks, CA, USA

**Ming Yao**, Bristol-Myers Squibb, Princeton, NJ, USA

**Richard A. Yost**, Department of Chemistry, University of Florida, Gainesville, FL, USA

**Ai-Ming Yu**, Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, The State University of New York, Buffalo, NY, USA

**Haoyu Zeng**, Safety Assessment, Merck Research Laboratories, West Point, PA, USA

**Donglu Zhang**, Bristol-Myers Squibb, Princeton, NJ, USA

**Yingru Zhang**, Bristol-Myers Squibb, Princeton, NJ, USA

**Zhoupeng Zhang**, Merck & Co. Inc., Rahway, NJ, USA

**Mingshe Zhu**, Bristol-Myers Squibb, Princeton, NJ, USA

**Peijuan Zhu**, Respiratory Therapeutic Area Unit, GlaxoSmithKline, King of Prussia, PA, USA

# CONTENTS

<b>FOREWORD</b>	<b>xxi</b>
<i>Lisa A. Shipley</i>	
<b>PREFACE</b>	<b>xxv</b>
<i>Donglu Zhang and Sekhar Surapaneni</i>	
<b>CONTRIBUTORS</b>	<b>xxvii</b>
<b>PART A ADME: OVERVIEW AND CURRENT TOPICS</b>	<b>1</b>
<b>1 Regulatory Drug Disposition and NDA Package Including MIST</b>	<b>3</b>
<i>Sekhar Surapaneni</i>	
1.1 Introduction	3
1.2 Nonclinical Overview	5
1.3 PK	5
1.4 Absorption	5
1.5 Distribution	6
1.5.1 Plasma Protein Binding	6
1.5.2 Tissue Distribution	6
1.5.3 Lacteal and Placental Distribution Studies	7
1.6 Metabolism	7
1.6.1 <i>In vitro</i> Metabolism Studies	7
1.6.2 Drug–Drug Interaction Studies	8
1.6.3 <i>In vivo</i> Metabolism (ADME) Studies	10
1.7 Excretion	11
1.8 Impact of Metabolism Information on Labeling	11
1.9 Conclusions	12
References	12
<b>2 Optimal ADME Properties for Clinical Candidate and Investigational New Drug (IND) Package</b>	<b>15</b>
<i>Rajinder Bhardwaj and Gamini Chandrasena</i>	
2.1 Introduction	15
2.2 NCE and Investigational New Drug (IND) Package	16

2.3	ADME Optimization	17
2.3.1	Absorption	18
2.3.2	Metabolism	20
2.3.3	PK	22
2.4	ADME Optimization for CNS Drugs	23
2.5	Summary	24
	References	25
<b>3</b>	<b>Drug Transporters in Drug Interactions and Disposition</b>	<b>29</b>
	<i>Imad Hanna and Ryan M. Pelis</i>	
3.1	Introduction	29
3.2	ABC Transporters	31
3.2.1	Pgp (MDR1, ABCB1)	31
3.2.2	BCRP (ABCG2)	32
3.2.3	MRP2 (ABCC2)	32
3.3	SLC Transporters	33
3.3.1	OCT1 (SLC22A1) and OCT2 (SLC22A2)	34
3.3.2	MATE1 (SLC47A1) and MATE2K (SLC47A2)	35
3.3.3	OAT1 (SLC22A6) and OAT3 (SLC22A8)	36
3.3.4	OATP1B1 (SLCO1B1, SLC21A6), OATP1B3 (SLCO1B3, SLC21A8), and OATP2B1 (SLCO2B1, SLC21A9)	37
3.4	<i>In vitro</i> Assays in Drug Development	39
3.4.1	Considerations for Assessing Candidate Drugs as Inhibitors	39
3.4.2	Considerations for Assessing Candidate Drugs as Substrates	39
3.4.3	Assay Systems	40
3.5	Conclusions and Perspectives	45
	References	46
<b>4</b>	<b>Pharmacological and Toxicological Activity of Drug Metabolites</b>	<b>55</b>
	<i>W. Griffith Humphreys</i>	
4.1	Introduction	55
4.2	Assessment of Potential for Active Metabolites	56
4.2.1	Detection of Active Metabolites during Drug Discovery	58
4.2.2	Methods for Assessing and Evaluating the Biological Activity of Metabolite Mixtures	58
4.2.3	Methods for Generation of Metabolites	59
4.3	Assessment of the Potential Toxicology of Metabolites	59
4.3.1	Methods to Study the Formation of Reactive Metabolites	60
4.3.2	Reactive Metabolite Studies: <i>In vitro</i>	61
4.3.3	Reactive Metabolite Studies: <i>In vivo</i>	61
4.3.4	Reactive Metabolite Data Interpretation	61
4.3.5	Metabolite Contribution to Off-Target Toxicities	62
4.4	Safety Testing of Drug Metabolites	62
4.5	Summary	63
	References	63
<b>5</b>	<b>Improving the Pharmaceutical Properties of Biologics in Drug Discovery: Unique Challenges and Enabling Solutions</b>	<b>67</b>
	<i>Jiwen Chen and Ashok Dongre</i>	
5.1	Introduction	67
5.2	Pharmacokinetics	68
5.3	Metabolism and Disposition	70

5.4	Immunogenicity	71
5.5	Toxicity and Preclinical Assessment	74
5.6	Comparability	74
5.7	Conclusions	75
	References	75
<b>6</b>	<b>Clinical Dose Estimation Using Pharmacokinetic/Pharmacodynamic Modeling and Simulation</b>	<b>79</b>
	<i>Lingling Guan</i>	
6.1	Introduction	79
6.2	Biomarkers in PK and PD	80
6.2.1	PK	80
6.2.2	PD	81
6.2.3	Biomarkers	81
6.3	Model-Based Clinical Drug Development	83
6.3.1	Modeling	83
6.3.2	Simulation	84
6.3.3	Population Modeling	85
6.3.4	Quantitative Pharmacology (QP) and Pharmacometrics	85
6.4	First-in-Human Dose	86
6.4.1	Drug Classification Systems as Tools for Development	86
6.4.2	Interspecies and Allometric Scaling	87
6.4.3	Animal Species, Plasma Protein Binding, and <i>in vivo</i> – <i>in vitro</i> Correlation	88
6.5	Examples	89
6.5.1	First-in-Human Dose	89
6.5.2	Pediatric Dose	90
6.6	Discussion and Conclusion	90
	References	93
<b>7</b>	<b>Pharmacogenomics and Individualized Medicine</b>	<b>95</b>
	<i>Anthony Y.H. Lu and Qiang Ma</i>	
7.1	Introduction	95
7.2	Individual Variability in Drug Therapy	95
7.3	We Are All Human Variants	96
7.4	Origins of Individual Variability in Drug Therapy	96
7.5	Genetic Polymorphism of Drug Targets	97
7.6	Genetic Polymorphism of Cytochrome P450s	98
7.7	Genetic Polymorphism of Other Drug Metabolizing Enzymes	100
7.8	Genetic Polymorphism of Transporters	100
7.9	Pharmacogenomics and Drug Safety	101
7.10	Warfarin Pharmacogenomics: A Model for Individualized Medicine	102
7.11	Can Individualized Drug Therapy Be Achieved?	104
7.12	Conclusions	104
	Disclaimer	105
	Contact Information	105
	References	105
<b>8</b>	<b>Overview of Drug Metabolism and Pharmacokinetics with Applications in Drug Discovery and Development in China</b>	<b>109</b>
	<i>Chang-Xiao Liu</i>	
8.1	Introduction	109

8.2	PK–PD Translation Research in New Drug Research and Development	109
8.3	Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADME/T) Studies in Drug Discovery and Early Stage of Development	110
8.4	Drug Transporters in New Drug Research and Development	111
8.5	Drug Metabolism and PK Studies for New Drug Research and Development	113
8.5.1	Technical Guidelines for PK Studies in China	113
8.5.2	Studies on New Molecular Entity (NME) Drugs	114
8.5.3	PK Calculation Program	117
8.6	Studies on the PK of Biotechnological Products	117
8.7	Studies on the PK of TCMs	118
8.7.1	The Challenge in PK Research of TCMs	118
8.7.2	New Concept on PK Markers	120
8.7.3	Identification of Nontarget Components from Herbal Preparations	122
8.8	PK and Bioavailability of Nanomaterials	123
8.8.1	Research and Development of Nanopharmaceuticals	123
8.8.2	Biopharmaceutics and Therapeutic Potential of Engineered Nanomaterials	123
8.8.3	Biodistribution and Biodegradation	123
8.8.4	Doxorubicin Polyethylene Glycol-Phosphatidylethanolamine (PEG-PE) Nanoparticles	124
8.8.5	Micelle-Encapsulated Alprostadil (M-Alp)	124
8.8.6	Paclitaxel Magnetoliposomes	125
	References	125

## **PART B ADME SYSTEMS AND METHODS** **129**

### **9 Technical Challenges and Recent Advances of Implementing Comprehensive ADMET Tools in Drug Discovery** **131**

*Jianling Wang and Leslie Bell*

9.1	Introduction	131
9.2	“A” Is the First Physiological Barrier That a Drug Faces	131
9.2.1	Solubility and Dissolution	131
9.2.2	GI Permeability and Transporters	136
9.3	“M” Is Frequently Considered Prior to Distribution Due to the “First-Pass” Effect	139
9.3.1	Hepatic Metabolism	139
9.3.2	CYPs and Drug Metabolism	140
9.4	“D” Is Critical for Correctly Interpreting PK Data	142
9.4.1	Blood/Plasma Impact on Drug Distribution	142
9.4.2	Plasma Stability	143
9.4.3	PPB	144
9.4.4	Blood/Plasma Partitioning	144
9.5	“E”: The Elimination of Drugs Should Not Be Ignored	145
9.6	Metabolism- or Transporter-Related Safety Concerns	146
9.7	Reversible CYP Inhibition	147
9.7.1	<i>In vitro</i> CYP Inhibition	147

9.7.2	Human Liver Microsomes (HLM) + Prototypical Probe Substrates with Quantification by LC-MS	147
9.7.3	Implementation Strategy	149
9.8	Mechanism-Based (Time-Dependent) CYP Inhibition	149
9.8.1	Characteristics of CYP3A TDI	150
9.8.2	<i>In vitro</i> Screening for CYP3A TDI	150
9.8.3	Inactivation Rate ( $k_{\text{obs}}$ )	150
9.8.4	IC <sub>50</sub> -Shift	151
9.8.5	Implementation Strategy	152
9.9	CYP Induction	152
9.10	Reactive Metabolites	153
9.10.1	Qualitative <i>in vitro</i> Assays	153
9.10.2	Quantitative <i>in vitro</i> Assay	154
9.11	Conclusion and Outlook	154
	Acknowledgments	155
	References	155
<b>10</b>	<b>Permeability and Transporter Models in Drug Discovery and Development</b>	<b>161</b>
	<i>Praveen V. Balimane, Yong-Hae Han, and Saeho Chong</i>	
10.1	Introduction	161
10.2	Permeability Models	162
10.2.1	PAMPA	162
10.2.2	Cell Models (Caco-2 Cells)	162
10.2.3	P-glycoprotein (Pgp) Models	162
10.3	Transporter Models	163
10.3.1	Intact Cells	164
10.3.2	Transfected Cells	165
10.3.3	<i>Xenopus</i> Oocyte	165
10.3.4	Membrane Vesicles	165
10.3.5	Transgenic Animal Models	166
10.4	Integrated Permeability–Transporter Screening Strategy	166
	References	167
<b>11</b>	<b>Methods for Assessing Blood–Brain Barrier Penetration in Drug Discovery</b>	<b>169</b>
	<i>Li Di and Edward H. Kerns</i>	
11.1	Introduction	169
11.2	Common Methods for Assessing BBB Penetration	170
11.3	Methods for Determination of Free Drug Concentration in the Brain	170
11.3.1	<i>In vivo</i> Brain PK in Combination with <i>in vitro</i> Brain Homogenate Binding Studies	171
11.3.2	Use of CSF Drug Concentration as a Surrogate for Free Drug Concentration in the Brain	171
11.4	Methods for BBB Permeability	172
11.4.1	<i>In situ</i> Brain Perfusion Assay	172
11.4.2	High-throughput PAMPA-BBB	173
11.4.3	Lipophilicity (LogD <sub>7.4</sub> )	173
11.5	Methods for Pgp Efflux Transport	173
11.6	Conclusions	174
	References	174



<b>12</b>	<b>Techniques for Determining Protein Binding in Drug Discovery and Development</b>	<b>177</b>
	<i>Tom Lloyd</i>	
12.1	Introduction	177
12.2	Overview	178
12.3	Equilibrium Dialysis	179
12.4	Ultracentrifugation	180
12.5	Ultrafiltration	181
12.6	Microdialysis	182
12.7	Spectroscopy	182
12.8	Chromatographic Methods	183
12.9	Summary Discussion	183
	Acknowledgment	185
	References	185
<b>13</b>	<b>Reaction Phenotyping</b>	<b>189</b>
	<i>Chun Li and Nataraj Kalyanaraman</i>	
13.1	Introduction	189
13.2	Initial Considerations	190
13.2.1	Clearance Mechanism	190
13.2.2	Selecting the Appropriate <i>in vitro</i> System	191
13.2.3	Substrate Concentration	191
13.2.4	Effect of Incubation Time and Protein Concentration	192
13.2.5	Determination of Kinetic Constant $K_m$ and $V_{max}$	192
13.2.6	Development of Analytical Methods	192
13.3	CYP Reaction Phenotyping	193
13.3.1	Specific Chemical Inhibitors	194
13.3.2	Inhibitory CYP Antibodies	195
13.3.3	Recombinant CYP Enzymes	196
13.3.4	Correlation Analysis for CYP Reaction Phenotyping	198
13.3.5	CYP Reaction Phenotyping in Drug Discovery versus Development	198
13.4	Non-P450 Reaction Phenotyping	199
13.4.1	FMOs	199
13.4.2	MAOs	200
13.4.3	AO	200
13.5	UGT Conjugation Reaction Phenotyping	201
13.5.1	Initial Considerations in UGT Reaction Phenotyping	202
13.5.2	Experimental Approaches for UGT Reaction Phenotyping	202
13.5.3	Use of Chemical Inhibitors for UGTs	203
13.5.4	Correlation Analysis for UGT Reaction Phenotyping	204
13.6	Reaction Phenotyping for Other Conjugation Reactions	204
13.7	Integration of Reaction Phenotyping and Prediction of DDI	205
13.8	Conclusion	205
	References	206
<b>14</b>	<b>Fast and Reliable CYP Inhibition Assays</b>	<b>213</b>
	<i>Ming Yao, Hong Cai, and Mingshe Zhu</i>	
14.1	Introduction	213
14.2	CYP Inhibition Assays in Drug Discovery and Development	215

14.3	HLM Reversible CYP Inhibition Assay Using Individual Substrates	217
14.3.1	Choice of Substrate and Specific Inhibitors	217
14.3.2	Optimization of Incubation Conditions	217
14.3.3	Incubation Procedures	217
14.3.4	LC-MS/MS Analysis	221
14.3.5	Data Calculation	221
14.4	HLM RI Assay Using Multiple Substrates (Cocktail Assays)	222
14.4.1	Choice of Substrate and Specific Inhibitors	222
14.4.2	Optimization of Incubations	223
14.4.3	Incubation Procedures	223
14.4.4	LC-MS/MS Analysis	224
14.4.5	Data Calculation	224
14.5	Time-Dependent CYP Inhibition Assay	226
14.5.1	IC <sub>50</sub> Shift Assay	226
14.5.2	K <sub>i</sub> and K <sub>inact</sub> Measurements	227
14.5.3	Data Calculation	228
14.6	Summary and Future Directions	228
	References	230
<b>15</b>	<b>Tools and Strategies for the Assessment of Enzyme Induction in Drug Discovery and Development</b>	<b>233</b>
	<i>Adrian J. Fretland, Anshul Gupta, Peijuan Zhu, and Catherine L. Booth-Genthe</i>	
15.1	Introduction	233
15.2	Understanding Induction at the Gene Regulation Level	233
15.3	<i>In silico</i> Approaches	234
15.3.1	Model-Based Drug Design	234
15.3.2	Computational Models	234
15.4	<i>In vitro</i> Approaches	235
15.4.1	Ligand Binding Assays	235
15.4.2	Reporter Gene Assays	236
15.5	<i>In vitro</i> Hepatocyte and Hepatocyte-Like Models	238
15.5.1	Hepatocyte Cell-Based Assays	238
15.5.2	Hepatocyte-Like Cell-Based Assays	239
15.6	Experimental Techniques for the Assessment of Induction in Cell-Based Assays	239
15.6.1	mRNA Quantification	240
15.6.2	Protein Quantification	241
15.6.3	Assessment of Enzyme Activity	244
15.7	Modeling and Simulation and Assessment of Risk	244
15.8	Analysis of Induction in Preclinical Species	245
15.9	Additional Considerations	245
15.10	Conclusion	246
	References	246
<b>16</b>	<b>Animal Models for Studying Drug Metabolizing Enzymes and Transporters</b>	<b>253</b>
	<i>Kevin L. Salyers and Yang Xu</i>	
16.1	Introduction	253
16.2	Animal Models of DMEs	253
16.2.1	Section Objectives	253
16.2.2	<i>In vivo</i> Models to Study the Roles of DMEs in Determining Oral Bioavailability	254