

Contents

List of Contributors	XV
Preface	XXI
A Personal Foreword	XXIII

Section 1 General Concept for Target-based Safety Assessment 1

1	Side Effects of Marketed Drugs: The Utility and Pitfalls of Pharmacovigilance 3
	<i>Steven Whitebread, Mateusz Maciejewski, Alexander Fekete, Eugen Lounkine, and László Urbán</i>
1.1	Introduction 3
1.2	Postmarketing Pharmacovigilance 6
1.3	Polypharmacy and Pharmacological Promiscuity of Marketed Drugs 9
	References 15
2	<i>In Silico</i> Prediction of Drug Side Effects 19
	<i>Michael J. Keiser</i>
2.1	Large-Scale Prediction of Drug Activity 20
2.1.1	Networks of Known and New Target Activity 21
2.1.1.1	Predicting Drug Off-Targets by Statistical Chemical Similarity 21
2.1.1.2	Representing Drugs Computationally for Rapid Comparison 23
2.1.2	Resources for Multiscale Inquiry 25
2.1.2.1	Ligands to Targets 25
2.1.2.2	Perturbing Biological Systems (Phenotypes) 25
2.1.2.3	Functional and Biological Annotations (Diseases) 27
2.1.2.4	Adverse Reactions as Drug-Induced Diseases 29
2.2	Multiscale Models of Adverse Drug Reactions 30
2.2.1	Inferring Adverse Reactions 31
2.2.1.1	From Off-Targets to Antitargets 31
2.2.1.2	Systematic Antitarget Prediction and Testing 32
2.2.1.3	Finding Side Effects sans Targets 33
2.2.2	Forward Perturbation and Prediction of Mechanisms 33

2.2.2.1	Forward Synthetic Behavior in Cell and Whole-Organism Model Systems	33
2.2.2.2	The Road Ahead	36
	References	36
3	Translational Value of Preclinical Safety Assessment: System Organ Class (SOC) Representation of Off-Targets	45
	<i>Mateusz Maciejewski, Eugen Lounkine, Andreas Hartmann, Steven Whitebread, and László Urbán</i>	
3.1	Introduction	45
3.2	Terminology: Medicinal Dictionary for Regulatory Activities (MedDRA)	46
3.2.1	Correct Use of MedDRA Terminology at Different Phases of Drug Discovery	48
3.2.2	Determination of Symptoms Associated with a Target	50
3.3	Data Interpretation: Modifying Factors	52
3.3.1	Access to Organs	52
3.3.2	Off-Target Promiscuity: Target Interactions (Synergies and Antagonism)	53
3.4	Conclusions	53
	References	54
4	Pathological Conditions Associated with the Disturbance of the 5-HT System	57
	<i>Daniel Hoyer</i>	
4.1	Introduction	57
4.2	From “St. Anthony’s Fire” to Ergot Alkaloids, the Serotonin Syndrome, and Modern 5-HT Pharmacology	59
4.3	Appetite-Reducing Agents, Fenfluramine, and Other 5-HT Releasers	61
4.4	Gastrointestinal and Antiemetic Indications, the 5-HT ₃ /5-HT ₄ Receptor Links	63
4.5	Antipsychotics and the 5-HT ₂ /Dopamine D ₂ Link (and Many Other 5-HT Receptors)	65
4.6	Antimigraine Medications of Old and New and the 5-HT _{1B/1D} Receptors	67
4.7	Antidepressants/Anxiolytics Acting at 5-HT and Other Transporters	69
4.8	Conclusions	71
	References	72
Section 2	Hepatic Side Effects	81
5	Drug-Induced Liver Injury: Clinical and Diagnostic Aspects	83
	<i>John R. Senior</i>	
5.1	Introduction	83
5.1.1	Postmarketing Hepatotoxicity versus Hepatotoxicity in Development	84

5.1.2	Isoniazid – If It Were Newly Discovered, Would It Be Approved Today? 85
5.2	Special Problems of Postmarketing Hepatotoxicity 89
5.2.1	Voluntary Monitoring after Approval for Marketing 90
5.2.2	Prediction of Serious, Dysfunctional Liver Injury 90
5.2.3	Severity of Liver Injury Is Not Measured by Aminotransferase Elevations 91
5.2.4	Attempts to Standardize Terminology 91
5.2.5	What Is the “Normal” Range, or the “Upper Limit of Normal”? 92
5.2.6	Diagnostic Test Evaluation 93
5.2.7	Determination of the Likely Cause of Liver Abnormalities 94
5.2.8	Treatment and Management of DILI in Practice 95
5.3	Special Problems for New Drug Development 95
5.3.1	How Many? 95
5.3.2	How Much? 96
5.3.3	How Soon? 97
5.3.4	How Likely? 97
5.3.5	Compared with What? 97
5.3.6	ROC Curves 98
5.3.7	eDISH: Especially for Controlled Trials 99
5.3.8	Test Validation and Qualification 100
5.4	Closing Considerations 101
5.4.1	A Handful of “Do Nots” 101
5.4.2	Need to Standardize ALT Measurement and Interpretation of Normal Ranges 102
5.4.3	Research Opportunities 102
	References 103
6	Mechanistic Safety Biomarkers for Drug-Induced Liver Injury 107
	<i>Daniel J. Antoine</i>
6.1	Introduction 107
6.2	Drug-Induced Toxicity and the Liver 110
6.3	Current Status of Biomarkers for the Assessment of DILI 111
6.4	Novel Investigational Biomarkers for DILI 113
6.4.1	Glutamate Dehydrogenase (GLDH) 114
6.4.2	Acylcarnitines 115
6.4.3	High-Mobility Group Box-1 (HMGB1) 116
6.4.4	Keratin 18 (K18) 116
6.4.5	MicroRNA-122 (miR-122) 117
6.5	Conclusions and Future Perspectives 118
	References 120

7	<i>In Vitro Models for the Prediction of Drug-Induced Liver Injury in Lead Discovery</i> 125
	<i>Frederic Moulin and Oliver Flint</i>
7.1	Introduction 125
7.2	Simple Systems for the Detection and Investigation of Hepatic Toxicants 130
7.2.1	Primary Hepatocytes 130
7.2.1.1	Cells 131
7.2.1.2	Cell Culture Conditions 131
7.2.1.3	Toxicity Endpoints 132
7.2.1.4	Limitations of Hepatocyte Cultures 133
7.2.2	Liver-Derived Cell Lines 135
7.2.2.1	HepG2 135
7.2.2.2	HepaRG 136
7.2.3	Differentiated Pluripotent Stem Cells 137
7.2.3.1	Embryonic Stem Cells 137
7.2.3.2	Induced Pluripotent Stem Cells 138
7.3	Models to Mitigate Hepatocyte Dedifferentiation 140
7.3.1	Liver Slices 140
7.3.2	Selective Engineering of Metabolism 141
7.4	Understanding Immune-Mediated Hepatotoxicity 144
7.4.1	Use of Inflammatory Cofactors 145
7.4.2	Innate Immune System and Inflammasome 147
7.5	Conclusions 148
	References 149
8	Transporters in the Liver 159
	<i>Bruno Stieger and Gerd A. Kullak-Ublick</i>
8.1	Introduction 159
8.2	Role of Organic Anion Transporters for Drug Uptake 159
8.3	Drug Interaction with the Bile Salt Export Pump 160
8.4	Susceptibility Factors for Drug–BSEP Interactions 161
8.5	Role of BSEP in Drug Development 162
	References 163
9	Mechanistic Modeling of Drug-Induced Liver Injury (DILI) 173
	<i>Kyunghee Yang, Jeffrey L. Woodhead, Lisl K. Shoda, Yuching Yang, Paul B. Watkins, Kim L.R. Brouwer, Brett A. Howell, and Scott Q. Siler</i>
9.1	Introduction 173
9.2	Mechanistic Modules in DILIsym® version 3A 175
9.2.1	Oxidative Stress-Mediated Toxicity 175
9.2.2	Innate Immune Responses 178
9.2.3	Mitochondrial Toxicity 179
9.2.4	Bile Acid-Mediated Toxicity 181
9.3	Examples of Bile Acid-Mediated Toxicity Module 184

9.3.1	Troglitazone and Pioglitazone	184
9.3.2	Bosentan and Telmisartan	187
9.4	Conclusions and Future Directions	190
	References	191

Section 3 Cardiovascular Side Effects 199

10	Functional Cardiac Safety Evaluation of Novel Therapeutics	201
	<i>Jean-Pierre Valentin, Brian Guth, Robert L. Hamlin, Pierre Lainée, Dusty Sarazan, and Matt Skinner</i>	
10.1	Introduction: What Is the Issue?	201
10.2	Cardiac Function: Definitions and General Principles	203
10.2.1	Definition and Importance of Inotropy and Difference from Ventricular Function	203
10.2.2	Definition and Importance of Lusitropy	207
10.2.3	Components and Importance of the Systemic Arterial Pressure	211
10.2.3.1	Afterload	212
10.3	Methods Available to Assess Cardiac Function	213
10.4	What Do We Know About the Translation of the Nonclinical Findings to Humans?	217
10.5	Risk Assessment	219
10.5.1	Hazard Identification	219
10.5.2	Risk Assessment	221
10.5.3	Risk Management	224
10.5.4	Risk Mitigation	225
10.6	Summary, Recommendations, and Conclusions	227
	References	228
11	Safety Aspects of the Ca_v1.2 Channel	235
	<i>Berengere Dumotier and Martin Traebert</i>	
11.1	Introduction	235
11.2	Structure of Ca _v 1.2 Channels	235
11.2.1	α-Subunit of Ca _v 1.2 Channel	236
11.2.2	β-Subunit of Ca _v 1.2 Channel	236
11.3	Function of Ca _v 1.2 Channels in Cardiac Tissue	237
11.3.1	Role in Conduction and Contractility	239
11.3.2	Modulation of Ca _v 1.2 Channels	240
11.3.2.1	Voltage- and Calcium-Dependent Facilitation	241
11.3.2.2	Sympathetic Stimulation and Kinase Regulation	241
11.3.2.3	Inactivation	242
11.3.2.4	Regulation by Calmodulin	242
11.3.2.5	Indirect Regulation of Ca _v 1.2 Channels	243
11.3.3	Ca _v 1.2 and Cardiac Diseases	244
11.4	Pharmacology of Ca _v 1.2 Channels: Translation to the Clinic	245
11.4.1	Ca _v 1.2 Antagonists: Impact on Electromechanical Functions	245
11.5	Prediction of Ca _v 1.2 Off-Target Liability	246
11.5.1	Ca _v 1.2 in Cardiomyocytes Derived from iPS Cells	246
	References	247

12	Cardiac Sodium Current ($I_{NaV1.5}$)	253
	<i>Gary Gintant</i>	
12.1	Background and Scope	253
12.2	Structure and Function	255
12.2.1	Molecular Biology	255
12.2.2	SCN5A Mutations Related to Congenital Long QT Syndromes	256
12.2.3	Evidence for Multiple Functional Types of Cardiac Sodium Channels and Heterogeneous Distribution	257
12.3	Physiological Role and Drug Actions	258
12.3.1	Fast Sodium Current (I_{NaF}): Conduction and Refractoriness	258
12.3.2	Late (or Residual or Slow) Sodium Current (I_{NaL})	259
12.3.3	Drug Effects on I_{NaF}	261
12.3.3.1	Voltage-Dependent Block	262
12.3.3.2	Use-Dependent Block (and Tonic Block)	262
12.3.3.3	Models of Block and Classification Schemes Based on Antiarrhythmic Drug Effects	263
12.3.4	Indirect Modulation of I_{NaF}	264
12.4	Methodology	265
12.4.1	Use of Human Stem Cell-Derived Cardiomyocytes	266
12.5	Translation of Effects on I_{NaF} : Relation to Conduction Velocity and Proarrhythmia	268
12.6	Conclusions	269
	References	270
13	Circulating Biomarkers for Drug-Induced Cardiotoxicity: Reverse Translation from Patients to Nonclinical Species	279
	<i>Gül Erdemli, Haisong Ju, and Sarita Pereira</i>	
13.1	Introduction	279
13.2	Cardiac Troponins	280
13.3	Natriuretic Peptides	282
13.4	Novel/Exploratory Biomarkers: H-FABP, miRNA, and Genomic Biomarkers	285
13.5	Regulatory Perspective	286
13.6	Conclusions and Future Perspectives	288
	References	289
14	The Mechanistic Basis of hERG Blockade and the Proarrhythmic Effects Thereof	295
	<i>Robert A. Pearlstein, K. Andrew MacCannell, Qi-Ying Hu, Ramy Farid, and José S. Duca</i>	
14.1	Introduction	295
14.1.1	The Role of hERG Dysfunction/Blockade in Promoting Early After Depolarizations	296
14.1.2	The Dynamics of hERG Blockade	301

14.1.3	Simulations of the Human Cardiac AP in the Presence of hERG Blockade	303
14.1.4	Estimation of Proarrhythmic hERG Occupancy Levels Based on AP Simulations	304
14.1.5	Novel Insights about the Causes of Inadvertent hERG Binding Function	305
14.1.6	Implications of Our Findings for hERG Safety Assessment	313
14.1.7	Conclusion and Future Directions	324
	References	324

Section 4 Kinase Antitargets 329

15	Introduction to Kinase Antitargets	331
	<i>Mark C. Munson</i>	
	References	360
16	Clinical and Nonclinical Adverse Effects of Kinase Inhibitors	365
	<i>Douglas A. Keller, Richard J. Brennan, and Karen L. Leach</i>	
16.1	Introduction	365
16.2	Perspectives on the Clinical Safety of Kinase Inhibitor Therapy	371
16.3	Adverse Effects of Kinase Inhibitor Drugs	372
16.3.1	Hepatic Toxicity	372
16.3.1.1	Role of Metabolism and Clearance Pathways in Hepatotoxicity	373
16.3.1.2	Genetic Risk Factors for Hepatotoxicity	375
16.3.1.3	Preclinical Evaluation of Hepatotoxicity	376
16.3.2	Thyroid Toxicity	377
16.3.2.1	Mechanistic Basis of Thyroid Toxicity	378
16.3.2.2	Clinical Management of Thyroid Toxicity	378
16.3.3	Bone and Tooth Toxicity	379
16.3.4	Cardiovascular Toxicity	380
16.3.5	Cutaneous Toxicity	380
16.3.5.1	Mechanistic Basis of Cutaneous Toxicity	381
16.3.5.2	Preclinical Evaluation of Cutaneous Toxicity	381
16.3.5.3	Clinical Management of Cutaneous Toxicity	383
16.3.6	Developmental and Reproductive Toxicity	383
16.3.6.1	Preclinical Evaluation of Reproductive Toxicity	384
16.3.6.2	Clinical Management of Reproductive Toxicity	384
16.3.7	Gastrointestinal Toxicity	385
16.3.8	Hematopoietic Toxicity	385
16.3.8.1	Mechanistic Basis of Hematopoietic Toxicity	385
16.3.8.2	Preclinical Evaluation of Hematopoietic Toxicity	387
16.3.9	Ocular Toxicity	387

16.3.9.1	Mechanistic Basis of Ocular Toxicity	387
16.3.9.2	Preclinical Evaluation of Ocular Toxicity	388
16.3.10	Pulmonary Toxicity	388
16.3.11	Renal Toxicity	389
16.4	Derisking Strategies for Kinase Inhibitor Toxicity	389
16.5	Concluding Remarks	391
	References	391
17	Cardiac Side Effects Associated with Kinase Proteins and Their Signaling Pathways	401
	<i>Roy J. Vaz and Vinod F. Patel</i>	
17.1	A Case Study	401
17.2	Introduction	402
17.3	Cardiac-Specific Kinase Antitargets	404
17.3.1	Preclinical Findings in Genetically Modified or KI-Treated Mice	404
17.3.2	Clinical Findings of Kinase Inhibitors on the Heart and Their Mechanistic Understandings	404
17.3.2.1	ErbB2 Inhibition	404
17.3.2.2	EGFR Inhibition	406
17.3.2.3	Dual EGFR/ErbB2 Inhibition	406
17.3.2.4	Raf Inhibition	407
17.3.2.5	MEK Inhibition	407
17.3.2.6	JAK/STAT Inhibition	407
17.3.2.7	Bcr–Abl Inhibition	408
17.3.2.8	PDGFR and c-Kit Inhibition	408
17.3.2.9	VEGFR Inhibition	408
17.4	Current and Future Directions	409
17.4.1	Preclinical Safety and Clinical Outcome Predictions	409
17.5	Conclusions	410
	References	411
18	Case Studies: Selective Inhibitors of Protein Kinases – Exploiting Demure Features	413
	<i>Ellen R. Laird</i>	
18.1	Introduction	413
18.2	Case I: Indane Oximes as Selective B-Raf Inhibitors	414
18.3	Case II: ARRY-380 (ONT-380) – an ErbB2 Agent that Spares EGFR	420
18.4	Case III: Discovery of GDC-0068 (Ipatasertib), a Potent and Selective ATP-Competitive Inhibitor of AKT	424
18.5	Concluding Remarks	428
	References	429

Section 5 Examples of Clinical Translation 435

- 19 Torcetrapib and Dalcetrapib Safety: Relevance of Preclinical *In Vitro* and *In Vivo* Models 437
Eric J. Niesor, Andrea Greiter-Wilke, and Lutz Müller
- 19.1 Introduction 437
- 19.2 Effect of Torcetrapib on Blood Pressure 437
- 19.3 *In Vitro* Studies 438
- 19.3.1 Direct Effect of Torcetrapib on Aldosterone Production *In Vitro* in Cultured H295R Adrenal Corticocarcinoma Cells 439
- 19.3.2 Molecular Mechanism of Torcetrapib Induction of Aldosterone Secretion 439
- 19.3.3 Development of Reproducible *In Vitro* Screening Models for Increase in Aldosterone and Cyp11B2 mRNA in a Human Adrenal Corticocarcinoma Cell Line 440
- 19.3.4 Application of *In Vitro* Models for the Successful Derisking of Dalcetrapib, Anacetrapib, and Evacetrapib 440
- 19.4 *In Vivo* Studies 441
- 19.4.1 Effect of Torcetrapib on Aldosterone and BP 441
- 19.4.1.1 Immediate Increase (Transient) in BP in Normotensive Wistar Rats 441
- 19.4.1.2 Sustained Increase in BP in Spontaneously Hypertensive and Zucker Diabetic Fatty Rats 441
- 19.4.1.3 Tissue mRNA Analysis Suggested Involvement of the Renin–Angiotensin–Aldosterone System (RAAS) 442
- 19.4.1.4 Increase in BP and Aldosterone with Torcetrapib in All Species Tested 443
- 19.4.2 Molecular Mechanisms of Torcetrapib-Induced BP Increase 444
- 19.4.2.1 Torcetrapib-Positive Inotropism and Increased Cardiac Work in a Dog Telemetry Study 446
- 19.4.2.2 A Common Molecular Mechanism for BP and Induction of Aldosterone Secretion? 447
- 19.5 General Safety Risk with Increased Aldosterone and BP 447
- 19.5.1 Inappropriate Increase in Aldosterone Secretion May Increase CV Risks 447
- 19.6 Relevance of BP and Aldosterone Preclinical Models to Clinical Observation with Dalcetrapib and Anacetrapib 448
- 19.7 Similarities between Potent CETPi and Halogenated Hydrocarbons 449
- 19.7.1 The Macrophage Scavenger Receptor MARCO, a Possible Antitarget for Dalcetrapib, and Its Relevance to Humans 450
- 19.8 Conclusions 451
References 451

20	Targets Associated with Drug-Related Suicidal Ideation and Behavior 457
	<i>Andreas Hartmann, Steven Whitebread, Jacques Hamon, Alexander Fekete, Christian Trendelenburg, Patrick Y. Müller, and László Urbán</i>
20.1	Introduction 457
20.2	Targets Associated with Increased Suicidal Intent and Behavior 458
20.2.1	G-Protein-Coupled Receptors 458
20.2.1.1	Dopamine D₁ and D₂ Receptors (DRD1 and DRD2) 458
20.2.1.2	Cannabinoid CB1 Receptor (CNR1) 462
20.2.1.3	Serotonin (5-HT_{1A}) Receptor (HTR1A) 464
20.2.1.4	5-HT_{2A} (HTR2A) 465
20.2.2	Transporters 466
20.2.2.1	Serotonin Transporter (SLC6A4) 466
20.2.2.2	Norepinephrine Transporter (SLC6A2) 468
20.2.2.3	Vesicular Monoamine Transporter, VMAT2 (SLC18A2) 468
20.2.3	Ion Channels 469
20.2.3.1	Neuronal Nicotinic $\alpha 4\beta 2$ Channel (CHRNA4) 469
20.2.3.2	Neural-Type Voltage-Gated Calcium Channel, Ca_v2.2 (CACNA1B) 471
20.3	Conclusions 472
	References 473
	Index 479