# Contents

**Foreword** *xiii*
**Preface** *xv*
**Acknowledgments** *xix*
**Epigraph** *xxi*

**1 The History of Molecular Medicine** *1*
1.1 Disease and Treatments in Antiquity *2*
1.2 Natural Products as Medicines *2*
1.3 Isolation of Active Ingredients and Chemical Synthesis *4*
1.4 The Receptor Theory of Drug Action *9*
1.5 Early Chemotherapy: Attempts to Mimic Nature *15*
1.6 Linus Pauling Establishes Sickle Cell Anemia as a Molecular Disease *19*
1.7 ACE Inhibitors: An Early Example of Targeted Therapeutics *21*
1.8 Genetic Alterations in Disease *26*
1.9 Structure-Based Drug Design *28*
1.10 Recombinant DNA and Protein Engineering *31*
1.11 Modern Drug Discovery *35*
1.12 Summary *38*
References *39*
Further Reading *43*

**2 Molecular Targeted Therapeutic Modalities** *45*
2.1 Candidate Drug Profile *48*
2.2 Biologic Therapeutics *49*
2.2.1 Therapeutic Antibody Discovery *52*
2.2.1.1 Target Selection and Antibody Generation *52*
2.2.1.2 Hit-Finding and Hit-to-Lead *53*
2.2.1.3 Lead Optimization *54*

2.3 Small-Molecule Drugs *55*
2.3.1 Target Druggability *55*
2.3.2 Reversible Inhibitors/Antagonists *56*
2.3.2.1 Orthosteric and Allosteric Binding Sites *61*
2.3.2.2 Inhibitor Modality is Not an Indication of Inhibitor-Binding Site *63*
2.3.3 Reversible Agonists and Activators *66*
2.3.4 Reversible Inhibitors of Protein–Protein Interactions *71*
2.3.5 Small-Molecule Modulators of Protein Homeostasis *77*
2.3.5.1 Small-Molecule Correctors and Potentiators of Misfolded Proteins *79*
2.3.5.2 Inducers of Protein–Protein Interactions *82*
2.3.6 Irreversible Covalent Inactivators *89*
2.3.6.1 *In Vitro* Analysis of Irreversible Covalent Inactivators *91*
2.3.6.2 $KRAS^{G12C}$ Inactivators: A Case Study *95*
2.4 Antibody–Drug Conjugates (ADCs) *98*
2.5 Summary *100*
References *100*

**3 Target Selection** *107*
3.1 Disease Relevance *108*
3.1.1 Genetic Alterations in Disease *110*
3.1.1.1 Differences in Orthologue Sequences *110*
3.1.1.2 Gene Amplification and Protein Overexpression *113*
3.1.1.3 Target Mutations in Disease *114*
3.1.1.4 Chromosomal Translocations in Disease *116*
3.1.1.5 Synthetic Lethality *117*
3.1.1.6 Disease Association Versus Disease Causality *121*
3.2 Selective Essentiality and Therapeutic Index *122*
3.2.1 RNA-Silencing Methods for Target Validation *123*
3.2.1.1 RNAi *124*
3.2.1.2 CRISPR-Cas9 *128*
3.2.2 Genetic Versus Disease Vulnerability Target Validation Funnels *130*
3.3 Actionability *132*
3.3.1 Existing Structures and/or Ligands *132*
3.3.2 Target Classes *133*
3.3.3 Sequence Alignment and Homology Modeling *133*
3.3.4 Other Computational Methods *135*
3.3.5 Target Vitality and Competition with Endogenous Ligands *135*
3.4 Unmet Medical Need *138*
3.5 DHX9: An Illustrative Example of Target Validation *140*
3.6 Summary *145*
References *145*

**4 Hit-Finding and Hit-Validation** *151*
4.1 Properties of Small-Molecule Therapeutics *152*
4.2 Hit-Validation Criteria *153*
4.3 Screening Strategies *155*
4.4 Protein Constructs for Screening *156*
4.5 Compound Sources for Screening *158*
4.5.1 Diversity Libraries *158*
4.5.2 Fragment-Based Libraries *159*
4.5.3 DNA-Encoded Libraries (DEL) *160*
4.5.4 Target Class-Specific Libraries *162*
4.5.5 Mechanism-Guided Design *163*
4.5.6 Structure-Based Design and Computational Docking Methods *168*
4.6 Assay Formats *169*
4.6.1 Binding Assays *169*
4.6.1.1 Competitive Displacement Assays *171*
4.6.2 Assays of Target Function *174*
4.6.3 Solution Conditions for Assays *177*
4.7 Measures of Assay Performance *178*
4.7.1 Calibration Curves *179*
4.7.2 Time-Dependent Background *180*
4.7.3 The Z′ Statistic *181*
4.7.4 Definition of Hits *182*
4.7.5 Post-Screening Prioritization of Reproducible Hits *183*
4.8 Orthogonal Assays and Mode of Modulation *186*
4.9 Confirmation of Chemical Purity and Identity *188*
4.10 Hit Expansion *188*
4.11 Hit-Validation Funnel *189*
4.12 Summary *191*
References *191*

**5 From Hits to Leads** *197*
5.1 The Project Matrix Team *198*
5.2 Target Potency and Selectivity *200*
5.2.1 Comparative Measures of Target Potency *203*
5.2.2 Selectivity Profiling *205*
5.2.3 Iterative Structural Biology *207*
5.2.4 Solubility and Lipophilicity *210*
5.3 Cellular Assays *212*
5.3.1 Caco-2 Permeability and Active Efflux *213*
5.3.2 Pharmacodynamic Assays *214*
5.3.3 Phenotypic Assays *218*

5.3.4 Intervening Biological Responses *221*
5.3.5 Characteristics of Useful Cellular Assays *222*
5.3.6 Positive and Negative Cell Types *224*
5.4 ADME Properties *225*
5.4.1 Plasma Stability *225*
5.4.2 Plasma Protein Binding *227*
5.4.3 Microsomal Stability: An Initial Indication of Hepatic Metabolism *229*
5.4.4 Assessment of hERG Channel Inhibition *230*
5.5 Chemical Tractability *232*
5.5.1 Chemical Stability *233*
5.5.2 Synthetic Route(s) *233*
5.5.3 Patent Assessment *234*
5.6 Lead Series *234*
5.6.1 The Elements of Translational Chemical Biology *235*
5.7 Case Studies *240*
5.7.1 DOT1L Inhibitors for Mixed Lineage Leukemia *240*
5.7.2 METTL3 Inhibitors for Acute Myeloid Leukemia (AML) *242*
5.7.3 EZH2 Inhibitors for Treatment of Non-Hodgkin's Lymphoma *244*
5.8 Summary *247*
References *247*

**6 Lead Optimization** *251*
6.1 Typical Criteria for Development Candidate Declaration *252*
6.2 Continued Optimization of Target Potency, Selectivity, and Cellular Potency *252*
6.2.1 Measuring Target Potency Under Tight-Binding Conditions *252*
6.2.2 The Impact of Protein Conformational Dynamics on SAR *256*
6.2.3 Extended Surveys of Target Selectivity *260*
6.2.3.1 Secondary Pharmacology Screening *261*
6.2.3.2 Assessing Affinity for Target Orthologues *262*
6.2.4 Optimization of Potency and Durability of Cellular Pharmacology *266*
6.2.5 Expanded Survey of Additional Cell Lines *269*
6.3 *In Vitro* ADME Properties *271*
6.3.1 CYP450-Mediated Metabolism *272*
6.3.2 CYP450 Inhibition and Drug–Drug Interactions *274*
6.3.2.1 Time-Dependent CYP450 Inhibition (TDI) *276*
6.3.3 CYP450 Induction *277*
6.3.4 Major Metabolite Identification *278*
6.4 *In Vivo* ADME Properties *280*

6.4.1 Oral Bioavailability *280*
6.4.2 Pharmacokinetic Profile of Orally Bioavailable Drugs *280*
6.4.3 Dose–Exposure Proportionality *282*
6.4.4 Relating Plasma Protein–Binding–Adjusted Exposure to Cellular $EC_{50}$ or $EC_{90}$ *282*
6.4.5 Relating PK and PD Time Courses *283*
6.4.6 Compartmental Modeling of Pharmacokinetics *285*
6.5 *In Vivo* Drug Efficacy *289*
6.5.1 Mouse Models of Efficacy in Cancer Drug Discovery *291*
6.5.1.1 Subcutaneous (Ectopic) Xenografts *291*
6.5.1.2 Relating Tumor Growth Inhibition to Systemic and Intratumoral Drug Exposure and PD *299*
6.5.1.3 Case Studies *299*
6.6 Allometric Scaling and Predicted Human Dose *309*
6.7 Preliminary Safety Assessment in Two Species *312*
6.8 Summary *313*
References *314*

**Appendix 1 Kinetic and Equilibrium Assessment of Receptor–Ligand Interactions** *319*
A1.1 The Equilibrium Dissociation Constant, $K_d$ *320*
A1.2 The Kinetics of Approach to Equilibrium *321*
A1.3 Kinetics of Irreversible or Pseudo-Irreversible Processes *323*
A1.4 Binding Measurements at Equilibrium *324*
A1.4.1 Derivation of the Hill–Langmuir Isotherm *324*
A1.4.2 Competition Among Ligands for a Common Target Receptor *326*
A1.5 Extension to Other Pharmacological Situations *327*

**Appendix 2 Investigational New Drug (IND) Applications** *331*
A2.1 Types of IND Applications *332*
A2.2 Key Content of an IND Application *333*
A2.3 Review by FDA *334*
A2.4 IND-Enabling Studies *335*
A2.4.1 CMC Studies *335*
A2.4.1.1 Drug Substance *335*
A2.4.1.2 Drug Product *336*
A2.4.1.3 Manufacturing Process *336*
A2.4.1.4 Specifications and Quality Control *336*
A2.4.1.5 Stability Testing *336*
A2.4.1.6 Environmental Impact Considerations *337*
A2.4.2 Pharmacology, Pharmacokinetics, and Toxicity Studies *337*

A2.4.2.1 Pharmacology Studies *337*
A2.4.2.2 PK Studies *337*
A2.4.2.3 General Toxicity Studies *338*
A2.4.2.4 Specialized Toxicity Studies *339*
References *340*

**Index** *341*