

Contents

Overview of Therapeutic Antibodies XXXI

A Greeting by the Editor XXXIII

Foreword XXXV

List of Authors XXXVII

Section I – Technologies

Introduction

- 1 Therapeutic Antibodies – From Past to Future** 3
Stefan Dübel
- 1.1 An Exciting Start – and a Long Trek 3
- 1.2 The Gold Rush 8
- 1.3 Success and Disappointment 9
- 1.4 The Gleaming Horizon 14
Further Reading 15
References 15

Part I Selecting and Shaping the Antibody Molecule

- 2 Selection Strategies I: Monoclonal Antibodies** 19
Gerhard Moldenhauer
- 2.1 Introduction 19
- 2.2 Historical Remarks 20
- 2.3 Antibody Structure and Function 21
- 2.3.1 Membrane-bound and Secreted Forms of Antibodies 21
- 2.3.2 Monoclonal Antibodies 23
- 2.4 Production of Monoclonal Antibodies 24
- 2.4.1 Immunization 24
- 2.4.2 Myeloma Cell Lines 25
- 2.4.3 Cell Fusion 25

2.4.4	Drug Selection of Hybridomas	28
2.4.5	Screening Hybridoma Cultures for Specific Antibody	29
2.4.5.1	Enzyme-linked Immunosorbent Assay (ELISA)	29
2.4.5.2	Flow Cytometry	31
2.4.5.3	Immunohistology and Immunocytology	31
2.4.5.4	Cytotoxicity Assays	32
2.4.5.5	Screening for Function	32
2.4.6	Cloning	33
2.4.7	Expansion and Freezing of Hybridoma Clones	33
2.5	Purification and Modification of Monoclonal Antibodies	34
2.5.1	Mass Culture and Purification of Monoclonal Antibody	34
2.5.2	Fragmentation of Monoclonal IgG Antibodies	34
2.5.3	Labeling of Monoclonal Antibodies	35
2.6	Monoclonal Antibodies for Tumor Therapy	35
2.6.1	Leukocyte Differentiation Antigens	35
2.6.2	Epithelial Differentiation Antigens	37
2.6.3	Mechanisms of Action of Monoclonal Antibodies	38
2.6.4	Human Monoclonal Antibodies	39
2.7	Outlook	40
	References	40
3	Selection Strategies II: Antibody Phage Display	45
	<i>Michael Hust, Lars Toleikis and Stefan Dübel</i>	
3.1	Introduction	45
3.2	The Phage Display System	48
3.3	Selection and Evaluation of Binders	50
3.4	Phage Display Vectors	52
3.5	Phage Display Libraries	57
3.6	Generation of Phage Display Libraries	61
	References	62
4	Selection Strategies III: Transgenic Mice	69
	<i>Marianne Brüggemann, Jennifer A. Smith, Michael J. Osborn, and Xiangang Zou</i>	
4.1	Introduction	69
4.2	Human Ig Genes and Loci	69
4.2.1	Minigene Constructs	71
4.2.2	Yeast Artificial Chromosomes (YACs)	74
4.2.3	Chromosome Fragments	76
4.3	Transgenic Ig Strains	77
4.3.1	Stability of the Transloci	78
4.3.2	Silenced Endogenous Loci	79
4.3.3	Immune Responses and Affinity of Human Ig	79
4.3.4	Ig Replacement	83

4.4	Complementary Strategies	84
4.4.1	H-chain-only Ig	84
4.4.2	<i>In vivo</i> Mutation	86
4.5	Outlook	86
	References	87
5	Bioinformatics Tools for Antibody Engineering	95
	<i>Andrew C.R. Martin and James Allen</i>	
5.1	Introduction	95
5.1.1	Brief Review of Antibody Structure	95
5.1.2	Conventions Used in this Chapter	96
5.2	Numbering Schemes for Antibodies	96
5.2.1	The Kabat Numbering Scheme	97
5.2.1.1	The Chothia Numbering Scheme	98
5.2.2	The IMGT Numbering Scheme	100
5.2.3	Honegger and Plückthun Numbering Scheme	100
5.3	Definition of the CDRs and Related Regions	100
5.4	Antibody Sequence Data	102
5.4.1	Antibody Sequence Databanks	102
5.4.2	Germline Sequence Databases	103
5.4.3	Web Resources for Sequence Analysis	104
5.4.3.1	Kabat Data	104
5.4.3.2	IMGT Data	105
5.5	Antibody Structure Data	105
5.6	Sequence Families	106
5.6.1	Families and Subgroups	106
5.6.2	Human Family Chronology	107
5.6.2.1	Human Heavy Chain Variable Genes (V_H)	107
5.6.2.2	Human Light Chain Variable Genes (V_K and V_L)	107
5.6.3	Mouse Family Chronology	108
5.6.3.1	Mouse Heavy Chain Variable Genes (V_H)	108
5.6.3.2	Mouse Light Chain Variable Genes (V_K and V_L)	108
5.6.4	Correspondence Between Human and Mouse Families	109
5.6.4.1	Heavy Chain Variable Genes (V_H)	109
5.6.4.2	Light Chain Variable Genes (V_K and V_L)	109
5.6.5	Tools for Assigning Subgroups	110
5.7	Screening new antibody sequences	111
5.8	Antibody Structure Prediction	111
5.8.1	Build the framework	112
5.8.2	Build the CDRs	112
5.8.3	Automated Modeling Tools	112
5.9	Summary	113
	References	113
	Websites	116
	Note added in proof	117

6	Molecular Engineering I: Humanization	119
	<i>José W. Saldanha</i>	
6.1	Introduction	119
6.2	History of Humanization	120
6.3	CDR Grafting	120
6.4	The Design Cycle	122
6.4.1	Analysis of the Source (Donor) Sequence	123
6.4.1.1	Complementarity Determining Regions (CDRs)	123
6.4.1.2	Canonical Residues	123
6.4.1.3	Interface Packing Residues	124
6.4.1.4	Rare Framework Residues	124
6.4.1.5	N- or O-Glycosylation Sites	125
6.4.2	Three-Dimensional Computer Modeling of the Antibody Structure	126
6.4.3	Choice of Human Framework Sequences	128
6.4.3.1	Fixed Frameworks or Best Fit?	128
6.4.3.2	V _L /V _H Frameworks from the Same or Different Clone?	130
6.4.3.3	Human Subgroup Consensus or Expressed Framework?	131
6.4.3.4	Germline Frameworks	131
6.4.3.5	Database Search	131
6.4.4	Identify Putative Backmutations	132
6.5	Other Approaches to Antibody Humanization	134
6.5.1	Resurfacing/Veneering	134
6.5.2	SDR Transfer	135
6.5.3	DeImmunization Technology	135
6.5.4	Phage Libraries	136
	References	137
7	Molecular Engineering II: Antibody Affinity	145
	<i>Lorin Roskos, Scott Klakamp, Meina Liang, Rosalin Arends, and Larry Green</i>	
7.1	Introduction	145
7.2	Affinity Maturation	145
7.2.1	Maturation <i>In Vivo</i>	145
7.2.2	Maturation <i>In Vitro</i>	147
7.3	Effect of Affinity on Antigen Binding and Antibody Potency	148
7.3.1	Binding and Potency <i>In Vitro</i>	150
7.3.2	Binding and Potency <i>In Vivo</i>	152
7.4	High-Throughput Selection of Hybridomas Secreting High-Affinity Antibodies	154
7.4.1	Soluble Antigens	154
7.4.2	Cell Surface Antigens	157
7.5	Kinetic and Equilibrium Determinations of Antibody Affinity	158
7.5.1	Biacore Technology	158
7.5.2	KinExA Technology	163

7.5.3 Cell-based K_D Titrations 166

7.6 Conclusions 167

References 168

8 Molecular Engineering III: Fc Engineering 171

Matthias Peipp, Thomas Beyer, Michael Dechant, and Thomas Valerius

8.1 Mechanisms of Action of Monoclonal Antibodies 171

8.1.1 Introduction 171

8.1.2 Preclinical Evidence 172

8.1.3 Clinical Evidence 173

8.2 Modifying Effector Functions 175

8.2.1 Antibody Isotype 175

8.2.1.1 IgG Antibodies 175

8.2.1.2 IgA Antibodies 177

8.2.2 Altered Fc Receptor Binding 179

8.2.2.1 Introduction 179

8.2.2.2 Glyco-Engineered Antibodies 180

8.2.2.3 Protein-Engineered Antibodies 183

8.2.3 Altered Complement Activation 188

8.3 Modifying the Pharmacokinetics of Antibodies 189

8.3.1 Introduction 189

8.3.2 Modifying Binding to FcRn 189

8.4 Summary and Conclusions 190

References 190

Part II The Way into the Clinic 197

9 Production and Downstream Processing 199

Klaus Bergemann, Christian Eckermann, Patrick Garidel, Stefanos Grammatikos, Alexander Jacobi, Hitto Kaufmann, Ralph Kempken, and Sandra Pisch-Heberle

9.1 Introduction 199

9.2 Upstream Processing 200

9.2.1 Expression System 200

9.2.2 Cell Culture Media 203

9.2.3 Cell Culture Process Design 204

9.2.4 Cell Culture Process Optimization 206

9.2.5 Scale-up, Economy of Scale 207

9.2.6 Harvest 208

9.3 Downstream Processing 210

9.3.1 Platform Technologies for Downstream Processing of Monoclonal Antibodies 210

9.3.2 Primary Recovery 211

9.3.2.1 Ultra-/Diafiltration (UF/DF) 211

9.3.2.2 Affinity Chromatography 212

9.3.3	Virus Clearance	212
9.3.4	Purification and Polishing	213
9.3.4.1	Hydrophobic Interaction Chromatography	213
9.3.4.2	Ion Exchange Chromatography	214
9.3.5	Final Formulation	215
9.3.6	Integrated Downstream Process Development	216
9.3.7	Future Perspectives	217
9.4	Formulation Development	217
9.4.1	Challenges during Early Formulation Development Phase of Biopharmaceuticals	217
9.4.2	Strategies and Analytical Tools for Rapid and Economic Formulation Development	218
9.4.3	Stabilization of Liquid Protein Formulations by Excipients	223
9.5	Protein Characterization and Quality Control Testing	224
9.5.1	Protein Characterization	224
9.5.1.1	Protein Variants	224
9.5.1.2	Overall Structural Confirmation (Higher Order Structure)	227
9.5.1.3	Relationship Between Physicochemical/Structural Properties and Biological Activity	227
9.5.2	Quality Control Testing	228
9.5.3	Stability Testing	231
9.6	Overall Development Strategy and Outlook	232
9.7	Outlook	233
	References	234
10	Pharmaceutical Formulation and Clinical Application	239
	<i>Gabriele Reich</i>	
10.1	Introduction	239
10.2	Clinical Application	240
10.2.1	Therapeutic Areas of Antibody Drugs	240
10.2.2	Antibody-Mediated Drug Delivery	241
10.2.3	PEGylated Antibodies and Antibody Fragments	243
10.2.4	Routes of Administration	244
10.3	Pharmaceutical Product Development	245
10.4	Stability Issues	246
10.4.1	Degradation Pathways	246
10.4.1.1	Chemical Degradation	247
10.4.1.2	Physical Degradation	248
10.4.2	Design of Stability Studies	249
10.4.2.1	Regulatory Aspects	249
10.4.2.2	Analytical Tools	250
10.4.2.3	Practical Approach	251
10.5	Formulation and Manufacturing of Parenteral Delivery Systems	252

10.5.1	Ready-to-Use Solutions and Concentrates	252
10.5.1.1	Appropriate Excipients	253
10.5.2	Freeze-Dried Powders	255
10.5.2.1	Appropriate Excipients	257
10.5.3	Crystalline Suspensions	258
10.5.4	Carrier-based Systems	259
10.6	Formulation and Manufacturing of Local Delivery Systems	260
10.6.1	Inhalation Powders	260
10.6.1.1	Spray Drying	260
10.6.1.2	Spray Freeze-drying	261
10.6.2	Various Dosage Forms	262
10.7	Outlook	262
	References	263
11	Immunogenicity of Antibody Therapeutics	267
	<i>Huub Schellekens, Daan Crommelin, and Wim Jiskoot</i>	
11.1	Introduction	267
11.2	Assays for Antibodies Induced by Monoclonal Antibodies	268
11.3	Mechanisms of Antibody Induction	269
11.4	Factors Influencing the Immunogenicity	270
11.5	Consequences of the Immunogenicity of Monoclonal Antibodies	272
11.6	Prediction of the Anti-mAb Response	273
11.7	Reduction of Immunogenicity of Monoclonal Antibodies	274
11.8	Conclusion	275
	References	275
12	Regulatory Considerations	277
	<i>Marjorie A. Shapiro, Patrick G. Swann, and Melanie Hartsough</i>	
12.1	Introduction	277
12.2	Regulatory Authority	280
12.3	Chemistry, Manufacturing, and Controls Considerations	282
12.3.1	Cell Line Qualification	282
12.3.2	Quality Control Testing	283
12.3.3	Transmissible Spongiform Encephalopathy (TSE)	285
12.3.4	Product Stability	286
12.3.5	Reference Standard	287
12.3.6	Virus Clearance and Inactivation Studies	287
12.3.7	Abbreviated Product Safety Testing for Feasibility Trials in Serious or Immediately Life-Threatening Conditions	288
12.3.8	Comparability	288
12.4	Considerations for Preclinical Testing	289
12.4.1	Tissue Cross-Reactivity	290
12.4.2	Relevant Species	291

12.4.3	Pharmacodynamic and Pharmacokinetic Studies	291
12.4.4	Toxicology	292
12.4.5	Immunogenicity	295
12.4.6	Comparability	296
12.5	Conclusions	297
	References	297
13	Intellectual Property Issues	301
	<i>Michael Braunagel and Rathin C. Das</i>	
13.1	Introduction	301
13.2	Why Intellectual Property Rights are Important	301
13.3	Recombinant Antibody Technologies	303
13.4	Antibody Humanization	304
13.5	Human Antibody Technology	304
13.6	Antibody Production	311
13.6.1	Genesis of New Cabilly	311
13.6.2	Xoma Patents	313
13.7	Litigations and Cross-licensing	314
13.8	Other Cross-licensing	316
13.9	Litigation between CAT and Abbott	316
13.10	Importation of Data	317
13.11	The Single-Chain Antibody Technology	318
13.12	US Patent Issued on Polyclonal Antibody Libraries	320
13.13	Conclusion	321

Section II – Emerging Developments

Part III Beyond IgG – Modified Antibodies

1	Immunoscintigraphy and Radioimmunotherapy	325
	<i>Jason L. J. Dearling and Alexandra Huhlov</i>	
1.1	Introduction	325
1.2	Solid Tumors as Targets for Antibody-Based Therapeutics	326
1.3	Antigen	326
1.4	Antibodies as Vehicles for Radionuclide Delivery	327
1.5	Radioisotope	334
1.6	Improving Radioimmunoscintigraphy and Therapy	337
1.7	Summary	338
	References	339
2	Bispecific Antibodies	345
	<i>Dafne Müller and Roland E. Kontermann</i>	
2.1	Introduction	345
2.2	The Generation of Bispecific Antibodies	346

2.3	Bispecific Antibodies and Retargeting of Effector Cells	354
2.4	Bispecific Antibodies and Retargeting of Effector Molecules	361
2.5	Bispecific Antibodies as Agonists or Antagonists	364
2.6	Bispecific Antibodies and Somatic Gene Therapy	366
2.7	Outlook	367
	References	368

3 Immunotoxins and Beyond: Targeted RNases 379

Susanna M. Rybak and Dianne L. Newton

3.1	Introduction	379
3.2	Immunotoxins	382
3.3	Targeted RNases	389
3.4	Targeted Onconase	394
3.5	Outlook	399
	References	400

Part IV Emerging Concepts

4 Automation of Selection and Engineering 413

Zoltán Konthur

4.1	Introduction	413
4.2	General Considerations for the Automation of Antibody Generation	415
4.3	Development of an Antibody Generation Pipeline	417
4.4	Conclusion	427
	References	428

5 Emerging Technologies for Antibody Selection 431

Mingyue He and Michael J. Taussig

5.1	Introduction	431
5.2	Display Technologies	432
5.3	Antibody Libraries	433
5.4	Antibody Selection and Maturation <i>In Vitro</i>	435
5.5	Linking Antibodies to mRNA: Ribosome and mRNA Display	436
5.6	Advantages of Ribosome Display	436
5.7	Ribosome Display Systems	437
5.8	Antibody Generation by Ribosome Display	440
5.9	Summary	440
	References	441

6 Emerging Alternative Production Systems 445

Thomas Jostock

6.1	Introduction	445
6.2	Production Systems	446
6.3	Outlook	458
	References	460

7	Non-Antibody Scaffolds	467
	<i>Markus Fiedler and Arne Skerra</i>	
7.1	Introduction	467
7.2	Motivation for Therapeutic Use of Alternative Binding Proteins	467
7.3	Single-Domain Immunoglobulins	473
7.4	Scaffold Proteins Presenting a Contiguous Hypervariable Loop Region	480
7.5	Scaffold Proteins for Display of Individual Extended Loops	484
7.6	Scaffold Proteins Providing a Rigid Secondary Structure Interface	488
7.7	Conclusions and Outlook: Therapeutic Potential and Ongoing Developments	491
	References	492
8	Emerging Therapeutic Concepts I: ADEPT	501
	<i>Surinder K. Sharma, Kerry A. Chester, and Kenneth D. Bagshawe</i>	
8.1	Introduction and Basic Principles of ADEPT	501
8.2	Preclinical Studies	503
8.3	Clinical Studies	505
8.4	Immunogenicity	506
8.5	Important Considerations/Outlook	508
	References	509
9	Emerging Therapeutic Concepts II: Nanotechnology	515
	<i>Dimiter S. Dimitrov, Igor A. Sidorov, Yang Feng, Ponraj Prabakaran, Michaela A.E. Arndt, Jürgen Krauss, and Susanna M. Rybak</i>	
9.1	Introduction	515
9.2	Nanoliposomes, Gold Nanoshells, Quantum Dots, and Other Nanoparticles with Biomedical Potential	516
9.3	Conjugation of Antibodies to Nanoparticles	518
9.4	Nanoparticle–Antibody Conjugates for the Treatment of Cancer and Other Diseases	520
9.5	Comparison Between Nanoparticle–Antibody Conjugates and Fusion Proteins for Cancer Diagnosis and Treatment	522
9.6	Conclusions	526
9.7	Summary	526
	References	527
	Appendix	529
10	Emerging Therapeutic Concepts III: Chimeric Immunoglobulin T Cell Receptors, T-Bodies	533
	<i>Thomas Schirrmann and Gabriele Pecher</i>	
10.1	Introduction	533
10.2	Chimeric Immunoglobulin T-Cell Receptors – “T-Bodies”	534

10.3	Preclinical Studies	543
10.4	Therapeutic Considerations	552
10.5	Perspectives	558
10.6	Conclusions	561
	References	561
11	Emerging Therapeutic Concepts IV: Anti-idiotypic Antibodies	573
	<i>Peter Fischer and Martina M. Uttenreuther-Fischer</i>	
11.1	Introduction	573
11.2	Definition of Anti-idiotypic Antibodies	573
11.3	Anti-idiotypic Antibodies as Autoantigens	575
11.4	Anti-idiotypes as a Tool for Generating Specific Antibodies	575
11.5	Intravenous Immunoglobulin Preparations (IVIG)	575
11.6	Anti-idiotypic Antibodies as Possible Superantigens?	577
11.7	Anti-idiotypic Antibodies in Cancer Therapy	578
11.8	Ab2 β Vaccine Trials Mimicking GD ₂	580
11.9	Molecular Characterization of the Anti-idiotypic Immune Response of a Relapse-free Cancer Patient	581
11.10	Conclusion	583
	References	583

Part V Ongoing Clinical Studies

12	Antibodies in Phase I/II/III: Cancer Therapy	593
	<i>P. Markus Deckert</i>	
12.1	Introduction	593
12.2	Novel Antibody Constructs	595
12.3	Specific Targeting and Effector Mechanisms	606
12.4	Antigens Without Known Effector Function	627
12.5	Disease-specific Concepts of Unknown Antigen Function and Structure	656
12.6	Summary	656
	References	659
13	Antibodies in Phase I/II/III: Targeting TNF	673
	<i>Martin H. Holtmann and Markus F. Neurath</i>	
13.1	Introduction	673
13.2	Inflammatory Bowel Disease	674
13.3	Pathophysiologic Role of T Cells	675
13.4	Tumor Necrosis Factor- α	675
13.5	TNF Receptors and Signaling	676
13.6	Anti-TNF Antibodies and Fusion Proteins in Clinical Testing	679
13.7	Mechanisms of Action	683
13.8	Other Anti-TNF Biologicals	683

13.9	Other Cytokine-based and Anti-CD4 ⁺ T-Cell Approaches	684
13.10	Perspective	685
	References	686

Section III – Approved Therapeutics

1	Adalimumab (Humira)	697
	<i>Hartmut Kupper, Jochen Salfeld, Daniel Tracey, and Joachim R. Kalden</i>	
1.1	Introduction	697
1.2	Pharmacology	698
1.3	Pharmacokinetics	700
1.4	Adalimumab Comparisons with Infliximab and Etanercept	701
1.5	Indications	703
1.5.1	Rheumatoid Arthritis	703
1.5.2	Psoriatic Arthritis	703
1.5.3	Ankylosing Spondylitis	704
1.5.4	Dosing and Administration	705
1.6	Clinical Experience	705
1.6.1	Studies in Rheumatoid Arthritis	705
1.6.1.1	Adalimumab in Combination with MTX	705
1.6.1.2	Adalimumab with Traditional DMARDs	708
1.6.1.2.1	Additional Experience: Adalimumab with Traditional DMARDs	709
1.6.1.3	Adalimumab Monotherapy	709
1.6.1.4	Pivotal Study in Early Rheumatoid Arthritis	710
1.6.1.5	Adalimumab in Patients Previously Treated with other Anti-TNF Agents	712
1.6.2	Pivotal Studies in Psoriatic Arthritis	713
1.6.3	Ankylosing Spondylitis	716
1.7	Review of Adalimumab Safety	717
1.7.1	Safety in Specific Indications	717
1.7.1.1	Long-Term Safety in Rheumatoid Arthritis	717
1.7.1.2	Safety Profile in Psoriatic Arthritis and Ankylosing Spondylitis	719
1.7.2	Immune System Function	719
1.7.3	Infections	719
1.7.3.1	Tuberculosis	720
1.7.3.2	Opportunistic Infections	720
1.7.4	Other Conditions	720
1.7.4.1	Lymphoma	720
1.7.4.2	Demyelinating Conditions	720
1.7.4.3	Autoantibodies and Autoimmune Diseases	721

1.8	Special Conditions and Populations	721
1.8.1	Pregnancy and Lactation	721
1.8.2	Elderly	721
1.8.3	Children and Adolescents	721
1.8.4	Impaired Renal and/or Hepatic Function	722
1.8.5	Congestive Heart Failure	722
1.9	Storage and Administration	722
1.10	Outlook and New Indications	723
1.10.1	Psoriasis	723
1.10.2	Crohn's Disease	723
1.10.3	Juvenile Rheumatoid Arthritis	726
1.11	Conclusions	727
	References	727
2	Alemtuzumab (MabCampath)	733
	<i>Thomas Elter, Andreas Engert, and Michael Hallek</i>	
2.1	Introduction	733
2.2	Basic Principles	734
2.2.1	Antibody Features and Production	734
2.2.1.1	Features of Alemtuzumab (Campath-1H)	734
2.2.1.2	Antibody Production	734
2.3	Mechanism of Action	734
2.3.1	Molecular Target and Target Expression	734
2.3.2	Mechanism of Cell Lysis	737
2.3.3	Immunogenicity and Antiglobulin Response	738
2.4	Clinical Studies with Alemtuzumab	738
2.4.1	Pharmacokinetic Studies	738
2.4.2	Chronic Lymphocytic Leukemia (CLL)	740
2.4.2.1	Relapsed/Refractory CLL	740
2.4.2.2	Minimal Residual Disease in CLL	746
2.4.2.3	Treatment-Naïve CLL	748
2.4.2.4	Chemoimmunotherapy Combinations	749
2.4.2.5	Immunotherapy Combination	753
2.4.2.6	Safety with Alemtuzumab in CLL	753
2.4.2.6.1	Infusion-related Adverse Events	754
2.4.2.6.2	Hematologic Toxicities	755
2.4.2.6.3	Immunosuppression and Infectious Events	756
2.4.3	T-Cell Leukemias and Lymphomas	758
2.4.3.1	T-Cell Lymphomas (Cutaneous/Peripheral T-Cell Lymphoma)	758
2.4.3.2	T-Cell Prolymphocytic Leukemia (T-PLL)	761
2.4.3.3	Adult T-Cell Leukemia	762
2.4.4	Non-Hodgkin's Lymphoma (NHL)	763
2.4.5	Stem Cell Transplantation (SCT)	765
2.4.5.1	Donor T-Cell Depletion (Prevention of GvHD) and Prevention of Graft Rejection	765

2.4.5.2	Reduced-Intensity/Non-Myeloablative Conditioning	766
2.4.5.3	Safety	768
2.5	The Future Outlook	769
2.5.1	Other Hematologic Malignancies (Multiple Myeloma, Acute Leukemias)	769
2.5.2	Prevention of GvHD in Solid Organ Transplantation	769
2.5.3	Applications in Autoimmune Disease	772
	References	772
3	Bevacizumab (Avastin)	779
	<i>Eduardo Diaz-Rubio, Edith A. Perez, and Guiseppe Giaccone</i>	
3.1	Introduction	779
3.1.1	Angiogenesis is Vital for Tumor Development	779
3.1.2	Vascular Endothelial Growth Factor: A Key Angiogenic Factor	780
3.1.3	Targeting Angiogenesis and VEGF: A Rational Treatment Option	781
3.2	The Development of Bevacizumab	783
3.2.1	Origin and Genetic Engineering	783
3.2.2	Mode of Action	784
3.2.3	Preclinical Activity of Bevacizumab	784
3.3	Clinical Trials of Bevacizumab in Key Tumor Types	785
3.3.1	Colorectal Cancer	786
3.3.1.1	Efficacy of Bevacizumab in CRC	786
3.3.1.2	Safety and Management of Bevacizumab in CRC	790
3.3.1.3	Future Use of Bevacizumab in CRC	792
3.3.2	Non-Small Cell Lung Cancer	795
3.3.2.1	Efficacy of Bevacizumab in NSCLC	795
3.3.2.2	Safety and Management of Bevacizumab in NSCLC	798
3.3.2.3	Future Use of Bevacizumab in NSCLC	799
3.3.3	Breast Cancer	799
3.3.3.1	Efficacy of Bevacizumab in Breast Cancer	799
3.3.3.2	Safety and Management of Bevacizumab in Breast Cancer	802
3.3.3.3	Future Use of Bevacizumab in Breast Cancer	802
3.4	The Potential of Bevacizumab	803
3.5	Conclusions	805
	References	806
4	Cetuximab (Erbix, C-225)	813
	<i>Norbert Schleucher and Udo Vanhoefer</i>	
4.1	Introduction	813
4.2	Preclinical Activity	815
4.3	Clinical Data I: Outcome of Monotherapy	816
4.4	Clinical Data II: Outcome of Combination Therapy	817
4.4.1	Colorectal Cancer	817

4.4.2	Head and Neck Cancer	819
4.4.3	Non-Small Cell Lung Cancer (NSCLC)	819
4.4.4	Other Tumors	820
4.5	Clinical Data III: Cetuximab and Radiotherapy/Chemo-Radiotherapy	820
4.5.1	The PARC Study	820
4.5.1.1	Dosing Schedule	821
4.5.1.2	Determinants of Cetuximab Efficacy	821
4.5.1.3	Treatment of Skin Toxicity	821
	References	822
5	Efalizumab (Raptiva)	827
	<i>Karlheinz Schmitt-Rau and Sigbert Jahn</i>	
5.1	Introduction	827
5.2	Development and Characterization of the Antibody	828
5.3	Efalizumab in the Treatment of Psoriasis	829
5.3.1	Psoriasis: Prevalence, Characteristics, and Therapeutic Options	829
5.3.2	Pathogenesis of Psoriasis	831
5.3.2.1	T-Cell Activation	831
5.3.2.2	T-Cell Migration and Extravasation	832
5.3.2.3	T-Cell Reactivation	832
5.3.3	Efalizumab: The Mechanism of Action	833
5.4	Pharmacology and Toxicology of Efalizumab	834
5.4.1	Preclinical Studies	834
5.4.2	Pharmacodynamics	834
5.4.3	Pharmacokinetics	835
5.4.3.1	Absorption	835
5.4.3.2	Distribution	836
5.4.3.3	Biotransformation	836
5.4.3.4	Elimination	836
5.5	Clinical Development of Efalizumab	837
5.5.1	Clinical Efficacy	837
5.5.1.1	Randomized, Placebo-Controlled, Double-Blind Studies	837
5.5.1.2	Extended Treatment and Re-Treatment	838
5.5.1.3	Long-Term Efficacy	840
5.5.2	Safety and Tolerability	841
5.5.3	Health-Related Quality of Life (HRQoL)	844
5.6	Practical Considerations for Therapy with Efalizumab	845
5.6.1	Managing Patients during Long-Term Efalizumab Treatment	845
5.6.2	Other Concerns	846
5.7	Summary	847
	References	847

6	^{99m}Tc-Fanolesomab (NeutroSpec) 851
	<i>Christopher J. Palestro, Josephine N. Rini, and Charito Love</i>
6.1	Introduction 851
6.2	The Agent 851
6.3	Pharmacokinetics/Dosimetry 852
6.4	Biodistribution 852
6.5	Technique 854
6.6	Indications 856
6.6.1	Appendicitis 856
6.6.2	Osteomyelitis 859
6.6.3	Other Infections 861
6.7	Adverse Side Effects and Safety 863
6.8	Summary 865
	References 865
7	Gemtuzumab Ozogamicin (Mylotarg) 869
	<i>Matthias Peipp and Martin Gramatzki</i>
7.1	Introduction 869
7.2	CD33 as a Target Antigen in Acute Leukemia Therapy 870
7.3	Gemtuzumab Ozogamicin 871
7.3.1	The IgG4 Moiety 872
7.3.2	Calicheamicin 872
7.3.3	The Design of Antibody-Calicheamicin Conjugates: Humanization and Choice of Linker 873
7.4	Mechanisms of Action 874
7.5	Potential Mechanisms of Resistance 874
7.6	Clinical Trials: The Data of GO 875
7.7	Summary and Conclusions 877
	References 878
8	Infliximab (Remicade) 885
	<i>Maria Wiekowski and Christian Antoni</i>
8.1	Antibody Characteristics 885
8.2	Preclinical Characterization 886
8.3	Pharmacokinetics 886
8.4	Clinical Response 887
8.4.1	Therapeutic Indications 887
8.4.1.1	Crohn's Disease 888
8.4.1.2	Rheumatoid Arthritis 889
8.4.1.3	Ankylosing Spondylitis 890
8.4.1.4	Psoriatic Arthritis 891
8.4.1.5	Psoriasis 892
8.4.1.6	Ulcerative Colitis 893

8.5	Safety	894
8.5.1	Serious Infections	894
8.5.1.1	Tuberculosis	894
8.5.2	Antibody Formation against Infliximab	895
8.5.3	Infusion Reactions/Delayed Hypersensitivity Reactions	896
8.5.4	Auto-Antibody Formation	896
8.5.5	Neurological Disorders/Demyelinating Disease	896
8.5.6	Malignancies/Lymphoma	897
8.5.7	Congestive Heart Failure	897
8.5.8	Other Adverse Events	898
8.5.8.1	Hepatic Events	898
8.5.8.2	Pregnancy Outcome	898
8.6	Summary	898
	References	899

9	Muromonab-CD3 (Orthoclone OKT3)	905
	<i>Harald Becker</i>	
9.1	Introduction	905
9.2	Production of the Monoclonal Antibody	906
9.3	The Pharmacology of Muromonab-CD3	908
9.3.1	Pharmacokinetic Properties of Muromonab-CD3	909
9.3.2	Pharmacodynamics of Muromonab-CD3	910
9.3.3	Activation of Human T Cells	911
9.3.4	Immunogenicity	912
9.3.5	Interactions	913
9.4	Therapeutic Use	914
9.4.1	Renal and or Renal-Pancreas Transplant Recipients	916
9.4.2	Liver Transplant Recipients	919
9.4.3	Cardiac Transplant Recipients	919
9.5	Cytokine Release Syndrome	924
9.5.1	The Pathophysiology of the Cytokine Release Syndrome	924
9.5.2	Symptoms of the Cytokine Release Syndrome	926
9.5.3	Muromonab-CD3 and the Cytokine Release Syndrome	927
9.5.4	Management of the Cytokine Release Syndrome	929
9.5.4.1	Methylprednisolone	929
9.5.4.2	Pentoxifylline	931
9.5.4.3	Indomethacin	932
9.5.4.4	Recombinant Human Soluble Tumor Necrosis Factor Receptor (TNFR: Fc)	932
9.5.4.5	Anti-TNF Monoclonal Antibodies	933
9.6	The Consequences of Immunosuppression	933
9.6.1	Infections	933
9.6.2	Neoplasia	934
	References	935

10	Natalizumab (Tysabri)	941
	<i>Sebastian Schimrigk and Ralf Gold</i>	
10.1	Introduction	941
10.2	Basic Principles	942
10.3	Mode of Action	943
10.3.1	Pharmacodynamic Profile	944
10.3.2	Pharmacokinetics	944
10.4	Technology	945
10.5	Clinical Findings	945
10.5.1	Phase I Clinical Trials	945
10.5.2	Phase II Clinical Trials	945
10.5.3	Phase III Clinical Trials	946
10.5.4	Adverse Side Effects	947
10.5.5	Neutralizing Antibodies	947
10.6	Indications for Tysabri	947
10.6.1	Clinical Applications	948
10.6.1.1	Preparation and Administration of Natalizumab	948
10.7	Outlook	949
	References	949
11	Omalizumab (Xolair)	
	Anti-Immunoglobulin E Treatment in Allergic Diseases	951
	<i>Claus Kroegel and Martin Foerster</i>	
11.1	Introduction	952
11.2	The Biology of the IgE Molecule	953
11.2.1	IgE Distribution and Blood Concentration	956
11.2.2	IgE Synthesis and Regulation	956
11.3	IgE Receptors	957
11.3.1	FcεRI (High-Affinity IgE Receptor)	957
11.3.2	FcεRII (Low-Affinity IgE Receptor, CD23)	959
11.4	Cell Distribution of IgE	962
11.4.1	Effector Cell-Associated IgE	962
11.4.2	Antigen-Presenting Cell-Associated IgE	962
11.5	Physiologic and Pathophysiologic Significance of IgE	963
11.6	The Concept of Anti-IgE-Based Treatment	964
11.7	Construction of the Monoclonal Anti-IgE Molecule	964
11.7.1	Antibody Generation	964
11.7.2	Complex Formation and Tissue Distribution	965
11.7.3	Preclinical Results	967
11.7.4	Clinical Studies	969
11.8	Anti-Inflammatory Effects of Omalizumab	973
11.8.1	Effects on Serum Free IgE Levels	974
11.8.2	Effect on Cytokines	974

11.8.3	Effects on FcεRI Cell Expression	975
11.8.4	Effect on Dendritic Cell APCs	975
11.8.5	Effect on Eosinophils	976
11.8.6	Effects on B Cells	977
11.9	Pharmacological Properties of Omalizumab	977
11.9.1	Pharmacodynamics	977
11.9.2	Pharmacokinetics	978
11.10	Adverse Effects	978
11.10.1	Systemic Side Effects	978
11.10.2	Local Reactions	979
11.10.3	Serious Adverse Effects	979
11.10.4	Immune Complex Diseases.	979
11.10.5	Long-Term Adverse Effects	980
11.11	Indications	980
11.12	Contraindications	981
11.13	Preparation for Use	982
11.14	Administration	982
11.15	Clinical Dosing	983
11.16	Dosing Adjustments	984
11.17	Precautions and Contraindications	984
11.17.1	Drug Interactions	984
11.17.2	Pregnancy and Lactation	984
11.18	Monitoring of Therapy	985
11.19	Cost	985
11.20	Response to Treatment	986
11.20.1	Onset of Action of Anti-Immunoglobulin E Effect	986
11.20.2	Duration of Treatment	986
11.21	Non-Approved Diseases	987
11.21.1	Allergic Rhinitis	987
11.21.2	Other Clinical Applications	987
11.22	Areas of Uncertainty	989
11.23	Outlook	990
	References	991
12	Palivizumab (Synagis)	999
	<i>Alexander C. Schmidt</i>	
12.1	Nature, Role in Disease, and Biology of the Target	999
12.1.1	Respiratory Syncytial Virus (RSV)-Induced Disease and RSV Epidemiology	999
12.1.2	The Target of the Antibody: The RSV Virion	1000
12.1.3	Correlates of Protection from Disease	1001
12.2	Origin, Engineering, and Humanization of the Antibody	1003
12.3	Mechanism of Action and Preclinical Results	1006

12.4	Production, Downstream Processing, and Galenics of the Antibody	1007
12.4.1	Production	1007
12.4.2	Downstream Processing	1008
12.4.3	Formulations	1008
12.4.4	Specifications	1009
12.5	Summary of Results from Clinical Studies	1010
12.5.1	Phase III Trials	1010
12.5.1.1	Palivizumab in Premature Infants and Children with BPD	1010
12.5.1.2	Palivizumab in Children with Significant CHD	1012
12.6	Indications and Usage	1014
12.7	Clinical Reports after Approval	1014
12.8	Is Protective Efficacy a Function of Palivizumab Serum Concentration?	1021
12.9	Post-Marketing Experience with Regard to Adverse Events	1023
12.10	Ongoing Clinical Studies and Outlook	1024
12.11	Summary	1025
	References	1026
13	Rituximab (Rituxan)	1033
	<i>Michael Wenger</i>	
13.1	Introduction	1033
13.1.1	Production, Design, and Structure of Rituximab	1033
13.1.2	CD20 as a Therapeutic Target	1034
13.1.3	Mode of Action	1035
13.1.4	Preclinical Studies	1037
13.1.5	Pharmacokinetic Studies	1038
13.2	Rituximab Clinical Data in NHL and CLL	1039
13.2.1	Overview of NHL and CLL	1039
13.2.2	Rituximab plus Chemotherapy Induction Therapy in Indolent NHL	1041
13.2.2.1	Rituximab plus Chemotherapy in Previously Untreated Indolent NHL	1041
13.2.2.1.1	Concurrent Rituximab and Chemotherapy: Phase II Studies	1042
13.2.2.1.2	Chemotherapy and Sequential Rituximab: Phase II Studies	1046
13.2.2.1.3	Chemotherapy plus Rituximab: Phase III Studies	1047
13.2.2.2	Rituximab plus Chemotherapy in Relapsed/Refractory Indolent NHL	1049
13.2.2.2.1	Rituximab and Chemotherapy: Phase II Trials	1050
13.2.2.2.2	Rituximab and Chemotherapy: Phase III Trials	1050
13.2.2.3	Meta-Analysis of Rituximab and Chemotherapy in Indolent NHL	1053
13.2.3	Induction Therapy with Rituximab plus Immune System Modulators in Indolent NHL	1053
13.2.3.1	Rituximab plus Immune System Modulators	1054

13.2.3.2	Rituximab plus Immune Modulators in Relapsed/Refractory Indolent NHL	1054
13.2.4	Induction with Rituximab Monotherapy in Indolent NHL	1055
13.2.4.1	Rituximab Monotherapy in Previously Untreated Indolent NHL	1055
13.2.4.2	Rituximab Monotherapy in Relapsed Indolent NHL	1055
13.2.5	Rituximab in Other Subtypes of Indolent Lymphoma	1056
13.2.5.1	Rituximab in Marginal Zone Lymphoma	1056
13.2.5.2	Rituximab in Small Lymphocytic Lymphoma	1057
13.2.5.3	Rituximab in Waldenström's Macroglobulinemia	1057
13.2.6	Rituximab Maintenance Therapy	1058
13.2.6.1	Rituximab Maintenance Therapy Following Monotherapy Induction	1058
13.2.6.2	Rituximab Maintenance Therapy Following Chemotherapy Induction	1061
13.2.6.3	Rituximab Maintenance Therapy Following Rituximab Chemotherapy Induction	1062
13.2.7	Rituximab Retreatment	1062
13.2.8	Rituximab in Aggressive NHL	1063
13.2.8.1	Rituximab plus Chemotherapy in Previously Untreated Aggressive NHL: Phase II Studies	1064
13.2.8.2	Rituximab plus Chemotherapy in Previously Untreated DLBCL: Phase III Studies and Population Analysis	1068
13.2.8.2.1	The GELA LNH98-5 Trial	1068
13.2.8.2.2	The Intergroup E4494 Trial	1069
13.2.8.2.3	The MInT Trial	1070
13.2.8.2.4	BCCA Population Analysis	1071
13.2.8.3	Rituximab plus Dose-Densified Chemotherapy in Previously Untreated Aggressive NHL: Phase III Studies	1072
13.2.8.4	Rituximab plus Chemotherapy in Relapsed Aggressive NHL	1072
13.2.8.5	Rituximab Monotherapy in Aggressive NHL	1073
13.2.8.6	Rituximab in Other Subtypes of Aggressive B-Cell NHL	1073
13.2.8.6.1	Rituximab in PMBCL	1073
13.2.8.6.2	Rituximab in Burkitt's and Burkitt-Like Lymphoma or Lymphoblastic Lymphoma/Leukemia	1074
13.2.9	Rituximab in MCL	1074
13.2.9.1	Rituximab plus Chemotherapy in Previously Untreated MCL	1074
13.2.9.2	Rituximab plus Chemotherapy in Relapsed MCL	1076
13.2.9.3	Rituximab Monotherapy in MCL	1077
13.2.10	Rituximab in CLL	1077
13.2.10.1	Rituximab plus Chemotherapy in Previously Untreated CLL	1077
13.2.10.2	Rituximab plus Chemotherapy in Relapsed CLL	1078
13.2.10.3	Rituximab with Immune System Modulators in CLL	1081
13.2.10.4	Rituximab Monotherapy in CLL	1082
13.2.11	Rituximab in the Transplant Setting	1082

13.2.12	Rituximab in Other Malignancies of B-Cell Origin	1083
13.2.12.1	Rituximab in PTLID	1084
13.2.12.2	Rituximab in HIV-Associated Lymphoma	1084
13.2.12.3	Rituximab in PCNSL	1084
13.2.12.4	Rituximab in HCL	1085
13.2.12.5	Rituximab in HD	1085
13.3	Rituximab in Autoimmune Disorders	1085
13.3.1	Rituximab in RA	1086
13.3.2	Rituximab in SLE	1087
13.3.3	Rituximab in Autoimmune Cytopenias and Hemophilia	1088
13.3.4	Rituximab in Chronic Graft-versus-Host Disease (GvHD)	1088
13.3.5	Rituximab in Other Autoimmune Disorders	1088
13.4	Summary and Conclusions	1089
	References	1091

14 Trastuzumab (Herceptin)

A Treatment for HER2-Positive Breast Cancer 1109

Paul Ellis

14.1	Introduction	1109
14.2	Metastatic Breast Cancer	1111
14.2.1	Trastuzumab Monotherapy	1111
14.2.2	Trastuzumab in Combination with Taxanes	1112
14.2.3	Trastuzumab in Combination with other Standard Chemotherapy	1114
14.2.4	Trastuzumab in Triple Combination	1115
14.2.5	Trastuzumab in Combination with Hormonal Therapies	1115
14.3	Early Breast Cancer	1115
14.4	Trastuzumab Treatment in other Tumor Types	1119
14.5	Safety	1120
14.5.1	Cardiac Adverse Events	1121
14.5.2	Infusion-related Reactions	1124
14.5.3	Age Considerations	1125
14.5.4	Patient Considerations	1125
14.6	Dosing/Scheduling	1126
14.7	Conclusions	1126
	References	1126

15 Abciximab, Arcitumomab, Basiliximab, Capromab, Cotara, Daclizumab, Edrecolomab, Ibritumomab, Igovomab, Nofetumomab, Satumomab, Sulesomab, Tositumomab, and Votumumab 1131

Christian Menzel and Stefan Dübel

15.1	Abciximab (Reopro)	1131
15.2	Arcitumomab (CEA-Scan)	1132
15.3	Basiliximab (Simulect)	1133
15.4	Capromab Pendetide (ProstaScint)	1135

15.5	^{131}I -chTNT-1/B (Cotara)	1136
15.6	Daclizumab (Zenapax)	1137
15.7	Edrecolomab (Panorex 17-1A)	1138
15.8	Gemtuzumab Ozogamicin (Mylotarg)	1139
15.9	Igovomab (Indimacis-125)	1140
15.10	Nofetumomab (Verluma)	1141
15.11	Satumomab (OncoScint/Oncorad: B72.3n)	1142
15.12	Sulesomab (LeukoScan, MN-3)	1142
15.13	Tositumomab; Iodine ^{131}I Tositumomab (Bexxar)	1144
15.14	Votumumab (Humaspect)	1145
16	Yttrium-90 Ibritumomab Tiuxetan (Zevalin®)	1147
	<i>Karin Hohloch, Björn Chapuy, and Lorenz Trümper</i>	
16.1	Introduction	1147
16.1.1	Epidemiology of Non-Hodgkin's Lymphoma	1147
16.1.2	Standard Therapy of NHL	1148
16.1.3	CD20-targeted Immunotherapy of NHL	1148
16.2	Basic Principles of Radioimmunotherapy	1149
16.3	Development and Advantages of ^{90}Y -Ibritumomab Tiuxetan	1150
16.3.1	Preparation of ^{90}Y -Ibritumomab Tiuxetan	1150
16.3.2	Dosing of ^{90}Y -Ibritumomab Tiuxetan	1152
16.4	Preclinical and Clinical Results	1153
16.4.1	Preclinical Results	1153
16.4.2	Clinical Therapeutic Efficacy	1153
16.4.3	Adverse Events	1157
16.4.4	Considerations for the Use of ^{90}Y -Ibritumomab Tiuxetan	1157
16.5	Outlook	1158
16.5.1	Novel Indications	1158
16.5.1.1	Aggressive (DLBCL) NHL	1158
16.5.1.2	RIT in Mantle Cell Lymphoma	1159
16.5.2	Combination Therapy	1159
16.5.3	^{90}Y -Ibritumomab Tiuxetan Consolidation of Front-line Chemotherapy	1159
16.5.4	^{90}Y -Ibritumomab Tiuxetan as Conditioning Regimen for Stem Cell Transplantation	1160
	References	1161
	Index	1165