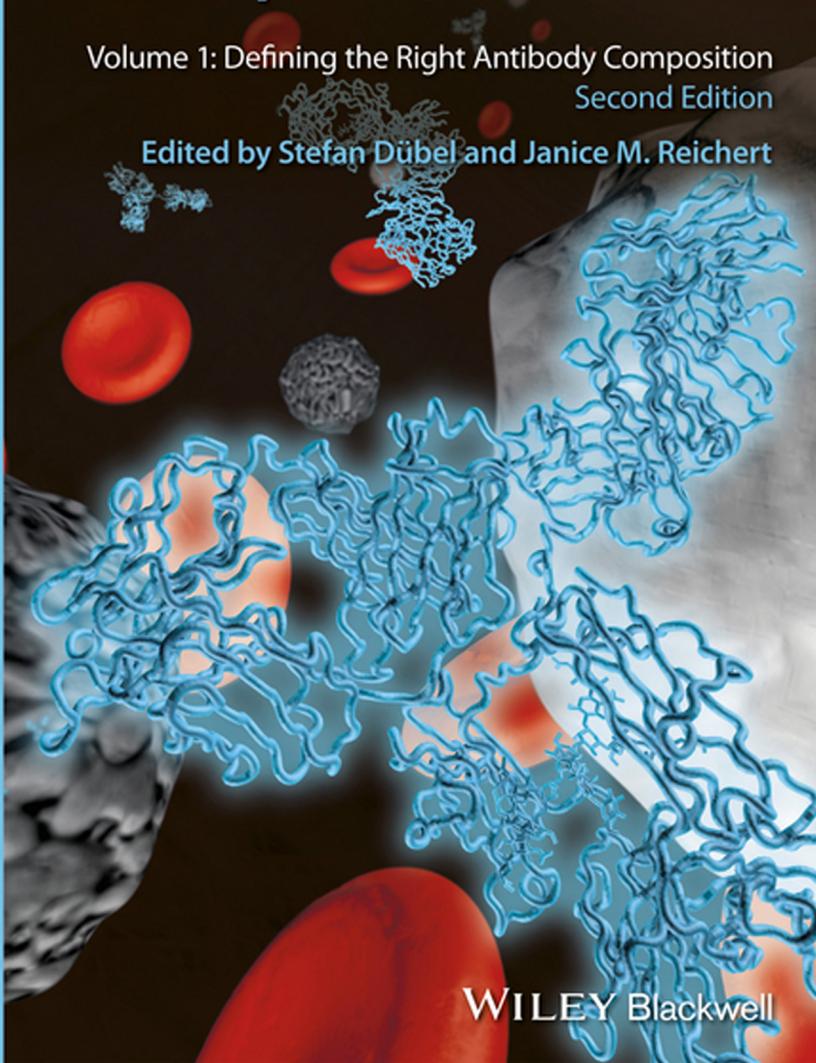


Handbook of Therapeutic Antibodies

Volume 1: Defining the Right Antibody Composition
Second Edition

Edited by Stefan Dübel and Janice M. Reichert



WILEY Blackwell

WILEY Blackwell

WILEY Blackwell

WILEY Blackwell

Edited by
Stefan Dübel and
Janice M. Reichert

Handbook of Therapeutic Antibodies

Related Titles

Chamow, S.M., Ryll, T., Lowman, H.B., Farson, D. (eds.)

Therapeutic Fc-Fusion Proteins

2014

Print ISBN: 978-3-527-33317-2, also available in digital formats

Knäblein, J. (ed.)

Modern Biopharmaceuticals

Recent Success Stories

2013

Print ISBN: 978-3-527-32283-1, also available in digital formats

Pathak, Y., Benita, S. (eds.)

Antibody-Mediated Drug Delivery Systems

Concepts, Technology, and Applications

2012

Print ISBN: 978-0-470-61281-1, also available in digital formats

Kratz, F., Senter, P., Steinhagen, H. (eds.)

Drug Delivery in Oncology

From Basic Research to Cancer Therapy

2011

Print ISBN: 978-3-527-32823-9, also available in digital formats

Tovey, M.G. (ed.)

Detection and Quantification of Antibodies to Biopharmaceuticals

Practical and Applied Considerations

2011

Print ISBN: 978-0-470-56666-4, also available in digital formats

Edited by Stefan Dübel and Janice M. Reichert

Handbook of Therapeutic Antibodies

Volume I: Defining the Right Antibody Composition

Second Edition

WILEY Blackwell

Edited by Stefan Dübel and Janice M. Reichert

Handbook of Therapeutic Antibodies

Volume II: Clinical Development of Antibodies

Second Edition

WILEY Blackwell

Edited by Stefan Dübel and Janice M. Reichert

Handbook of Therapeutic Antibodies

Volume III: Approved Therapeutic Antibodies

Second Edition

WILEY Blackwell

Edited by Stefan Dübel and Janice M. Reichert

Handbook of Therapeutic Antibodies

Volume IV: Approved Therapeutic Antibodies and *in vivo*
Diagnostics

Second Edition

WILEY Blackwell

Editors

Prof. Dr. Stefan Dübel

Technische Universität Braunschweig
Institute of Biochemistry
Biotechnology and Bioinformatics
Spielmannstr. 7
38106 Braunschweig
Germany

Dr. Janice M. Reichert

Reichert Biotechnology Consulting LLC
Prospect Street 247
Framingham, MA
USA

Cover

Antibodies have become standard therapy in many therapeutic areas including cancer, inflammation, osteoporosis, autoimmune, cardiovascular, ophthalmic and infectious diseases. Early successes in the treatment of leukemia and lymphoma by rituximab and alemtuzumab spawned the development of ofatumumab and obinutuzumab, antibodies that kill tumor cells more potently via diverse mechanisms. The cover is an artist's impression of lymphocytic leukemia cells under therapeutic antibody attack. The image was developed by Joost M. Bakker, www.scicomvisuals.com.

■ 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 can 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 authors shall be liable for any loss of profit or any other commercial damages, including but not limited to special, incidental, consequential, or other damages.

Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library.

Bibliographic information published by the Deutsche Nationalbibliothek

The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet at <<http://dnb.d-nb.de>>.

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Boschstr. 12, 69469 Weinheim, Germany

Wiley-Blackwell is an imprint of John Wiley & Sons, formed by the merger of Wiley's global Scientific, Technical, and Medical business with Blackwell Publishing.

All rights reserved (including those of translation into other languages). No part of this book may be reproduced in any form – by photostriping, microfilm, or any other means – nor transmitted or translated into a machine language without written permission from the publishers. Registered names, trademarks, etc. used in this book, even when not specifically marked as such, are not to be considered unprotected by law.

Print ISBN: 978-3-527-32937-3

ePDF ISBN: 978-3-527-68245-4

ePub ISBN: 978-3-527-68244-7

Mobi ISBN: 978-3-527-68243-0

oBook ISBN: 978-3-527-68242-3

Cover Design Formgeber, Mannheim, Germany

Typesetting Laserwords Private Limited, Chennai, India

Printing and Binding Markono Print Media, Pte Ltd., Singapore

Printed on acid-free paper

Contents

Volume I: Defining the Right Antibody Composition

Quick Reference List of Antibodies by International Nonproprietary Name XXIII

Quick Reference List of Antibodies by Brand Name XXV

A Greeting by the Editors XXVII

Foreword to the First Edition XXIX

Foreword to the Second Edition XXXI

List of Contributors XXXIII

Abbreviations LI

Appendix: Marketed Monoclonal Antibodies Compendium LXXXIII

1 Therapeutic Antibodies – from Past to Future 1

Stefan Dübel and Janice M. Reichert

1.1 An Exciting Start – and a Long Trek 1

1.2 The Gold Rush 6

1.3 Success and Setbacks 7

1.4 The Gleaming Horizon 10

References 12

Further Reading 12

Part I: Selecting and Shaping the Antibody Molecule 15

2 Selection Strategies for Monoclonal Antibodies 17

Gerhard Moldenhauer

2.1 Introduction 17

2.2 Historical Remarks 18

2.3 Antibody Structure and Function 19

2.3.1 Membrane-Bound and Secreted Forms of Antibodies 19

2.3.2 Monoclonal Antibodies 21

2.4 Production of Monoclonal Antibodies 21

2.4.1 Immunization 21

2.4.2	Myeloma Cell Lines	22
2.4.3	Cell Fusion	23
2.4.4	Drug Selection of Hybridomas	25
2.4.5	Screening Hybridoma Cultures for Specific Antibody	26
2.4.5.1	Enzyme-Linked Immunosorbent Assay (ELISA)	27
2.4.5.2	Flow Cytometry	27
2.4.5.3	Immunohistology and Immunocytology	28
2.4.5.4	Cytotoxicity Assays	29
2.4.5.5	Screening for Function	30
2.4.6	Cloning	30
2.4.7	Expansion and Freezing of Hybridoma Clones	30
2.5	Purification and Modification of Monoclonal Antibodies	31
2.5.1	Mass Culture and Purification of Monoclonal Antibody	31
2.5.2	Fragmentation of Monoclonal IgG Antibodies	32
2.5.3	Labeling of Monoclonal Antibodies	32
2.6	Monoclonal Antibodies for Tumor Therapy	33
2.6.1	Leukocyte Differentiation Antigens	33
2.6.2	Epithelial Differentiation Antigens	33
2.6.3	Mechanisms of Action of Monoclonal Antibodies	34
2.6.4	Human Monoclonal Antibodies	35
2.7	Outlook	36
	References	37
3	Antibody Phage Display	43
	<i>Michael Hust, André Frenzel, Florian Tomszak, Jonas Kügler, and Stefan Dübel</i>	
3.1	Introduction	43
3.2	Phage Display	45
3.3	Selection and Screening	46
3.4	Phage Display Vectors	48
3.5	Phage Display Libraries	57
3.6	Construction of Phage Display Libraries	58
	Acknowledgments	65
	References	65
4	Transgenic Animals Derived by DNA Microinjection	77
	<i>Marianne Brüggemann, Michael J. Osborn, Biao Ma, Suzanne Avis, Ignacio Anegon, and Roland Buelow</i>	
4.1	Introduction	77
4.2	Construction of Human Ig Transloci	78
4.2.1	IgH	78
4.2.2	Igκ	80
4.2.3	Igλ	80
4.3	BAC Integration	81

4.4	Designer Zinc Finger Endonucleases to Silence Endogenous Ig Loci	82
4.5	Expression Comparison of Fully Human and Chimeric IgH Loci	83
4.6	Outlook	85
	References	85
5	Humanization Strategies	89
	<i>José W. Saldanha</i>	
5.1	Introduction	89
5.2	History of Humanization	89
5.3	CDR-Grafting	90
5.4	The Design Cycle	92
5.4.1	Analysis of the Source (Donor) Sequence	92
5.4.1.1	Complementarity-Determining Regions (CDRs)	92
5.4.1.2	Canonical Residues	93
5.4.1.3	Interface Packing Residues	93
5.4.1.4	Rare Framework Residues	94
5.4.1.5	N- or O-Glycosylation Sites	95
5.4.2	Three-Dimensional Computer Modeling of the Antibody Structure	95
5.4.3	Choice of Human Framework Sequences	97
5.4.3.1	Fixed Frameworks or Best-Fit?	100
5.4.3.2	VL/VH Frameworks from the Same or Different Clone?	100
5.4.3.3	Human Subgroup Consensus or Expressed Framework?	101
5.4.3.4	Germline Frameworks	101
5.4.3.5	Database Search	101
5.4.4	Identifying Putative Backmutations	102
5.4.5	Stability	104
5.5	Other Approaches to Antibody Humanization	104
5.5.1	Resurfacing/Veneering	104
5.5.2	SDR-Transfer	105
5.5.3	Removal of T- and B-Cell Epitopes	106
5.5.4	Phage Libraries	106
	References	107
6	Antibody Affinity	115
	<i>André Frenzel, Lorin Roskos, Scott Klakamp, Meina Liang, Rosalin Arends, and Larry Green</i>	
6.1	Introduction	115
6.2	Affinity Maturation	115
6.2.1	Affinity Maturation <i>In Vivo</i>	115
6.2.2	Affinity Maturation <i>In Vitro</i>	117
6.3	Antibody Affinity: Antigen Binding and Potency	120
6.4	Binding and Potency <i>In Vitro</i>	121
6.5	Binding and Potency <i>In Vivo</i>	123

6.6	Selection of High-Affinity Antibodies from Hybridoma Cell Lines	126
6.7	Generation of Antibodies against Soluble Antigens	126
6.8	Generation of Antibodies against Cell Surface Antigens	127
6.9	Determination of Antibody Affinity	128
6.10	Surface Plasmon Resonance	128
6.11	Other Methods for Determining Antibody Affinity	131
6.12	Conclusion	134
	References	134
7	Fc Engineering	141
	<i>Matthias Peipp, Stefanie Derer, Stefan Lohse, Christian Kellner, and Thomas Valerius</i>	
7.1	Mechanisms of Action of Monoclonal Antibodies	141
7.1.1	Introduction	141
7.1.2	Preclinical Evidence	142
7.1.3	Clinical Evidence	144
7.2	Modifying Effector Functions	145
7.2.1	Antibody Isotype	145
7.2.1.1	IgG Antibodies	145
7.2.1.2	IgA Antibodies	149
7.2.2	Altered Fc Receptor Binding	151
7.2.2.1	Introduction	151
7.2.2.2	Protein-Engineered Antibodies	151
7.2.3	Altered Complement Activation	157
7.3	Modifying Antibodies' Pharmacokinetics	158
7.3.1	Introduction	158
7.3.2	Modifying Binding to FcRn	159
7.4	Summary and Conclusions	160
	References	160
8	Glycosylation of Antibody Molecules	171
	<i>Roy Jefferis</i>	
8.1	Introduction	171
8.2	Overview of the IgG Molecule	172
8.3	Quaternary Structure of IgG-Fc: The Protein Moiety	174
8.4	The IgG-Fc Oligosaccharide Moiety	176
8.5	IgG-Fc Protein/Oligosaccharide Interactions	177
8.6	Protective Mechanisms Activated by Immune Complexes	180
8.7	Role of IgG Glycoforms in Recognition by Cellular F γ Rs	180
8.8	The Influence of Fucose and Bisecting N-Acetylglucosamine on IgG-Fc Activities	180
8.9	The Influence of Galactosylation on IgG-Fc Activities	182
8.10	Sialylation of IgG-Fc Oligosaccharides	184
8.11	Chemo-Enzymatic Synthesis of Novel IgG-Fc Glycans	185

8.12	Restoration of Functionality to Aglycosylated IgG-Fc	186
8.13	IgG-Fab Glycosylation	187
8.14	Conclusion	189
	References	189
9	Bioinformatics Tools for Analysis of Antibodies	201
	<i>Andrew C.R. Martin and James Allen</i>	
9.1	Introduction	201
9.1.1	Brief Review of Antibody Structure	201
9.1.2	Conventions Used in this Chapter	202
9.2	Numbering Schemes for Antibodies	202
9.2.1	The Kabat Numbering Scheme	203
9.2.1.1	The Chothia Numbering Scheme	204
9.2.2	The IMGT Numbering Scheme	206
9.2.3	Honegger and Plückthun (Aho) Numbering Scheme	206
9.2.4	Enhanced Chothia (Martin) Numbering Scheme	206
9.2.5	Numbering Scheme Summary	206
9.3	Definition of the CDRs and Related Regions	208
9.4	Antibody Sequence Data	209
9.4.1	Antibody Sequence Databanks	210
9.4.2	Germline Sequence Databases	211
9.4.3	Web Resources for Analyzing Antibody Sequence Data	211
9.4.3.1	Kabat Data	211
9.4.3.2	IMGT Data	212
9.5	Antibody Structure Data	213
9.6	Screening New Antibody Sequences	213
9.6.1	Tools for Assigning Subgroups	213
9.6.2	Identifying Germline Components	214
9.6.3	Identifying Unusual Features	214
9.6.4	Assessing “Humanness” of Sequences	214
9.7	abYsis – An Integrated Antibody Sequence and Structure Resource	215
9.8	Antibody Structure Prediction	216
9.8.1	Build the Framework	216
9.8.2	Build the CDRs	216
9.8.3	Automated Modeling Tools	217
9.9	Sequence Families	218
9.9.1	Families and Subgroups	218
9.9.2	Human Family Chronology	219
9.9.2.1	Human Heavy Chain Variable Genes (V_H)	219
9.9.2.2	Human Light Chain Variable Genes ($V\lambda$ and $V\kappa$)	219
9.9.3	Mouse Family Chronology	220
9.9.3.1	Mouse Heavy Chain Variable Genes (V_H)	220
9.9.3.2	Mouse Light Chain Variable Genes ($V\kappa$ and $V\lambda$)	220
9.9.4	Correspondence between Human and Mouse Families	221

9.9.4.1	Heavy Chain Variable Genes (V_H)	221
9.9.4.2	Light Chain Variable Genes ($V\kappa$ and $V\lambda$)	221
9.10	Summary	222
	References	223
	Websites	226
10	How to Use IMGT® for Therapeutic Antibody Engineering	229
	<i>Marie-Paule Lefranc</i>	
10.1	Introduction	229
10.2	Fundamental Information from IMGT-ONTOLOGY Concepts	232
10.2.1	IDENTIFICATION: IMGT® Standardized Keywords	232
10.2.2	DESCRIPTION: IMGT® Standardized Labels	233
10.2.3	CLASSIFICATION: IMGT® Standardized Genes and Alleles	233
10.2.4	NUMEROTATION: IMGT Unique Numbering and IMGT Colliers de Perles	236
10.2.4.1	IMGT Unique Numbering for V and C Domains	236
10.2.4.2	IMGT Collier de Perles	237
10.3	IMGT® Tools and Databases	241
10.3.1	IMGT/Collier-de-Perles Tool	241
10.3.2	IMGT/3Dstructure-DB	241
10.3.3	IMGT/2Dstructure-DB	244
10.3.4	IMGT/DomainGapAlign	244
10.3.5	IMGT/V-QUEST	245
10.3.6	IMGT/HighV-QUEST	246
10.4	Examples of IMGT® Web Resources for Antibody Engineering and Humanization	246
10.4.1	Antibody V Domain Humanization	246
10.4.1.1	CDR-IMGT Grafting	246
10.4.1.2	Amino Acid Interactions between FR-IMGT and CDR-IMGT	247
10.4.2	Only-Heavy-Chain Antibodies	247
10.4.2.1	Dromedary IgG2 and IgG3	247
10.4.2.2	Human Heavy Chain Diseases (HCD)	248
10.4.2.3	Nurse Shark IgN	248
10.4.3	IGHG CH Amino Acid Positions	249
10.4.3.1	N-Linked Glycosylation Site CH2 N84.4	249
10.4.3.2	Knobs-into-Holes CH3 T22 and Y86	249
10.4.3.3	Interface Ball-and-Socket-Like Joints	251
10.4.3.4	IGHG1 Alleles and G1m Allotypes	251
10.5	Conclusions	253
	Acknowledgments	255
	Abbreviations	257
	References	257
	Website	263

Part II: Modified Antibodies 265

11	Bispecific Antibodies 267
	<i>Dafne Müller and Roland E. Kontermann</i>
11.1	Introduction 267
11.2	The Generation of Bispecific Antibodies 268
11.2.1	Somatic Hybridization 268
11.2.2	Chemical Conjugation 269
11.2.3	Recombinant Bispecific Antibody Molecules 271
11.2.3.1	Small Recombinant Bispecific Antibody Formats Derived from the Variable Domain 272
11.2.3.2	Recombinant Bispecific Antibody Formats Generated by Fusing an Antigen-Binding Site to an IgG 275
11.2.3.3	Recombinant Bispecific Antibody Formats Containing Asymmetric Heterodimerization Domains 276
11.3	Bispecific Antibodies and Retargeting of Effector Cells 278
11.3.1	Retargeting of Cytotoxic T Lymphocytes 279
11.3.2	Retargeting of Fc Receptor Bearing Effector Cells 283
11.4	Bispecific Antibodies and Retargeting of Effector Molecules 285
11.4.1	Bispecific Antibodies and Radioimmunotherapy 286
11.4.2	Bispecific Antibodies and Targeting of Toxins and Drugs 288
11.5	Dual Targeting Strategies with Bispecific Antibodies 289
11.6	Bispecific Antibodies and Somatic Gene Therapy 291
11.7	Outlook Update 293
	References 293
12	Single-Domain Antibodies: An Overview 311
	<i>Carrie Enever, Edward Coulstock, Małgorzata Pupecka-Swidler, and Bruce Hamilton</i>
12.1	Introduction 311
12.2	Historical Perspective 312
12.2.1	Overview 312
12.2.2	Companies 312
12.2.3	Assets in the Clinic 314
12.3	How are sdAbs Isolated? 314
12.3.1	Introduction 314
12.3.2	Single-Domain Antibody Library Generation 317
12.3.2.1	Immune Library Generation 317
12.3.2.2	Naïve Library Generation 317
12.3.2.3	Synthetic Library Generation 317
12.3.2.4	Transgenic Animals 318
12.3.3	Selection Technologies 319
12.3.3.1	Phage Display 319
12.3.3.2	Yeast and Bacterial Display 319
12.3.3.3	Alternative Display Methods 320

12.3.4	Affinity Maturation	321
12.4	Target Space	321
12.4.1	Structural Differences	322
12.4.2	Cryptic and Conformational Epitopes	323
12.4.3	Routes of Administration	324
12.4.4	Modularity	324
12.4.5	Tissue Penetration	325
12.4.6	Diagnostic Application	325
12.5	Bi-specifics and Targeted Payloads	326
12.6	Pharmacokinetics/Biodistribution and Half-Life Extension Technologies	328
12.6.1	PEGylation	328
12.6.2	Fc-Fusion	329
12.6.3	Albumin Binding	330
12.7	Imaging	332
12.8	Outlook	334
	Acknowledgments	334
	References	334
13	Antibody–Drug Conjugates: New Frontier in Cancer Therapeutics	341
	<i>Rajeeva Singh, John M. Lambert, and Ravi V. J. Chari</i>	
13.1	Introduction	341
13.2	Currently Approved ADCs for Cancer Treatment	344
13.3	Cytotoxic Compounds in ADCs	346
13.3.1	Microtubule-Targeted Cytotoxic Agents	346
13.3.2	DNA- or DNA-Topoisomerase-Targeted Cytotoxic Agents	352
13.4	Linkers in ADCs	353
13.4.1	Noncleavable Thioether Linkers	354
13.4.2	Disulfide Linkers	355
13.4.3	Peptide Linkers	356
13.4.4	Hydrazone Linkage	356
13.4.5	Carbonate Linkage	356
13.4.6	Site of Linkage	357
13.5	Antibody in ADCs	358
13.6	Conclusions	358
	References	359
14	Antibody-Targeted Drugs: From Chemical Immunoconjugates to Recombinant Fusion Proteins	363
	<i>Athanasis Mavratzas, Michaela A.E. Arndt, Stefan Kiesgen, and Jürgen Krauss</i>	
14.1	Introduction	363
14.2	Lessons Learned from Chemical Immunoconjugates	363
14.2.1	Evolution	363
14.2.2	Linker Stability	364

14.2.3	Cross-Linkage Heterogeneity	369
14.2.4	Characteristics of Target Antigens	370
14.2.5	Characteristics of Effector Moieties	372
14.3	Recombinant Cytotoxic Fusion Proteins	374
	References	378
	Part III: Emerging Technologies	391
15	Emerging Technologies for Antibody Selection	393
	<i>Mingyue He and Michael J. Taussig</i>	
15.1	Introduction	393
15.2	Display Technologies	394
15.3	Antibody Libraries	395
15.4	Antibody Selection and Maturation <i>In vitro</i>	397
15.5	Linking Antibodies to mRNA: Ribosome and mRNA Display	398
15.6	Advantages of Ribosome Display	399
15.7	Ribosome Display Systems	399
15.7.1	Prokaryotic: <i>E. coli</i> S30	399
15.7.2	Eukaryotic: Rabbit Reticulocyte	400
15.7.3	Ribosome Display Constructs	400
15.7.4	Monosome versus Polysome Display	401
15.8	Antibody Generation by Ribosome Display	402
15.9	Summary	402
	References	402
16	Anti-Idiotypic Antibodies	407
	<i>Alejandro López-Requena, Oscar R. Burrone, and Rolando Pérez</i>	
16.1	Introduction	407
16.2	Basic Concepts	408
16.3	Physiological Role of Anti-idiotypic Antibodies	412
16.3.1	Self/Non-self Discrimination	412
16.3.2	Therapeutic Effect of the Pool of Intravenous Immunoglobulins (IVIg) on Autoimmune Diseases	413
16.4	Anti-Idiotypic Antibody Responses	414
16.5	Anti-Idiotypic Antibodies in Cancer	415
16.6	Anti-idiotypic Antibodies in Other Diseases	417
16.7	Concluding Remarks	418
	References	419
17	Non-Antibody Scaffolds as Alternative Therapeutic Agents	435
	<i>Markus Fiedler and Arne Skerra</i>	
17.1	Introduction	435
17.2	Motivation for Therapeutic Use of Alternative Binding Proteins	437
17.3	Single Domain Immunoglobulins	448

17.4	Scaffold Proteins Presenting a Contiguous Hypervariable Loop Region	450
17.5	Scaffold Proteins for Display of Individual Extended Loops	454
17.6	Scaffold Proteins Providing a Rigid Secondary Structure Interface	457
17.7	Non-Antibody Scaffolds Stepping into the Clinic	461
17.8	Conclusions and Outlook: Therapeutic Potential and Ongoing Developments	463
	References	464
18	Antibody-Directed Enzyme Prodrug Therapy (ADEPT)	475
	<i>Surinder K. Sharma, Kerry A. Chester and Kenneth D. Bagshawe</i>	
18.1	Introduction and Basic Principles of ADEPT	475
18.2	Pre-clinical Studies	477
18.2.1	CPG2 and Benzoic Mustard Prodrugs	477
18.2.2	Other Enzyme/Prodrug Systems	478
18.2.3	Catalytic Antibodies	478
18.3	Clinical Studies	479
18.3.1	F(ab)2 Fragments Conjugated to CPG2	479
18.3.2	Recombinant scFv-CPG2 Fusion Protein	479
18.4	Immunogenicity	480
18.5	Important Considerations/Outlook	481
	Acknowledgments	482
	Abbreviations	482
	References	482
19	Engineered Antibody Domains as Candidate Therapeutics	487
	<i>Weizao Chen, Ponraj Prabakaran, and Dimiter S. Dimitrov</i>	
19.1	Introduction	487
19.2	eAd Structure and Function	489
19.2.1	V _H H	492
19.2.2	VNAR	492
19.2.3	VH and VL	494
19.2.4	CH2	495
19.3	eAd Libraries	495
19.3.1	Generation of eAd Libraries from Naturally Occurring HCabs	495
19.3.2	Generation of Semi-Synthetic and Synthetic eAd Libraries	496
19.3.3	Generation of eAd Libraries with Grafted <i>In Vivo</i> Formed CDRs	497
19.4	eAds against HIV-1	498
19.4.1	eAds to the CoRbs of HIV-1 gp120	499
19.4.2	eAds to the CD4bs of HIV-1 gp120	500
19.4.3	eAds to the MPER of HIV-1 gp41	500
19.4.4	eAds to HIV-1 Coreceptors	501
19.4.5	Implications for HIV-1 Vaccine Immunogen Design	501
19.5	eAds Targeting Cancer	502

19.5.1	eAds for Cancer Imaging	502
19.5.2	eAds for Cancer Therapy	503
19.5.2.1	eAds Blocking Cancer Cell Signaling	503
19.5.2.2	eAds for Cancer Drug Targeting	503
19.5.2.3	eAds Targeting Cancer-Related Soluble Ligands for Their Irreversible Removal	504
19.6	eAds against Inflammation	505
19.6.1	eAds against Rheumatoid Arthritis (RA)	505
19.6.2	eAds against Inflammatory Bowel Disease (IBD)	507
19.7	eAds against Hematological Disorders	507
19.8	Conclusions	508
	Acknowledgments	508
	References	508
20	Chimeric Antigen Receptors –“CARs”	519
	<i>Ulf Petrusch and Thomas Schirrmann</i>	
20.1	Introduction	519
20.2	Chimeric Antigen Receptors –“CARs”	521
20.2.1	Antigen Recognition of Antibodies and T Cell Receptors	521
20.2.2	General Design of Chimeric Immunoglobulin T Cell Receptors	522
20.2.3	Double Chain CARs	523
20.2.4	Single-Chain CARs	524
20.2.5	The First Signal of the CAR	525
20.2.6	Signal Domains Employing Downstream Signal Molecules	526
20.2.7	The Transmembrane Domain – More Than Only a Membrane Anchor?	528
20.2.8	Extracellular Spacer Domains Promote CAR Expression and Function	528
20.2.9	The Second and Third Signals of the CAR	529
20.3	Preclinical Studies	530
20.3.1	Retroviral Gene Transfer into T Lymphocytes	530
20.3.2	“Naked” Gene Delivery Systems	532
20.3.3	Enrichment of CAR Transfected Effector Cells	532
20.3.4	Effector Functions of CAR Gene-Modified Effector Lymphocytes	533
20.3.5	Memory Function of Redirected T Cells	533
20.3.6	Animal Models	537
20.4	Therapeutic Considerations	538
20.4.1	Adoptive Cellular Immunotherapy	538
20.4.2	Clinical Studies with CAR-Modified T Lymphocytes	540
20.5	Perspectives	545
20.5.1	Tumor Taxis and Application of the CAR ⁺ Effector Cells	545
20.5.2	Neovascularization of Solid Tumors – Barrier or Target?	546
20.5.3	Rejection of Receptor Gene-Modified Effector Lymphocytes	546
20.6	Conclusions	547
	References	547

21	Emerging Alternative Production Systems	561
	<i>Benjamin Sommer, Holger Laux, Andre Frenzel, and Thomas Jostock</i>	
21.1	Introduction	561
21.2	Production Systems	562
21.2.1	Prokaryotic Expression Systems	562
21.2.1.1	<i>Escherichia coli</i>	562
21.2.1.2	<i>Pseudomonas fluorescens</i>	564
21.2.1.3	<i>Bacillus</i> Species	564
21.2.2	Eukaryotic Expression Systems	565
21.2.2.1	Yeast	565
21.2.2.2	Filamentous Fungi	569
21.2.2.3	Insect Cells	570
21.2.2.4	Mammalian Cells	571
21.2.2.5	Plants	579
21.2.2.6	Transgenic Animals	580
21.3	Outlook	581
	Abbreviations	583
	References	583

Volume II: Clinical Development of Antibodies

Quick Reference List of Antibodies by International Nonproprietary Name	XXIII
Quick Reference List of Antibodies by Brand Name	XXV
A Greeting by the Editors	XXVII
Foreword to the First Edition	XXIX
Foreword to the Second Edition	XXXI
List of Contributors	XXXIII
Abbreviations	LI
Appendix: Marketed Monoclonal Antibodies Compendium	LXXXIII

Part IV: The Way into the Clinic 601

22	Process Development and Manufacturing of Therapeutic Antibodies	603
	<i>Alexander Jacobi, Barbara Enenkel, Patrick Garidel, Christian Eckermann, Mathias Knappenberger, Ingo Presser, and Hitto Kaufmann</i>	
23	The Immunogenicity of Therapeutic Antibodies	665
	<i>Melody Sauerborn</i>	
24	Biosimilar Monoclonal Antibodies	681
	<i>Susanne D. Pippig, Carsten Brockmeyer, and Robert E. Zoubek</i>	
25	Patent Issues Relating to Therapeutic Antibodies	705
	<i>Barbara Rigby, Michael Braunagel, and Deborah Owen</i>	

Part V: Therapeutic Antibody Pipeline 735

- 26 **Monoclonal Antibody Cancer Treatments in Phase III Clinical Trials 737**
Ulf Petrausch and Peter Markus Deckert
- 27 **Antibodies in Cancer Treatment: Early Clinical Development 787**
Matthew Zibelman, Hossein Borghaei, and Anthony J. Olszanski
- 28 **Targeting Angiogenesis by Therapeutic Antibodies 823**
Onat Kadioglu, Ean Jeong Seo, and Thomas Efferth
- 29 **Antibodies in Phase III Studies for Immunological Disorders 851**
Penelope Ward and Mark Bodmer
- 30 **Monoclonal Antibodies in Phase 1 and 2 Studies for Immunological Disorders 927**
Frank R. Brennan
- 31 **MAbs Targeting Soluble Mediators in Phase 1 and 2 Clinical Studies Immunological Disorders 969**
Frank R. Brennan
- 32 **T Cell Inhibitors in Phase 1 and 2 Clinical Studies for Immunological Disorders 1079**
Frank R. Brennan
- 33 **B-Cell Inhibitors in Phase 1 and 2 Clinical Studies for Immunological Disorders 1115**
Frank R. Brennan
- 34 **Inhibitors of Leukocyte Adhesion and Migration in Phase 1 and 2 Clinical Studies for Immunological Disorders 1127**
Frank R. Brennan
- 35 **Toll-Like Receptor Inhibitors in Phase 1 and 2 Clinical Studies for Immunological Disorders 1145**
Frank R. Brennan
- 36 **IgE Inhibitors in Phase 1 and 2 Clinical Studies for Immunological Disorders 1159**
Frank R. Brennan
- 37 **Complement Inhibitors in Phase 1 and 2 Clinical Studies for Immunological Disorders 1165**
Frank R. Brennan

- 38 mAbs Targeting Apoptosis, Angiogenesis Inhibitors, and Other mAbs in Phase 1 and 2 Clinical Studies for Immunological Disorders 1175
Frank R. Brennan

- 39 *In vitro* Studies and Clinical Trials about Monoclonal Antibodies Used in Infectiology 1195
Guillaume Desoubeaux

- 40 Immunotherapeutics for Neurological Disorders 1215
Anne Messer, Kevin Manley, and Cynthia A. Lemere

Part VI: Gaining Marketing Approval 1231

- 41 Regulatory Considerations in the Development of Monoclonal Antibodies for Diagnosis and Therapy 1233
Marjorie A. Shapiro, Patrick G. Swann, and M. Stacey Ricci

- 42 Regulatory Review: Clinical to Market Transition 1263
Gabriele Dallmann

- 43 Monoclonal Antibody Nomenclature for Clinical Studies (USA) 1283
Stephanie C. Shubat

Volume III: Approved Therapeutic Antibodies

Quick Reference List of Antibodies by International Nonproprietary Name XXIII

Quick Reference List of Antibodies by Brand Name XXV

A Greeting by the Editors XXVII

Foreword to the First Edition XXIX

Foreword to the Second Edition XXXI

List of Contributors XXXIII

Abbreviations LI

Appendix: Marketed Monoclonal Antibodies Compendium LXXXIII

Part VII: Approved Therapeutic Antibodies 1289

- 44 Oligoclonal and Polyclonal Antibody Preparations 1291
Rishab K. Gupta and Mark C. Glassy

- 45 Adalimumab (Humira®) 1309
Janice M. Reichert

- 46 Alemtuzumab (Lemtrada, MabCampath) 1323
Thomas Elter, Michael Hallek, and Janice M. Reichert

- 47 **Basiliximab (Simulect®) and Daclizumab (Zenapax®)** 1375
Nadim Mahmud, Burcin Taner, and Nasimul Ahsan
- 48 **Belimumab (Benlysta®)** 1405
Pamela M. K Lutalo, Natasha Jordan, Thi-Sau Migone, and David P. D'Cruz
- 49 **Brentuximab Vedotin (Adcetris®) for the Treatment of CD30-Positive Hematologic Malignancies** 1417
Niels W.C.J. van de Donk and Eugen Dhimolea
- 50 **Canakinumab (ILARIS®)** 1445
Hermann Gram
- 51 **Catumaxomab (Removab) –Trifunctional Antibodies: Combining Direct Tumor Cell Killing with Therapeutic Vaccination** 1463
Horst Lindhofer, Michael Stanglmaier, Raymund Buhmann, Michael Jäger, Daniel Klunker, Peter Ruf, and Juergen Hess
- 52 **Cetuximab (Erbitux)** 1501
Sonja Wilke and Michael Hust
- 53 **Denosumab (Prolia®)** 1521
Torsten Meyer
- 54 **Efalizumab (Raptiva)** 1531
Karlheinz Schmitt-Rau
- 55 **Calicheamicin Conjugates: Gemtuzumab Ozogamicin (Mylotarg), Inotuzumab Ozogamicin** 1545
Matthias Peipp and Martin Gramatzki
- 56 **Golimumab (Simponi®)** 1565
Sohini Mazumdar and Janice M. Reichert
- 57 **Yttrium-90 Ibritumomab Tiuxetan (Zevalin®)** 1579
Karin Hohloch, Björn Chapuy, and Lorenz Trümper
- 58 **Infliximab (Remicade®)** 1599
Christian Antoni and Maria Wiekowski
- 59 **Ipilimumab (Yervoy®)** 1619
Teresa Alonso Gordoa, Javier Puente Vázquez, and Eduardo Díaz-Rubio

- 60 **Muromonab-CD3 (Orthoclone OKT[®]3)** 1645
Harald Becker and Janice M. Reichert
- 61 **Nimotuzumab: A Humanized Anti-EGFR Antibody** 1679
Tania Crombet Ramos
- 62 **Obinutuzumab (Gazyva[®]), a Novel Glycoengineered Type II CD20 Antibody for the Treatment of Chronic Lymphocytic Leukemia and Non-Hodgkin's Lymphoma** 1695
Christian Klein, Marina Bacac, Pablo Umaña, and Michael Wenger

Volume IV: Approved Therapeutic Antibodies and in vivo Diagnostics

Quick Reference List of Antibodies by International Nonproprietary Name XXIII

Quick Reference List of Antibodies by Brand Name XXV

A Greeting by the Editors XXVII

Foreword to the First Edition XXIX

Foreword to the Second Edition XXXI

List of Contributors XXXIII

Abbreviations LI

Appendix: Marketed Monoclonal Antibodies Compendium LXXXIII

- 63 **Ofatumumab (Arzerra[®]): a Next-Generation Human Therapeutic CD20 Antibody with Potent Complement-Dependent Cytotoxicity** 1733
Margaret A. Lindorfer, Joost M. Bakker, Paul W.H.I. Parren, and Ronald P. Taylor
- 64 **Omalizumab (Xolair) – Anti-Immunoglobulin E Treatment in Allergic Diseases** 1775
Claus Kroegel and Martin Foerster
- 65 **Palivizumab (Synagis[®])** 1825
Louis Bont
- 66 **Panitumumab (Vectibix[®]): A Treatment for Metastatic Colorectal Cancer** 1855
Jonas Kügler
- 67 **Pertuzumab (Perjeta[®])** 1871
Jose Angel García-Saénz, Fernando Moreno Anton, and Coralia Bueno Muñoz

- 68 **Ranibizumab (Lucentis): a New Anti-Angiogenic Treatment in Ophthalmology** 1883
Nicolas Leveziel, Marc Ohresser, and Gilles Paintaud
- 69 **Raxibacumab, Human Monoclonal Antibody against Anthrax Toxin** 1899
Sally D. Bolmer and Thi-Sau Migone
- 70 **Rituximab (Rituxan®)** 1909
Axel Böhnke and Michael Wenger
- 71 **Tocilizumab (Actemra®)** 2023
Graeme Jones and Changhai Ding
- 72 **Trastuzumab (Herceptin®) and Ado-Trastuzumab Emtansine (Kadcyla®): Treatments for HER2-Positive Breast Cancer** 2041
Ruhe Chowdhury and Paul Ellis
- 73 **Ustekinumab (Stelara®)** 2069
Oya Cingoz, Stefan Dübel, and Janice M. Reichert
- 74 **Abciximab (Reopro®), Bevacizumab (Avastin®), Certolizumab Pegol (Cimzia®), Eculizumab (Soliris®), Natalizumab (Tysabri®)** 2087
Janice M. Reichert
- 75 **Itolizumab (Alzumab®), Mogamulizumab (Poteligeo®), and Tositumomab (Bexxar®)** 2113
Stefan Dübel
- Part VIII: In vivo Diagnostics** 2121
- 76 **Radiolabeled Antibodies for Diagnostic Imaging** 2123
Christopher J. Palestro
- Index** 2143

Contents

Volume I: Defining the Right Antibody Composition

Quick Reference List of Antibodies by International Nonproprietary Name XXIII

Quick Reference List of Antibodies by Brand Name XXV

A Greeting by the Editors XXVII

Foreword to the First Edition XXIX

Foreword to the Second Edition XXXI

List of Contributors XXXIII

Abbreviations LI

Appendix: Marketed Monoclonal Antibodies Compendium LXXXIII

- 1 **Therapeutic Antibodies – from Past to Future** 1

Stefan Dübel and Janice M. Reichert

Part I: Selecting and Shaping the Antibody Molecule 15

- 2 **Selection Strategies for Monoclonal Antibodies** 17

Gerhard Moldenhauer

- 3 **Antibody Phage Display** 43

Michael Hust, André Frenzel, Florian Tomszak, Jonas Kügler, and Stefan Dübel

- 4 **Transgenic Animals Derived by DNA Microinjection** 77

Marianne Brüggemann, Michael J. Osborn, Biao Ma, Suzanne Avis, Ignacio Anegon, and Roland Buelow

- 5 **Humanization Strategies** 89

José W. Saldanha

- 6 **Antibody Affinity** 115

André Frenzel, Lorin Roskos, Scott Klakamp, Meina Liang, Rosalin Arends, and Larry Green

- 7 **Fc Engineering** 141
Matthias Peipp, Stefanie Derer, Stefan Lohse, Christian Kellner, and Thomas Valerius
- 8 **Glycosylation of Antibody Molecules** 171
Roy Jeffries
- 9 **Bioinformatics Tools for Analysis of Antibodies** 201
Andrew C.R. Martin and James Allen
- 10 **How to Use IMGT® for Therapeutic Antibody Engineering** 229
Marie-Paule Lefranc
- Part II: Modified Antibodies** 265
- 11 **Bispecific Antibodies** 267
Dafne Müller and Roland E. Kontermann
- 12 **Single-Domain Antibodies: An Overview** 311
Carrie Enever, Edward Coulstock, Małgorzata Pupecka-Swidler, and Bruce Hamilton
- 13 **Antibody–Drug Conjugates: New Frontier in Cancer Therapeutics** 341
Rajeeva Singh, John M. Lambert, and Ravi V.J. Chari
- 14 **Antibody-Targeted Drugs: From Chemical Immunoconjugates to Recombinant Fusion Proteins** 363
Athanasis Mavratzas, Michaela A.E. Arndt, Stefan Kiesgen, and Jürgen Krauss
- Part III: Emerging Technologies** 391
- 15 **Emerging Technologies for Antibody Selection** 393
Mingyue He and Michael J. Taussig
- 16 **Anti-Idiotypic Antibodies** 407
Alejandro López-Requena, Oscar R. Burrone, and Rolando Pérez
- 17 **Non-Antibody Scaffolds as Alternative Therapeutic Agents** 435
Markus Fiedler and Arne Skerra
- 18 **Antibody-Directed Enzyme Prodrug Therapy (ADEPT)** 475
Surinder K. Sharma, Kerry A. Chester and Kenneth D. Bagshawe

- 19 **Engineered Antibody Domains as Candidate Therapeutics** 487
Weizao Chen, Ponraj Prabakaran, and Dimiter S. Dimitrov
- 20 **Chimeric Antigen Receptors –“CARs”** 519
Ulf Petrausch and Thomas Schirrmann
- 21 **Emerging Alternative Production Systems** 561
Benjamin Sommer, Holger Laux, Andre Frenzel, and Thomas Jostock

Volume II: Clinical Development of Antibodies

Quick Reference List of Antibodies by International Nonproprietary Name XXIII

Quick Reference List of Antibodies by Brand Name XXV

A Greeting by the Editors XXVII

Foreword to the First Edition XXIX

Foreword to the Second Edition XXXI

List of Contributors XXXIII

Abbreviations LI

Appendix: Marketed Monoclonal Antibodies Compendium LXXXIII

Part IV: The Way into the Clinic 601

- 22 **Process Development and Manufacturing of Therapeutic Antibodies** 603
Alexander Jacobi, Barbara Enenkel, Patrick Garidel, Christian Eckermann, Mathias Knappenberger, Ingo Presser, and Hitto Kaufmann
- 22.1 Introduction 603
- 22.2 Upstream Processing 604
- 22.2.1 Expression Systems 605
- 22.2.2 Cell Culture Media 614
- 22.2.3 Cell Culture Process Design 614
- 22.2.4 Cell Culture Process Optimization 617
- 22.3 Downstream Processing 618
- 22.3.1 Platform Technologies for Downstream Processing of Monoclonal Antibodies 620
- 22.3.2 Primary Recovery 622
- 22.3.2.1 Ultra/Diafiltration (UF/DF) 622
- 22.3.2.2 Affinity Chromatography 622
- 22.3.3 Purification and Polishing 623
- 22.3.3.1 Hydrophobic Interaction Chromatography 623
- 22.3.3.2 Ion-Exchange Chromatography 623
- 22.3.3.3 Cation-Exchange Chromatography 624
- 22.3.3.4 Anion-Exchange Chromatography 624
- 22.3.4 Validation of DNA Removal and Virus Clearance 624