RECOMBINANT ANTIBODIES FOR IMMUNOTHERAPY

Recombinant Antibodies for Immunotherapy provides a comprehensive overview of the field of monoclonal antibodies (mAbs), a market that has grown tremendously in recent years. Twenty-four chapters by experienced and innovative authors cover the isolation of specific human mAbs, humanization, immunogenicity, technologies for improving efficacy, ''arming'' mAbs, novel alternative Ab constructs, increasing half-lives, alternative concepts employing non-immunoglobulin scaffolds, novel therapeutic approaches, a market analysis of therapeutic mAbs, and future developments in the field.

The concepts and technologies are illustrated by examples of recombinant antibodies being used in the clinic or in development. This book will appeal to both newcomers and experienced scientists in the field, biology and biotechnology students, research and development departments in the pharmaceutical industry, medical researchers, clinicians, and biotechnology investors.

Melvyn Little's research group at the German Cancer Research Center (DKFZ) in Heidelberg was one of the first to develop methods for making and screening antibody libraries. After co-founding Affitech (Oslo, Norway) in 1997, he left the DKFZ in 2000 to found Affimed Therapeutics, a biotechnology company in Heidelberg specializing in the isolation and engineering of human antibodies to treat various diseases, especially cancer. He has been an extracurricular professor of biochemistry at the University of Heidelberg since 1986.

RECOMBINANT ANTIBODIES FOR IMMUNOTHERAPY

Edited by

Melvyn Little

Affimed Therapeutics

CAMBRIDGE UNIVERSITY PRESS

Shaftesbury Road, Cambridge CB2 8EA, United Kingdom

One Liberty Plaza, 20th Floor, New York, NY 10006, USA

477 Williamstown Road, Port Melbourne, VIC 3207, Australia

314–321, 3rd Floor, Plot 3, Splendor Forum, Jasola District Centre, New Delhi – 110025, India

103 Penang Road, #05–06/07, Visioncrest Commercial, Singapore 238467

Cambridge University Press is part of Cambridge University Press & Assessment, a department of the University of Cambridge.

We share the University's mission to contribute to society through the pursuit of education, learning and research at the highest international levels of excellence.

www.cambridge.org
Information on this title: www.cambridge.org/9780521887328

First published 2009

A catalogue record for this publication is available from the British Library

Library of Congress Cataloging-in-Publication data
Recombinant antibodies for immunotherapy / edited by Melvyn Little.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-0-521-88732-8 (hardback)
1. Monoclonal antibodies – Therapeutic use. I. Little, Melvyn, 1945– II. Title.
[DNLM: 1. Immunotherapy – methods. 2. Antibodies, Monoclonal – therapeutic use.
3. Recombinant Proteins – therapeutic use. QW 940 R311 2009]

RM282.M65R43 2009
615´.37 – dc22 2008044201

ISBN 978-0-521-88732-8 Hardback

Contents

Color plates follow page xvi.

Contributors

Jan Terje Andersen
Department of Molecular Biosciences and Centre for Immune Regulation
University of Oslo
Oslo, Norway

Kenneth D. Bagshawe
Department of Oncology
Imperial College London
London, U.K.

Matthew P. Baker
Antitope Ltd.
Babraham, Cambridge, U.K.

Claude Beltejar
Raven Biotechnologies
San Francisco, California, U.S.

Gerald Beste
formerly MedImmune Ltd.
Cambridge, U.K.
now Ablynx NV
Ghent, Belgium

Christophe Bourrilly
ABN AMRO Corporate Finance Ltd.
London, U.K.

Peter Brünker
Glycart Biotechnology (Roche Group)
Schlieren, Switzerland

Frank J. Carr
Antitope Ltd.
Babraham, Cambridge, U.K.

Aaron K. Chamberlain
Xencor
Monrovia, California, U.S.

Kerry A. Chester
CR UK Targeting & Imaging Group
Department of Oncology
UCL Cancer Institute
University College London
London, U.K.

Nigel S. Courtenay-Luck
Antisoma Research Ltd.
London, U.K.

Agamemnon A. Epenetos
Trojantec Ltd.
Nicosia, Cyprus

Claudia Fieger
Raven Biotechnologies
San Francisco, California, U.S.

Daron Forman
Tolerx
Cambridge, Massachusetts, U.S.

Yannick Gansemans
Algonomics NV
Gent-Zwijnaarde, Belgium

Gholamreza Hassanzadeh Ghassabeh
Laboratory of Cellular and Molecular Immunology
Vrije Universiteit
and Department of Molecular and Cellular Interaction
VIB
Brussels, Belgium

Sam P. Heywood
Antibody Research
UCB-Celltech
Slough, U.K.

David P. Humphreys
Antibody Research
UCB-Celltech
Slough, U.K.

Aya Jakobovits
Agensys, Inc.
Santa Monica, California, U.S.

David Jones
Antisoma Research Ltd.
London, U.K.

David J. King
formerly Medarex
Sunnyvale, California, U.S.
now AnaptysBio Inc.
San Diego, California, U.S.

Kathleen L. King
Raven Biotechnologies
San Francisco, California, U.S.

Ingo M. Klagge
MorphoSys AG
Martinsried/Planegg, Germany

Stefan Knackmuss
Affimed Therapeutics AG
Heidelberg, Germany

Christina A. Kousparou
Trojantec Ltd.
Nicosia, Cyprus

Ignace Lasters
Algonomics NV
Gent-Zwijnaarde, Belgium

Greg A. Lazar
Xencor
Monrovia, California, U.S.

Fabrice Le Gall
Affimed Therapeutics AG
Heidelberg, Germany

Jonathan Li
Raven Biotechnologies
San Francisco, California, U.S.

Tony W. Liang
Raven Biotechnologies
San Francisco, California, U.S.

Monica Licea
Raven Biotechnologies
San Francisco, California, U.S.

Melvyn Little
Affimed Therapeutics AG
Heidelberg, Germany

Deryk Loo
Raven Biotechnologies
San Francisco, California, U.S.

David Lowe
MedImmune Ltd.
Cambridge, U.K.

Helen L. Lowe
CR UK Targeting & Imaging Group
Department of Oncology
UCL Cancer Institute
University College London
London, U.K.

Jennie P. Mather
Raven Biotechnologies
San Francisco, California, U.S.

Devangi Mehta
Tolerx
Cambridge, Massachusetts, U.S.

Vera Molkenthin
Affimed Therapeutics AG
Heidelberg, Germany

Andrew Murphy
Regeneron
Tarrytown, New York, U.S.

Serge Muyldermans
Laboratory of Cellular and Molecular Immunology
Vrije Universiteit
and Department of Molecular and Cellular Interaction
VIB
Brussels, Belgium

Jurgen Pletinckx
Algonomics NV
Gent-Zwijnaarde, Belgium

Andreas Plückthun
Department of Biochemistry
University of Zürich
Zurich, Switzerland

Paul Ponath
Tolerx
Cambridge, Massachusetts, U.S.

Joe Ponte
Tolerx
Cambridge, Massachusetts, U.S.

Beverly Potts
Raven Biotechnologies
San Francisco, California, U.S.

Patricia Rao
formerly Tolerx
Cambridge, Massachusetts, U.S.
now Synta Pharmaceutical Corp.
Lexington, Massachusetts, U.S.

Michael Rosenzweig
Tolerx
Cambridge, Massachusetts, U.S.

Florian Rüker
Department of Biotechnology
University of Natural Resources
and Applied Life Sciences
Vienna, Austria

Dirk Saerens
Laboratory of Cellular and Molecular
Immunology
Vrije Universiteit
and Department of Molecular and Cellular
Interaction
VIB
Brussels, Belgium

José W. Saldanha
Division of Mathematical Biology
National Institute for Medical Research
Mill Hill, London, U.K.

Inger Sandlie
Department of Molecular
Biosciences and Centre for Immune Regulation
University of Oslo
Oslo, Norway

Surinder K. Sharma
CR UK Targeting & Imaging Group
Department of Oncology
UCL Cancer Institute
University College London
London, U.K.

Jessica Snyder
Tolerx
Cambridge, Massachusetts, U.S.

Peter Sondermann
Glycart Biotechnology (Roche Group)
Schlieren, Switzerland

Philippe Stas
Algonomics NV
Gent-Zwijnaarde, Belgium

Ross Stewart
MedImmune Ltd.
Cambridge, U.K.

Pablo Umaña
Glycart Biotechnology (Roche Group)
Schlieren, Switzerland

Herman Waldmann
formerly Tolerx
Cambridge, Massachusetts, U.S.
now Sir William Dunn School of Pathology
Oxford, U.K.

Carl Webster
MedImmune Ltd.
Cambridge, U.K.

Gordana Wozniak-Knopp
Department of Biotechnology
University of Natural Resources
and Applied Life Sciences
Vienna, Austria

Peter Young
Raven Biotechnologies
San Francisco, California, U.S.

Foreword

Antibodies were discovered in 1890 but remained on the periphery of the pharmaceutical industry for more than 100 years. Yet within the last 15 years, a succession of antibodies has been approved for therapy by the United States Food and Drug Administration (FDA). Unlike natural antibodies which are polyclonal and directed against infectious disease, almost all those approved by the FDA are monoclonal antibodies directed against human self-antigens and used for treatment of cancer and diseases of the immune system.

Two major breakthroughs proved necessary to launch this antibody revolution. The first breakthrough was rodent hybridoma technology in the 1970s. Antibodies could now be made against single antigens in complex mixtures and used to identify the molecular targets of disease. In some cases this allowed disease intervention by blocking the antigen or by killing a class of cells (such as cancer cells) bearing the antigen. However, hybridoma technology provided only part of the solution; the rodent antibodies proved immunogenic and often did not trigger human effector functions efficiently. The second breakthrough, in the 1990s, was protein engineering; its application allowed the creation of chimeric and humanized antibodies from rodent monoclonal antibodies; not only were these less immunogenic than rodent antibodies, but they more efficiently triggered human effector functions. These chimeric and humanized antibodies now account for the majority of the currently approved therapeutic antibodies.

Nevertheless the field continued to embrace new technologies and to spawn new approaches, most notably the development of genuine human antibodies in the 1990s. Human therapeutic antibodies were made by selection from highly diverse antibody repertoires displayed on filamentous phage, and then from mice transgenic with human antibody genes. The pace of innovation continued in the new millennium; antibodies were built from single domains, endowed with enhanced effector functions or prolonged serum half-life, and even tailored to bind antigen via engineered constant domains. Earlier approaches, for example those based on cytotoxic drugs or radio-immune conjugates, were also re-evaluated. In a field with few clinically validated targets and a thicket of intellectual property, technological innovation has offered freedom for new biotechnology companies to develop therapeutics based on antibodies or antibody mimics.

Recombinant Antibodies for Immunotherapy, edited by Professor Melvyn Little, covers both the fundamentals of the technology and the current state of its

development and concludes with a section on novel therapeutic approaches and an overview of the market that has driven, and continues to drive, the field. The book promises to be an essential and most convenient guide to the field.

Sir Gregory Winter FRS
Deputy Director
Laboratory of Molecular Biology and MRC Centre for Protein Engineering
Cambridge, UK

Preface

The potential of antibodies as magic bullets for curing disease has excited the imagination of medical researchers ever since this phrase was first coined by Paul Ehrlich about a century ago. Seventy-five years after the publication of Ehrlich's side-chain theory to explain antibody-antigen reactions in 1900, Georges Köhler and César Milstein invented a means of cloning antibodies with defined specificity that paved the way for major advances in cell biological and clinical research. They were awarded the Nobel Prize in Medicine in 1984 for this ground-breaking research. In 1986, the first monoclonal antibody, the murine mAb OKT3 for preventing transplant rejection, was approved for clinical use, and although many other murine mAbs were subsequently investigated as therapeutic agents, most of them had a disappointing clinical profile largely due to their immunogenicity. This situation improved dramatically with the advent of techniques to humanize existing mAbs, followed by technologies that sought to imitate the generation of specific antibodies by the immune system *in vitro*. For example, the expression of antibody fragments in *E. coli* using bacterial leader sequences and the use of phage display and later ribosome display facilitated the selection of specific human antibodies from extremely large libraries. The process of somatic hypermutation to increase antibody affinity was mimicked by introducing random mutations. Another major advance for obtaining human antibodies was the creation of transgenic mice carrying a large part of the human antibody gene repertoire, which could be used to produce human antibodies by standard hybridoma technology. The success of these novel technologies resulted in a first generation of recombinant antibodies that now account for a large proportion of the market for biopharmaceuticals, with annual growth rates of almost 40%.

Therapeutic antibodies for cancer rely to a large extent on the recruitment of other elements in the immune system for their effect; very few of them function as magic bullets in the sense of ''target and destroy.'' For example, although antibody binding to a specific epitope of a cell surface receptor can directly induce strong apoptotic signals, the effect is usually amplified by cross-linking of the antibody Fc domains through binding to Fc receptors on immune effector cells such as macrophages and natural killer cells. Concomitantly, the immune effector cells are activated by the engagement of the Fc receptors, resulting in an attack on the cells to which they are bound, a process known as antibody-dependent cell cytolysis (ADCC). The Fc domains can also activate the complement system, causing complement-dependent cytolysis (CDC). To what extent cell lysis is caused by direct binding and how much is due to the recruitment of immune effector cells and complement is difficult to quantify, especially in an *in vivo* system, and in many cases the mechanism of action of

antitumor antibodies remains ill defined. For the action of most cytolytic antibodies, all three mechanisms are probably involved to a lesser or greater extent. Furthermore, recent findings suggest that ADCC also contributes to the efficacy of those antibodies that were previously thought to cause tumor regression solely by blocking the ligand-binding site of growth hormone receptors.

In the second generation of therapeutic recombinant antibodies now in various stages of development, novel techniques and creative antibody engineering have evolved to optimize pharmokinetic and pharmodynamic properties. For example, the affinity of antibody Fc domains for their receptors on immune effector cells or to complement has been improved by both random and targeted mutagenesis. Cell lines have also been generated for altering the glycosyl side chains on the Fc domains for better Fc-receptor binding. Algorithms and *in vitro* techniques have been devised for predicting immunogenicity and selecting the best variants. In addition, the cytotoxic potential of antibodies and antibody fragments has been increased by arming them with toxins, radionuclides, or immune effector molecules such as cytokines. A large number of novel antibody formats ranging from single variable domains of approximately 13kDa to full-length antibodies with multiple variable domains of approximately 200kDa have been constructed to enable a variety of different functions. For example, bispecific antibodies for recruiting T cells to lyse tumor cells have been engineered without constant domains, thus reducing the risk of cytokine storms due to extensive cross-linking with Fc receptors. Finally, a variety of novel protein scaffolds are being investigated as alternatives to immunoglobulin fragments for the generation of libraries of highly diverse binding molecules that could result in novel therapeutic drugs. However, as nearly all of the alternative binding molecules are the same size as or even smaller than single immunoglobulin domain antibodies, their serum half-lives will probably have to be significantly extended using techniques such as pegylation or fusion to serum proteins such as albumin.

All of the recombinant antibody technologies just described are covered by the 24 articles in this book, written by recognized experts in their field, many of whom have pioneered important new techniques. Starting with a description of the technologies used to generate recombinant antibodies, the following chapters provide a fairly comprehensive overview, with examples and background information, on how antibody efficacy is being improved by decreasing immunogenicity, increasing effector function through increased Fc-receptor binding, conjugating with cytolytic agents, using novel formats and scaffolds with multiple valencies and specificities, and increasing serum half-life. Several promising therapeutic approaches have been included, such as a novel method for selecting antibodies that specifically lyse tumor cells, the development of a recombinant antibody prodrug, and the use of novel recombinant antibodies that target T cells for the treatment of autoimmune disease. Last but not least, an attempt to forecast future developments in the field of therapeutic recombinant antibodies has been made on the basis of an excellent market analysis of this rapidly growing field.

M.L.