Methods in Molecular \mathbf{B} iology

Series Editor John M. Walker School of Life Sciences University of Hertfordshire Hatfield, Hertfordshire, AL10 9AB, UK

For further volumes: http://www.springer.com/series/7651

Antibody-Drug Conjugates

Edited by

Laurent Ducry

Lonza Ltd, Visp, Switzerland

╬ Humana Press

Editor Laurent Ducry Lonza Ltd Visp, Switzerland

ISSN 1064-3745 ISSN 1940-6029 (electronic) ISBN 978-1-62703-540-8 DOI 10.1007/978-1-62703-541-5 (eBook) Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2013943693

© Springer Science+Business Media, LLC 2013

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed. Exempted from this legal reservation are brief excerpts in connection with reviews or scholarly analysis or material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work. Duplication of this publication or parts thereof is permitted only under the provisions of the Copyright Law of the Publisher's location, in its current version, and permission for use must always be obtained from Springer. Permissions for use may be obtained through RightsLink at the Copyright Clearance Center. Violations are liable to prosecution under the respective Copyright Law.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

While the advice and information in this book are believed to be true and accurate at the date of publication, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Printed on acid-free paper

Humana Press is a brand of Springer Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Antibody–drug conjugates (ADCs) represent a promising therapeutic approach for cancer patients by combining the antigen-targeting specificity of monoclonal antibodies (mAbs) with the cytotoxic potency of chemotherapeutic drugs. The FDA approval of Adcetris[®] (brentuximab vedotin) in 2011 and Kadcyla[®] (trastuzumab emtansine or T-DM1) in 2013 has validated the idea of making "armed" antibodies, attracting a lot of attention into this field. ADC technology has been an active area of research in recent years, resulting in a number of ADCs in development for various tumor types. The number of immunoconjugates or ADCs undergoing clinical trial will thus further increase, possibly replacing some of the existing naked monoclonal antibodies, and becoming the next generation of anticancer biotherapeutics.

Although the ADC concept is quite simple, successfully designing and developing such a "smart bomb" is a complex task. Despite a tremendous increase in our understanding in recent years, a lot of work is necessary in order to identify a suitable target; properly design the mAb, the linker, and the payload; as well as conjugate them in a reproducible and scalable fashion.

The success of the current conjugation technologies has been achieved thanks to the development of new methodologies. The aim of this book is to provide detailed protocols for many of the key ADC techniques necessary for working in the field. Each method is described by an author who has regularly used the technique in his or her laboratory. In addition, several review chapters are included to summarize the current knowledge and results in the ADC area. These should make this book useful to readers with no previous ADC experience as well as those already working in the field. It is my hope that this publication will further drive ADC development and thus help towards improving cancer treatments of the future.

Visp, Switzerland

Laurent Ducry

Contents

	face tributors	v ix
1	Antibody–Drug Conjugate (ADC) Clinical Pipeline: A Review Ingrid Sassoon and Véronique Blanc	1
2	Antibody–Drug Conjugate Target Selection: Critical Factors Neil H. Bander	29
3	Selecting an Optimal Antibody for Antibody–Drug Conjugate Therapy: Internalization and Intracellular Localization Jay Harper, Shenlan Mao, Patrick Strout, and Adeela Kamal	41
4	Antibody–Drug Conjugate Payloads Jan Anderl, Heinz Faulstich, Torsten Hechler, and Michael Kulke	51
5	Linker Technologies for Antibody–Drug Conjugates	71
6	In Vivo Testing of Drug-Linker Stability Pierre-Yves Abecassis and Céline Amara	101
7	Pharmacokinetics and ADME Characterizations of Antibody–Drug Conjugates	117
8	Safe Handling of Cytotoxic Compounds in a BiopharmaceuticalEnvironmentMiriam I. Hensgen and Bernhard Stump	133
9	Micro- and Mid-Scale Maleimide-Based Conjugation of Cytotoxic Drugs to Antibody Hinge Region Thiols for Tumor Targeting James E. Stefano, Michelle Busch, Lihui Hou, Anna Park, and Diego A. Gianolio	145
10	Protocols for Lysine Conjugation Marie-Priscille Brun and Laurence Gauzy-Lazo	173
11	Engineering THIOMABs for Site-Specific Conjugation of Thiol-Reactive Linkers Sunil Bhakta, Helga Raab, and Jagath R. Junutula	189
12	Enzymatic Antibody Modification by Bacterial Transglutaminase Patrick Dennler, Roger Schibli, and Eliane Fischer	205
13	Formulation Development of Antibody–Drug Conjugates	217
14	Conjugation Process Development and Scale-UpBernhard Stump and Jessica Steinmann	235
15	Methods for Conjugating Antibodies to Nanocarriers Anil Wagh and Benedict Law	249
16	Drug-to-Antibody Ratio (DAR) by UV/Vis Spectroscopy	267

17	Drug-to-Antibody Ratio (DAR) and Drug Load Distribution by Hydrophobic Interaction Chromatography and Reversed Phase High-Performance Liquid Chromatography	275
18	Drug-to-Antibody Ratio (DAR) and Drug Load Distribution by LC-ESI-MS	285
19	Determination of Charge Heterogeneity and Level of Unconjugated Antibody by Imaged cIEF Joyce Lin and Alexandru C. Lazar	295
20	Risk-Based Scientific Approach for Determination of Extractables/Leachables from Biomanufacturing of Antibody–Drug Conjugates (ADCs) <i>Weibing Ding</i>	303
Index		313

Contributors

PIERRE-YVES ABECASSIS • DSAR (Disposition, Safety & Animal Research), Sanofi, Vitry-sur-Seine, France Céline Amara • DSAR (Disposition, Safety & Animal Research), Sanofi, Vitry-sur-Seine, France JAN ANDERL • Heidelberg Pharma GmbH, Ladenburg, Germany NEIL H. BANDER • Weill-Cornell Medical College, Cornell University, New York, NY, USA LOUISETTE BASA • Protein Analytical Chemistry, Genentech Inc., South San Francisco, CA, USA SUNIL BHAKTA • Genentech Inc., South San Francisco, CA, USA Véronique Blanc • Sanofi Oncology, Vitry-sur-Seine, France MARIE-PRISCILLE BRUN • Natural Product and Protein Chemistry, Sanofi, Vitry-sur-Seine, France MICHELLE BUSCH • Transitional Research, Genzyme, a Sanofi Company, Framingham, MA, USA YAN CHEN • Protein Analytical Chemistry, Genentech Inc., South San Francisco, CA, USA PATRICK DENNLER • Center for Radiopharmaceutical Sciences, Paul Scherrer Institute, Villigen, Switzerland WEIBING DING • SLS Global Technical Support, Pall Corporation, Port Washington, NY, USA HEINZ FAULSTICH • Heidelberg Pharma GmbH, Ladenburg, Germany ELIANE FISCHER • Center for Radiopharmaceutical Sciences, Paul Scherrer Institute, Villigen, Switzerland WILLIAM J. GALUSH • Early Stage Pharmaceutical Development, Genentech Inc., South San Francisco, CA, USA LAURENCE GAUZY-LAZO • Natural Product and Protein Chemistry, Sanofi, Vitry-sur-Seine, France DIEGO A. GIANOLIO • Drugs and Biomaterials R&D, Genzyme, a Sanofi Company, Cambridge, MA, USA JAY HARPER • Oncology Research, MedImmune Inc., Gaithersburg, MD, USA Torsten Hechler • Heidelberg Pharma GmbH, Ladenburg, Germany MIRIAM I. HENSGEN • Bioconjugates R&D, Lonza Ltd, Visp, Switzerland LIHUI HOU • Transitional Research, Genzyme, a Sanofi Company, Framingham, MA, USA JAGATH R. JUNUTULA • Genentech Inc., South San Francisco, CA, USA ADEELA KAMAL • Oncology Research, MedImmune Inc., Gaithersburg, MD, USA MICHAEL KULKE • Heidelberg Pharma GmbH, Ladenburg, Germany BENEDICT LAW • Department of Pharmaceutical Sciences, College of Pharmacy, Nursing and Allied Sciences, North Dakota State University, Fargo, ND, USA ALEXANDRU C. LAZAR • Analytical & Pharmaceutical Sciences, ImmunoGen, Inc., Waltham, MA, USA

- JOYCE LIN Analytical & Pharmaceutical Sciences, ImmunoGen, Inc., Waltham, MA, USA
- KEDAN LIN Early Development Pharmacokinetics and Pharmacodynamics, Genentech Inc., South San Francisco, CA, USA
- SHENLAN MAO Oncology Research, MedImmune Inc., Gaithersburg, MD, USA
- BIRTE NOLTING Biotherapeutics Research and Development, Pfizer, Pearl River, NY, USA
- JUN OUYANG Pharma Technical Regulatory, Genentech Inc., South San Francisco, CA, USA
- ANNA PARK Transitional Research, Genzyme, a Sanofi Company, Framingham, MA, USA
- HELGA RAAB Genentech Inc., South San Francisco, CA, USA
- INGRID SASSOON Sanofi Oncology, Vitry-sur-Seine, France
- ROGER SCHIBLI Center for Radiopharmaceutical Sciences, Paul Scherrer Institute, Villigen, Switzerland
- BEN-QUAN SHEN Early Development Pharmacokinetics and Pharmacodynamics, Genentech Inc., South San Francisco, CA, USA
- JAMES E. STEFANO Transitional Research, Genzyme, a Sanofi Company, Framingham, MA, USA
- JESSICA STEINMANN Bioconjugates R&D, Lonza Ltd, Visp, Switzerland
- PATRICK STROUT Oncology Research, MedImmune Inc., Gaithersburg, MD, USA
- BERNHARD STUMP Bioconjugates R&D, Lonza Ltd, Visp, Switzerland
- JAY TIBBITTS Drug Metabolism and Pharmacokinetics, UCB Celltech Slough, Berkshire, UK
- ANIL WAGH Department of Pharmaceutical Sciences, College of Pharmacy, Nursing and Allied Sciences, North Dakota State University, Fargo, ND, USA
- ADITYA A. WAKANKAR Late Stage Pharmaceutical Development, Genentech Inc., South San Francisco, CA, USA