Bispecific Antibodies

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Editor

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Springer

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ISBN 978-3-642-20909-3 e-ISBN 978-3-642-20910-9
DOI 10.1007/978-3-642-20910-9
Springer Heidelberg Dordrecht London New York

Library of Congress Control Number: 2011933913

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

The first description of bispecific antibodies dates back 50 years, when Nisonoff and Rivers described the recombination of a mixture of univalent antibody fragments of different specificity. However, it took another 20 years before bispecific antibodies were proposed for therapeutic applications, mainly for the retargeting of effector T cells to tumor cells. These early studies implied already the use of bispecific antibodies to extend the functions beyond that normally executed by antibodies. Limited by the availability of monoclonal antibodies obtained from animal sources, bispecific antibodies of this early phase were generated by somatic hybridization of two hybridomas or by chemical conjugation of two IgG molecules. The high expectations on these bispecific antibodies were, however, not fulfilled, mainly because of low efficacy, immunogenicity, and severe adverse effects seen in clinical trials. This resulted in a loss of interest in this kind of molecules during the last decade of the last century. However, with advancements in antibody engineering and the establishment of novel applications, bispecific antibodies experienced a revival at the beginning of this century. Besides effector cell retargeting for cancer immunotherapy, applications include, among others, pre-targeting strategies in radioimmunotherapy and more recently dual-targeting strategies simultaneously attacking two disease-relevant targets. Genetic engineering allows nowadays to generate recombinant bispecific antibodies of defined composition, as well as with improved stability and producibility. Hence, bispecific antibodies have regained interest by the pharmaceutical industry, and many companies have meanwhile established their own bispecific antibody program. The first bispecific antibody for the retargeting of effector cells to EpCAM-positive tumor cells was approved in 2009 for the treatment of malignant ascites, and an increasing number of bispecific antibodies is currently in preclinical and clinical development.

Today, approaches to generate bispecific antibodies cover a broad spectrum including chemical conjugation, somatic hybridization, and genetic engineering. The latter has resulted in a multitude of recombinant IgG-like but also small-size bispecific antibody formats. Importantly, applications have been extended in the same manner. With many bispecific antibodies, especially of the second generation,

in development, this class of molecules is rapidly advancing. This book is intended to provide a comprehensive overview of the various techniques and formats to generate bispecific antibodies and to give insights into the various applications which have emerged during the last two decades and which are actively explored for therapeutic and diagnostic purposes.

Stuttgart, Germany Roland E. Kontermann

Foreword

The development of monoclonal antibodies (mAbs) as therapeutics is an evolving discipline that relies heavily on innovation in biological engineering for its advancement. Therapeutic mAbs first entered clinical study in the early 1980s, soon after the hybridoma technology used to generate them was first described. Substantial improvements in the safety and efficacy of mAbs were a direct result of the advances in biological engineering that led to production of chimeric, humanized, and human antibody therapeutics. Advancement of technologies to design, engineer, and manufacture antibodies in the 1990s, as well as increases in the global sales of therapeutic mAbs, led to substantial commercial interest and investment in the 2000s. One measure of this investment is the number of novel mAbs that entered clinical study each year, which averaged approximately 20 candidates per year during the late 1990s and early 2000s but rose to nearly 50 per year in 2009. As of 2010, over 30 mAbs have been approved for marketing. Limitations inherent in the canonical monospecific IgG antibody prompted exploration of alternative molecular formats, including numerous bispecific versions of antibodies. This avenue of research recently yielded the first bispecific antibody to be approved, catumaxomab (Removab®), which is marketed in Europe for treatment of malignant ascites in epithelial cell adhesion molecule (EpCAM)-positive cancer patients.

The development of bispecific antibodies is a microcosm that has mimicked the past trends observed for overall antibody development and seems likely to share in a bright future. Historically, about half of all antibodies that entered clinical study sponsored by commercial firms were developed for cancer indications. Bispecific antibodies, with their inherent ability to bring two different targets into proximity, have been clinically studied almost exclusively in cancer patients. In order of frequency (first to fourth), the tumor-associated antigens most frequently targeted by anticancer antibodies evaluated in clinical studies sponsored by commercial firms to date are EpCAM, CD20, epithelial growth factor receptor (EGFR), and HER2/neu. Following these, insulin-like growth factor-1 receptor and carcinoembryonic antigen (CEA) were tied for fifth; CD30 and MUC1 were sixth;

and CD33, prostate-specific membrane antigen, tumor-related apoptosis-inducing ligand-receptor 2, and CD19 were seventh in frequency.

The tumor-associated antigens targeted by bispecific antibodies have mirrored these selections, with HER2/neu as the most frequent target followed by EpCAM, CEA, and CD30. A design challenge of the bispecific antibodies has been in the selection of the second specificity, which enables the cell-killing functionality of the anticancer molecules. Bispecific antibodies began entering clinical study in the early 1990s; the focus then was on CD64 (also known as FcγR1; found on macrophages and monocytes) and CD16 (T-cell co-receptor) as the second target of the molecules. At that time, Medarex had a robust bispecific antibody development program that focused on antigen-binding fragments (Fab) targeting CD64 that could be combined with Fab molecules targeting tumor-associated antigens such as HER2/neu, CD30, EGFR, or TAG72. However, all the bispecific molecules that entered clinical study in that period were ultimately terminated. The complexity of the biology, production issues, and competition from full-size IgG1 antibodies targeting the same tumor-associated antigens that were also in development at the same time were contributing factors in the decisions to discontinue development of these early bispecific candidates. The competitive IgG1 molecules proved to be superior in two cases – the humanized anti-HER2/neu trastuzumab (Herceptin®) was first approved in 1998 and the chimeric anti-EGFR cetuximab (Erbitux®) was first approved in 2003.

Although no therapeutic products resulted from the early work on bispecific antibodies, the ideas underlying it were sound and the research provided a foundation for advancement of the technology and improvement of the candidates. The combination of past experience with the recent increase in investments in antibody development has led to a notable revival of interest in bispecific formats. A path to approval for therapeutic bispecific antibodies has already been established by the work of Fresenius, the commercial sponsor of the approved bispecific antibody, catumaxomab. Numerous targets have now been clinically validated and bispecific molecules that might show enhanced efficacy compared with canonical full-size therapeutic antibodies are now entering clinical study in increasing numbers. The bispecific T-cell engager (BiTE) antibodies from Micromet are an excellent example of the potential of bispecific therapeutics. These molecules target CD3 on T cells, as well as tumor-associated antigens. Encouraging Phase 2 clinical results have been reported for blinatumomab, which targets CD3 and CD19 and is undergoing evaluation in patients with B-precursor acute lymphoblastic leukemia.

Considering the recent advances in bispecific antibody engineering and the obvious promise of the molecules as therapeutics, the publication of "Bispecific Antibodies," edited by Roland Kontermann, is timely. The book provides comprehensive coverage of the past, present, and probable future of bispecific antibody research and development. The available formats of these molecules have expanded rapidly and many of these, e.g., diabodies, dual variable domains, two-in-one antibodies, BiTEs, are discussed in detail. The potential of bispecific antibodies in gene therapy and their use as diagnostics is also explained. "Bispecific

Antibodies" contains a vast trove of in-depth knowledge about these versatile molecules and will thus be an invaluable resource for both experts and those new to the field.

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