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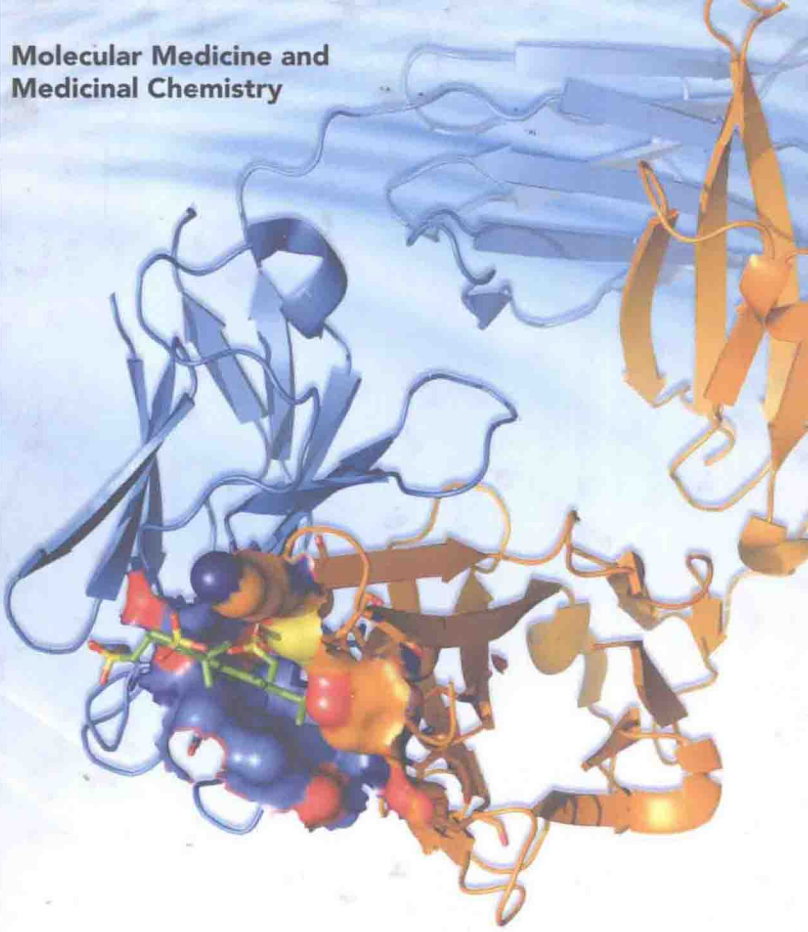

Antibody Drug Discovery

Clive R Wood

Editor

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Editor

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Foreword

*John McCafferty**

In 1996 only two antibodies were approved for therapeutic use in humans. In the years that have followed, however, 26 more antibody products have been approved with hundreds more entering clinical trials[1]. Antibodies are the fastest growing class of drugs, and the factors behind this success are clear. Their long half-lives allow relatively infrequent dosing, and they are well-tolerated and highly specific for their targets. Their basic properties can even be improved by engineering binding or effector functions. Simply stated, antibodies make good drugs, and they have become accepted by drug developers, regulators and users alike.

Monoclonal antibodies were first described in 1975, so why did it take so long for their potential to be realized in the clinic? Initially, there was great optimism around the clinical use of antibodies as "magic bullets" for targeting tumors or for modifying immunological function. However, this initial optimism soon gave way to pessimism when murine antibodies were found to induce unwanted immune responses in human recipients. Additionally, poor engagement with human effector function limited their potency. The answer to the problem was to either develop antibodies directly from a human source or make murine antibodies more "human-like". Over the last two decades a number of solutions have emerged to allow generation of antibody drugs which are accepted by the patients' immune system. It was these technological developments that unlocked the potential of monoclonal antibodies and drove the "antibody revolution", leading to the expansion of antibody drugs in the world's dispensary.

Advanced molecular biology techniques allowed the creation of chimeric antibodies by grafting variable domains from mouse monoclonal antibodies onto human constant regions. The reduction in immunogenicity was taken a step

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further by "CDR grafting" (also called "humanization") where the individual binding loops from murine variable domains were grafted onto human variable region frameworks. These approaches circumvented the problems associated with using murine antibodies in humans, and many of the clinical candidates from the 1990s were generated by this route. The current profile of approved drugs reflects this pedigree, with chimeric or CDR grafted antibodies accounting for two thirds of all antibody-based drugs currently marketed in the USA. Other technology developments during the early 1990s enabled direct isolation of human antibodies. The demonstration 20 years ago that functional antibodies could be displayed on the surface of filamentous bacteriophage opened the door to a powerful method for isolating or improving human antibodies using large antibody display libraries. Fully human antibodies could also be directly isolated from transgenic mice in which murine antibody genes were replaced with human antibody genes, allowing the generation of high affinity human antibodies by immunization. Over the last decade an increasing proportion of antibodies in development have been derived from phage display or transgenic technologies, with 10 such antibodies already approved or undergoing review by the FDA and 88 more currently undergoing clinical trials[1].

Technological developments have not only driven our ability to generate desired binding specificities but have also helped enhance clinical effects and outcomes by allowing flexibility in the choice of antibody class, isotype and mode of action. In particular, engineering of antibody constant domains has played an important role over recent years in increasing half-life or controlling engagement with effector functions.

These combined developments over the last two decades have moved antibody drugs from being on the fringes of medical practice to becoming a major weapon in combating disease, a trend that is likely to continue in the coming years. In *Antibody Drug Discovery* Clive Wood has assembled a number of excellent overviews and expositions of the transforming technologies that have driven this revolution. This book is a valuable resource for those seeking to understand and benefit from these mature technologies. Through their efforts, the contributors have distilled extensive "real world" knowledge and experiences to create a volume that will hopefully inform and inspire the next generation of drug developers.

John McCafferty, Cambridge, 2011

[1] Nelson A.L., Dhimolea E., Reichert J.M. (2010). Development trends for human monoclonal antibody therapeutics. *Nat Rev Drug Discov* 9: 767–74.

Preface

*Clive R. Wood**

The recombinant DNA-based antibody technologies crafted during the last few decades have allowed us to exploit the natural defense mechanisms that antibodies provide. We can identify antibodies that bind almost any target with high affinity and exquisite specificity, and in an IgG scaffold antibodies have the benefits of bivalent binding, effector mechanisms and a long plasma half-life. Since the market launch of two humanized antibodies in 1997, these drugs have found a wide range of clinical uses. In 2009, only fourteen years later, four IgG1antibodies and one IgG1 fusion protein were represented in the list of prescription drugs with the twenty largest worldwide markets. This productive trend is forecast to continue with the market for antibody drugs having a compound adjusted growth rate (2008–2014) of 10.3% (PharmaVitae: Monoclonal Antibodies: 2009 Update, Datamonitor), dramatically outpacing that of small molecule drugs. This success is based on delivering value to patients that is not provided by other means. Antibodies are firmly embedded in our armory of approaches against disease.

This book was developed as a place to capture an overview of the foundations of antibody drug discovery technology, with each chapter written by leaders in the field. The guiding principle for these authors was that they should clarify how each area has developed and emphasize key points for readers working in this area to consider. These foundations are well established and will serve us well for years to come. Their general accessibility is increasing today as patents covering some areas have expired or are close to expiring. Many of us in the field are excited by emerging new areas of antibody technology and novel scaffolds that may have antibody-like properties. Nonetheless, the relative ease of use of

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existing technologies and their increasing availability with patent expiration will increase the demands on new approaches to deliver benefits over and above those of the foundation technologies.

Three core technologies are used for identifying antibody leads: humanization of mouse monoclonal antibodies (mAbs), human immunoglobulin transgenic mice, and *in vitro* display. Once a murine mAb has been isolated with the desired properties, the fastest route to a drug candidate can be by grafting the murine mAb CDR sequences into a human scaffold. The 'Humanization of Antibodies' chapter (Olivier Léger and José Saldanha) provides a succinct overview of the different humanization technologies that are currently used. The two other core technologies do not use a murine mAb as a starting point. In the transgenic approach, a number of mouse strains have been engineered that block the ability of the endogenous murine immunoglobulin genes to contribute to an antibody response, while introducing human immunoglobulin genes that can rearrange, be used in the murine B-cell response and isolated via conventional hybridoma technology. The chapter on 'Transgenic Mice Rearranging Human Antibodies' (Sean Stevens) addresses key advances in this field. Additionally, *in vitro* display with combinatorial libraries of antibody fragments has proven very successful. Display systems based on phage particles and yeast cells are the most extensively employed. Among other advantages, they allow the identification of antibodies with epitope specificities that would be silenced by immunologic tolerance *in vivo*. Therefore, with well-designed selection strategies, there is a higher probability of finding leads with unique specificities, including the ability to bind both the human target antigen and its orthologs in preclinical species, thereby accelerating pharmacological and toxicological testing. I am particularly pleased that the chapter on 'Selection and Screening of Antibody Phage Display Libraries' (David Buckler *et al.*) is a unique collaboration between two of the pioneering organizations in phage display and represents a summation of many decades of experience. It is followed by a chapter on how antibodies from different approaches, especially from phage display, have been affinity optimized (David Lowe *et al.*). Most antibody phage display libraries have used immunoglobulin sequences from naïve donors, so the resulting leads have not been through affinity maturation *in vivo*. Thus, the leads may have low affinities, and in some cases affinity maturation is necessary to match antibodies from *in vivo* immunization approaches.

Once an antibody lead is identified, the structure of the Fc must be selected, expressed and then purified to provide supply to the research team for further characterization. The majority of marketed antibody therapeutics are of the human IgG1 isotype, which has proven to be a very successful scaffold for a wide variety of antigen specificities and clinical uses. This success justifies treating it as a preferred isotype unless the effector properties of IgG1 are verified as being unsuitable for a particular use. In recent years, a number of antibodies with other isotypes have been approved. The 'Isotype Selection and Fc Engineering' chapter (William Strohl) provides a comprehensive review of Fc isotypes and engineering

strategies. The 'Antibody Expression from Bacteria to Transgenic Animals' chapter (Paul Stephens and Bernie Sweeney) provides an overview of the current systems used for antibody expression and an evaluation of some of the alternative systems that are being developed. In addition to expressing the antibody, a purification process is needed for research supply and an evaluation of the feasibility of a development process. 'Current Trends in Antibody Purification and Lead Selection' (Jie Chen and Andrew Nixon) provides an overview of key purification approaches and describes how the feasibility of development processes is increasingly playing a role in selecting lead candidates.

Two chapters address distinct and important themes in antibody therapeutic discovery. 'Design and Application of Immunoconjugates for Cancer Therapy' (Sherif El Sheikh *et al.*) provides an update on the field of conjugating antibodies to cytotoxic agents. This exciting concept developed in the early days of antibody technology and has had a relatively slow development since. However, many pivotal clinical studies are now being reported and are anticipated to be completed in the next few years. "Likewise, the development of multispecific antibodies has been much anticipated but slow to develop momentum. In 'Dual-Targeting Bispecific Antibodies as New Therapeutic Modalities for Cancer' (Zhenping Zhu) an update on the progress and options in this area is described." This book does not aim to cover in depth the wide range of alternative technologies designed to supplement or replace what is possible with antibodies. However, in 'Antibody Fragments and Alternate Protein Scaffolds,' (Lioudmila Tchistiakova *et al.*) there is a valuable perspective on how one biologics discovery organization has approached these areas.

I am very grateful to all of the authors of this volume for their willingness to share their expertise and perspectives. In addition, I thank Britta Werthwein for her excellent administrative assistance; the Sarkis Science Editing team for their superb editorial support; and many colleagues for their reviews and input, especially René Hoet, Ulrich Haupts, Wayne Coco, Jörg Willuda, Heiner Apeler, John Murphy and Rick Harkins. The advances described in this book are based on the contributions of a wide range of scientists and inventors. I salute these pioneers who have made antibody therapeutics possible. They can take pride in both having delivered drugs to patients today and having provided the basic tools to build new drugs as we gain insights into disease and identify innovative targets.

Clive R. Wood, Gloucester, MA, 2011

A number of antibody drugs have been approved for marketing during the preparation of this volume. Any statements on the exact number are bound to be outdated quickly in this fast-moving field. For current information, Janice Reichert's list at http://www.landesbioscience.com/journals/mabs/about/#background is recommended as a regularly revised catalog.

Contents

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Humanization of Antibodies

Olivier Léger and José W. Saldanha†*

1.1 Introduction

Antibody humanization utilizes proven strategies for lessening the immunogenicity of well-characterized monoclonal antibodies (mAbs) from animal sources (commonly mice) and for ameliorating their activation of the human immune system, thus producing clinical diagnostics and therapeutics. At the time of writing, there were thirty approved antibodies listed at the Food and Drug Administration (FDA) website and over a hundred humanized antibodies in clinical trials.

Antibodies produced from rudimentary forms of inoculation are likely to have been used for centuries before the English scientist Edward Jenner pioneered vaccination in the late-eighteenth century. However, the concept of using antibodies as drugs stems from their discovery in 1890, when von Behring and Kitasato reported that small doses of diphtheria toxin injected into animals yielded sera containing an active ingredient called "antitoxin," which we now know as "antibody." This antiserum could protect against lethal doses of toxins in humans, and since the late 1800s antisera have been used to treat acute disease and in prophylaxis, as well as being used *in vitro* as diagnostic tools to establish and monitor disease. Because antiserum is a crude extract containing not only animal antibodies but also other animal proteins, it invariably induces complications, particularly upon multiple administrations. The resulting immune complex disease and anaphylaxis were termed "serum sickness." The active animal antibody apparently needed to be produced separately from the other animal proteins. This came about when Köhler and Milstein (1975) at the Medical Research Council

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Laboratory in Cambridge (UK) published their method of producing custom-built antibodies *in vitro*. They fused antibody producing spleen cells from an immunized rodent with immortal tumor cells (myelomas) from the bone marrow of mice to produce hybridomas. A hybridoma combines the cancer cell's ability to reproduce almost indefinitely with the immune cell's ability to produce antibodies. Once screened to isolate the hybridoma yielding antibodies of the required antigen specificity and affinity, a hybridoma will grow and divide, mass-producing antibodies of a single type (monoclonal). Nearly a century previously, the German scientist Paul Ehrlich envisaged that such entities could be used as "magic bullets" to target and destroy human diseases, and hybridomas were the factories for producing these magic bullets.

An early clinical success of mAb use was in the treatment of organ transplant rejection with a mouse IgG2a antibody reactive against the cell–surface receptor CD3 (Goldstein, 1987). However, exposing humans to mouse antibodies provoked a response similar to the serum sickness of antisera therapy, namely that when mouse mAbs are administered in multiple doses, the patient almost invariably raised an immune response to the mAbs. This human anti-mouse antibody response (HAMA) (Schroff *et al.*, 1985) can develop shortly after initiation of treatment and precludes long-term therapy. Antibodies produced by the HAMA response can neutralize or result in clearance of the therapeutic antibody in immune complexes, or can sensitize the patient to allergic reactions on re-administration, thus compromising the efficacy, biological half-life or safety of the mAb. Moreover, mouse mAbs are inefficient activators of the human effector functions that result in a therapeutic effect. Although they may bind an antigen, it may not be targeted for clearance. As such, although mAbs from hybridoma technology are immensely useful as scientific research and diagnostic reagents, they have not completely fulfilled the possibilities inherent in Ehrlich's vision. Ideally, human antibodies should be used in human therapy. However, immortalization of human antibody-producing cells has had limited success, and besides being difficult to prepare, human hybridomas are unstable and generally secrete low levels of mAbs.

1.1.1 *First step: chimerization*

An alternative approach to mouse antibodies is chimeric antibodies, where animal variable domains are joined to human constant domains (Boulianne *et al.*, 1984; Morrison *et al.*, 1984; Neuberger *et al.*, 1985). This can be achieved by linking the animal and human genes coding for each domain and then expressing the engineered, recombinant antibody gene in rodent myeloma cell lines. In mouse/human chimeric antibodies, the immunogenic mouse constant domains are replaced with their human counterparts to reduce the likelihood of a HAMA response, while retaining the mouse variable domains that allows the