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An integrated science-based approach to drug development

Editorial overview

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Paul Parren was trained in molecular immunology at the University of Amsterdam, where he studied the effector functions of recombinant antibodies. He was an associate professor at The Scripps Research Institute in La Jolla, California. Here he unraveled mechanisms of antibody-mediated protection against viral infections. Since 2002, he has been at Genmab in Utrecht, the Netherlands, where he serves as Sr. Vice President, Research and Pre-Clinical development and works on the research and development of human antibody therapeutics. He is dedicated to advancing the field of therapeutic antibodies by the science-based development of novel and/or improved antibody-based therapies for combating human disease.

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Jan van de Winkel received his MSc degree in Biology and a PhD in Immunology from the University of Nijmegen and conducted postdoctoral work in the Netherlands and USA. Over the past decades his work focused on the biological role and clinical application of antibodies. He is President Research and Development and Chief Scientific Officer of Genmab, which he co-founded in 1999. He has overseen the development of a number of human antibody products. Before joining Genmab, he served as Vice President and Scientific Director of Medarex Europe. He is also a professor of Immunotherapy at the University Medical Center Utrecht.

Monoclonal antibodies and related molecules (including antibody fragments, fusion proteins, and conjugates) currently represent the largest group of therapeutic proteins in development. The number of applications for regulatory approval of such molecules is growing steadily as reviewed by Swann et al., in this issue of *Current Opinion in Immunology*. To date approximately two dozen monoclonal antibodies have been approved worldwide in various therapeutic areas (Figure 1). Technologies to generate therapeutic antibodies have matured from fully mouse (e.g. muromonab-CD3 (Orthoclone)) to fully human molecules (e.g. panitimumab (Vectibix)). This technological advance has crucially contributed to reducing immunogenicity,[1] enabling the long-term use of antibody therapeutics. Human IgG1 antibodies, which benefit from a very long *in vivo* half-life of up to ~3 weeks and an ability to recruit immune effector functions,[2] have been particularly successful. IgG1-based therapeutics are effectively applied in daily clinical practice in the treatment of lymphomas, several solid cancers, as well as inflammatory diseases (Figure 1). Antibody fragments[3] or antibodies of different subclasses[4] are used where IgG1 qualities are undesired.

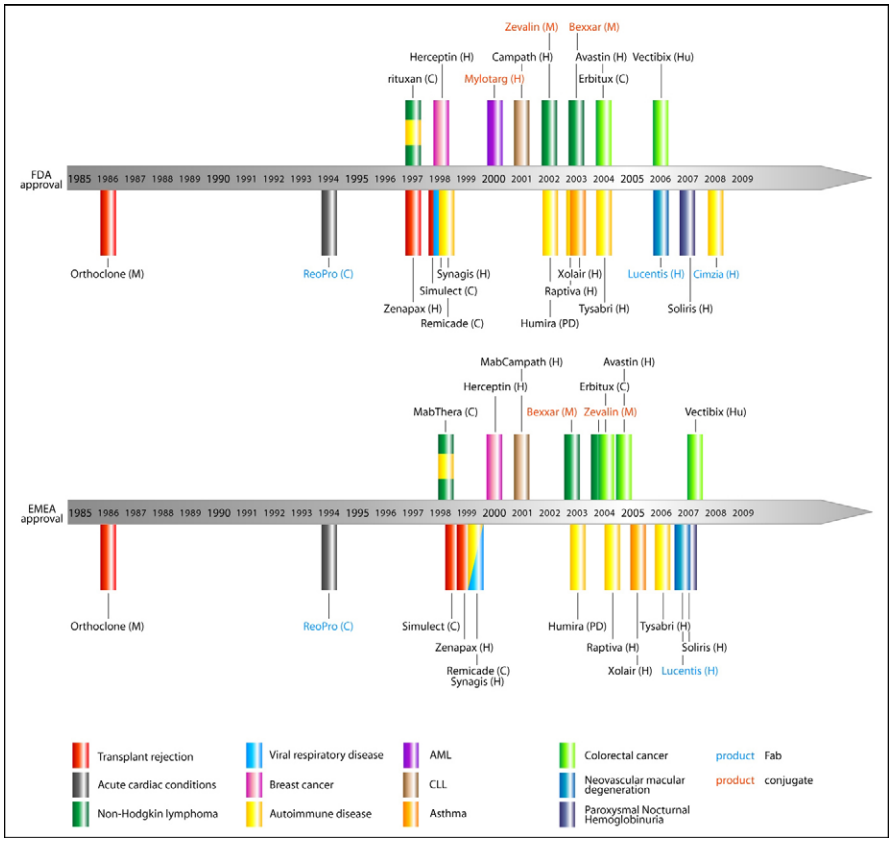
The development of novel human antibody therapeutics centers around three main players: the target, the antibody, and the patient. To successfully develop a therapy, these three need not only be considered separately, but also in an integrated manner (Figure 2). Unfortunately, the relevant interconnections are often considered too late or not at all, leading to drug development failures or suboptimal therapies.

The target

The key attribute of antibody-based therapies is the exquisite target specificity of antibodies. This high specificity is directly reflected in predictable *in vivo* characteristics and favorable toxicology profiles. However, target selectivity comes with a price: It requires a knowledge-based approach to select targets that will enable the antibody to significantly modulate disease outcome. Target selection, directly followed by validation of its drugability by passive antibody transfer experiments in a relevant animal model, thus becomes the first crucial step in successfully developing a novel therapy. Generally speaking, it is thought that drugable targets are those that play a critical role in the biology of the diseased cell or tissue (or pathogen for infectious diseases). Many clinically validated targets represent cell surface receptors or their ligands, such as ErbB family members (i.e. the EGF receptor

[1] The generation of patient antibody responses against non-self parts of a therapeutic antibody.
[2] The IgG1 Fc fragment interacts with immune receptors on blood cells and the reticulo-endothelium system, and may engage cytotoxic proteins of the complement system.
[3] Fab fragments that only contain the binding domain.
[4] For example, in which the Fc fragment is derived of a mouse IgG comprising a short half-life, or human IgG4 comprising reduced effector function.

Figure 1



Marketed antibody products. Therapeutic antibodies approved for human use by the American (Food and Drug Administration, FDA) and European (European Medicines Agency, EMEA) regulatory authorities are shown by their time of first registration. Note that Orthoclone and Reopro were approved in the EU before the establishment of centralized procedures and EMEA in 1994. The therapeutic areas for which the antibodies are currently approved have been indicated by colors as described. Letters in parenthesis indicate the technology applied for generating the antibody: M: mouse; C: chimeric; H: humanized; PD: human (phage display); Hu: human (transgenic mouse). Some antibody products have first been registered in local markets before their introduction into the US and EU. Actemra (tocilizumab, humanized anti-IL6R), for example, is marketed for treating Castleman's disease (2005) and rheumatoid arthritis (2008) in Japan and is currently awaiting US and EU regulatory approval. TheraCIM h-R3 (nimotuzumab, chimeric anti-EGFr) is approved for treating head and neck cancer and/or glioma in Argentina, China, Columbia, Cuba, India, Peru, and Ukraine and is currently in clinical trials in EU, US, and Japan. Reditux is a generic form of the chimeric antibody rituximab and was approved in India in 2007. Reditux is the first marketed 'biosimilar' therapeutic antibody. Biosimilars generate considerable interest, but their potential is being questioned as they represent copies of products derived from older technologies and because of difficulties to ensure product comparability that makes registration in highly regulated markets problematic.

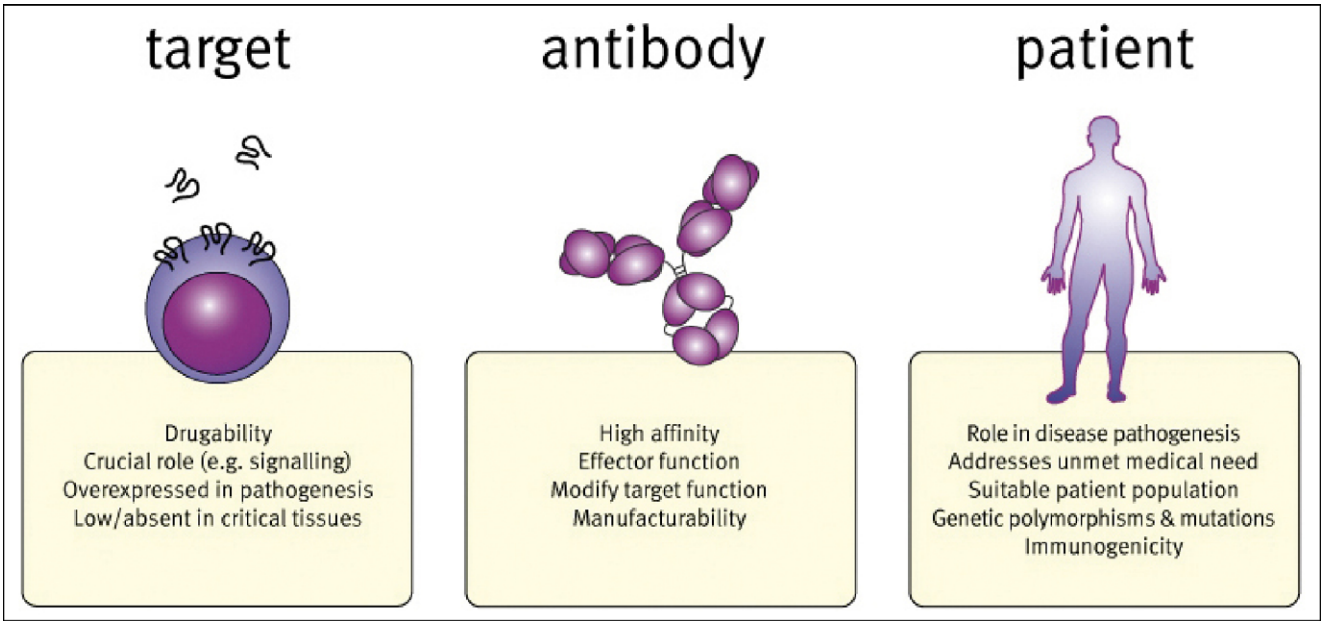
or Her-2) or TNFα, which play key roles in cell signaling and inflammation. The ideal target is also overexpressed in pathogenesis and has low or absent expression in crucial organs and tissues. Arguably, the therapeutic potential and benefit/risk ratio of a target increases with a more restricted expression pattern. The CD20 target for example is exclusively expressed on B cells and B cell related malignancies. Therapeutic anti-CD20 antibodies that heavily rely on antibody mediated effector functions (e.g. rituximab (Rituxan, MabThera)) or on deploying a radioactive payload (e.g. ibritumomab tiuxetan (Zevalin; ^{90}Y-labeled) and tositumomab (Bexxar; ^{131}I-labeled)) to kill tumor cells can thus be highly effective with limited toxicity.

The reviews by Aarden et al., Peipp et al., and Taylor and Lindorfer in this issue highlight some of the features of the important therapeutic targets indicated above. It is expected that the increasing insight in target biology, as well as the identification of targets with even more selective expression patterns (such as tumor testis antigens, and antigens aberrantly expressed or processed by diseased tissues), will drive the development of novel and improved antibody-based therapies and potentially enable the application of antibodies in novel therapeutic areas.

The antibody

Identifying and designing the right antibody represents the second crucial step in developing immunotherapy. The therapeutic antibody should bind its target with high affinity (typically in the low nM to sub-nM range) and high specificity, as reviewed by Lonberg and Presta in this

Figure 2



The trinity of therapeutic antibody development. The development of therapeutic antibodies is crucially dependent on three key factors: the target, the antibody, and the patient that during drug design and development should not only be considered individually, but also as an integrated whole. Each should be validated for suitability for the intended immunotherapeutic application. In general terms, the target should be drugable and play an important role in pathogenesis. The therapeutic antibody should have high specificity and affinity for the target and should be able to modulate target function. For the antibody Fc fragment there is the choice for an activating Fc-tail, which can recruit antibody-mediated immune effector function or, for other applications, a non-activating Fc-tail may be required. The ability to manufacture a well-behaved and consistent antibody product is essential and should be validated. It is essential to perform clinical trials in a suitable patient population for which there is an unmet medical need. The role of the target in disease pathogenesis should be validated. Genetic polymorphisms between patients and specific mutations in diseased tissues may impact therapy and should be investigated. Finally, immunogenicity that is dependent on antibody sequence but strongly modulated by patient, disease, and treatment factors may impact long-term therapy. An integrated science-based approach in which knowledge of target and antibody biology, as well as insights in disease pathology and patient heterogeneity are taken into account maximizes the chances for the successful development of novel antibody drugs.

issue. These two important characteristics are often considered to be co-dependent, which, however, is an incorrect view as highlighted by Presta. Thus, affinity maturation of the anti-RSV antibody Synagis (palivizumab) generated an antibody with greatly increased affinity but decreased specificity, leading to aberrant *in vivo* behavior.

Next to high affinity and specificity, a therapeutic antibody should also effectively modulate target function. Increasingly, screening for antibodies that harbor all desired characteristics takes place very early in drug development. High-throughput approaches that allow parallel screening for antibody affinity, specificity, and functional activity facilitate a knowledge-based approach. Thus, unique and optimal antibodies closely matching a pre-determined product profile can be isolated.

Two major technologies to generate human therapeutic antibody lead candidates, phage display and human immunoglobulin transgenic mice, are predominantly employed. Optimization in the form of affinity maturation of lead candidates is typically performed for antibodies identified by phage display, whereas it is unusual for those derived from transgenic mice, as discussed by Lonberg.

Following selection of the lead candidate, it is essential to select an antibody format appropriate for the intended mechanism of action and clinical application. Research to improve on the IgG1 therapeutic antibody format has been a hot area of research in recent years, in which two major routes for optimization have been followed [1]. The first route, reviewed by Presta, is based on protein engineering and aims to improve therapeutic antibody-induced cytoxicity by optimizing the interaction with activating Fc receptors and/or complement factors [2]. In addition, mutagenesis is applied to modulate the *in vivo* half-life of antibodies. The second route, reviewed by Raju, has been termed glyco-engineering, and is based on the observation that the specific composition of sugar chains present on the antibody Fc fragment strongly impact antibody binding to certain activating Fc receptors [3–5]. A number of therapeutic antibody candidates

have been optimized/engineered using both methods and are presently evaluated in clinical trials.

Whereas the IgG1 antibody format is highly appropriate for the treatment of diseases such as cancer, in which antibody-mediated activation of immune effector functions is advantageous, other applications may require a distinct, non-activation (or inert) format. Historically, antibodies were re-formatted into other subclasses such as IgG2 and IgG4 to prevent immune activation [6]. Recent studies, however, have identified significant complications with respect to the *in vivo* stability of these antibody classes [7,8]. Novel solutions to counter these limitations are being sought and are leading to the identification of novel antibody formats such as the UniBody® molecule. A state-of-the-art overview of the relatively novel field of non-activating antibody formats is provided by Labrijn et al., 2008.

Insights into therapeutic mechanisms of action can be exploited to isolate novel antibodies in which improvements in biological activity translate to increased *in vivo* function. An example is the therapeutic antibody ofatumumab, which targets a novel small loop epitope on CD20, resulting in a highly potent ability to kill tumor cells via complement-mediated cytoxicity.[5] Taylor and Lindorfer 2008 provide insight into the contribution of immune effector function to antibody therapy. Here, they also discuss a phenomenon, in which the exhaustion of the body's immune effector mechanisms may impact the *in vivo* activity of certain therapeutic antibodies. Indeed, the activity of cytotoxic leukocytes, the phagocytic system as well as crucial components of the complement system may be compromised or consumed as a result of antibody therapy or disease. As a consequence, antibodies that harbor an increased ability to engage immune effector function may have improved clinical efficacy. In addition, therapeutic regimens combining therapeutic antibodies with immune modifying drugs that enhance or replenish immune effector function, bear promise for improvement of clinical outcome and should be explored.

A classical role of antibodies is their activity in combating viral infections. The current insight as reviewed by Law and Hangartner, 2008, shows that protection against infection is not only dependent on direct virus neutralization, but antibody-mediated effector functions are also crucial. A thorough understanding of mechanisms of neutralization, as well as the ability to improve immune effector function may lead to a more prominent therapeutic impact of antibodies against infectious agents.

[5] Activation of the complement system by antibody leads to formation of a membrane attack complex that mediates cell lysis.

Another area of research that is becoming increasingly important for therapeutic antibody development is the area of manufacturability and the control of drug product quality, some aspects of which are addressed by Swann et al., 2008.

The patient

Novel drugs, including antibodies, typically go through various phases of evaluation in patients, addressing safety (Phase I), proof of concept (Phase II), and efficacy (Phase III). Subsequently, an application for marketing approval can be submitted to the regulatory authorities such as to the FDA (for the United States) and EMEA (for Europe).[6] Already in early clinical development a good understanding of the major mechanism(s) of action of candidate therapeutic antibodies is crucially important for determining optimal dosing regimens and for making appropriate risk assessments. Inter-individual genetic heterogeneity, or polymorphisms, in patients, as well as disease-associated mutations may significantly impact the outcome and success of clinical trials, as reviewed by Peipp et al., 2008. For example, it has been shown that the antibody Herceptin is only effective against breast cancers that profoundly overexpress the ErbB2 (or Her-2) target. Anti-ErbB1 (or EGF receptor) directed therapy has also been shown to be sensitive to target molecule expression levels on tumor cells, and was found to be inefficacious in patients with tumors containing a mutated KRAS [9]. Leukocyte Fc receptor polymorphisms, which modulate the ability of patients' immune effector cells to interact with therapeutic antibodies have shown to impact the efficacy of antibody treatments for cancer, as shown for CD20 and anti-ErbB antibody therapeutics [10]. Target expression levels, tumor mutations, as well as patient genotype thus represent biomarkers that may be used for predicting the probability of therapeutic success.

The immunogenic potential of therapeutic antibodies has been strongly limited by the coming of age of fully human antibodies, as reviewed by Lonberg and Aarden et al., in this issue. Patient characteristics, as well as treatment design are becoming an increasingly important factor in determining immunogenicity. Careful trial designs with optimized dosing regimens and appropriate concomitant treatment may further decrease antibody immunogenicity and contribute to the long-term efficacy of therapeutic antibodies.

Trends in therapeutic antibody development

Antibodies have reached center stage and have become a mainstay in the therapeutic arsenal in which they increasingly overtake the role of more traditional (small

[6] Monoclonal antibodies for *in vivo* use are reviewed and regulated by the Center for Drug Evaluation and Research (CDER) of the Food and Drug Administration (FDA) in the United States and the European Medicines Agency (EMEA) in Europe.

molecule) drugs. Antibodies are demonstrating efficacy in first-line as well as in long-term treatments [11,12]. Therapeutic antibodies are also increasingly being used as cocktails that may benefit, similar to classical polyclonal antibody preparations, from the ability to target multiple epitopes or targets. Combinations of antibodies may thus be more effective against disease targets that are commonly heterogeneous and may thereby limit resistance or escape [13].

The trinity of target, antibody and patient: three-way interactions impacting the success of therapeutic antibodies

Understanding the mechanism(s) of action of a therapeutic antibody relative to the pathogenic profile of the target, as well as patient characteristics becomes an increasingly important intertwined area of study. Research on the identification of biomarkers related to target expression or antibody mechanisms of action (such as Fc receptor polymorphisms) that allow identification of responding patients is becoming a central focus. Indeed, careful matching of therapeutic antibodies with patients that are likely to respond is leading to strong benefits in terms of increased efficacy and reduced cost. *By* rational discovery and selection of targets, *by* exploiting the rapidly increasing knowledge of antibody biology to identify therapeutic antibodies with tailored mechanism(s) of action, *by* developing cocktails of antibodies with additive and synergistic activities, and *by* using biomarkers to match patient and therapy, the field of immunotherapy will continue to revolutionize the treatment of human disease.

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