The role of therapeutic antibodies in drug discovery

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Abstract

The last 5 years have seen a major upturn in the fortune of therapeutic monoclonal antibodies (mAbs), with nine mAbs approved for clinical use during this period and more than 70 now in clinical trials beyond phase II. Sales are expected to reach $4 billion per annum worldwide in 2002 and $15 billion by 2010. This success can be related to the engineering of mouse mAbs into mouse/human chimaeric antibodies or humanized antibodies, which have had a major effect on immunogenicity, effector function and half-life. The issue of repeated antibody dosing at high levels with limited toxicity was essential for successful clinical applications. Emerging technologies (phage display, human antibody-engineered mice) have created a vast range of novel, antibody-based therapeutics, which specifically target clinical biomarkers of disease. Modified recombinant antibodies have been designed to be more cytotoxic (toxin delivery), to enhance effector functions (bivalent mAbs) and to be fused with enzymes for prodrug therapy and cancer treatment. Antibody fragments have also been engineered to retain specificity and have increased the penetrability of solid tumours (single-chain variable fragments). Radiolabelling of antibodies has now been shown to be effective for cancer imaging and targeting. This article focuses on developments in the design and clinical use of recombinant antibodies for cancer therapy.

Introduction

Monoclonal antibodies (mAbs) have revolutionized biological research and clinical diagnostics. However, any suggestions that mAbs could ever realize potential as anticancer therapeutics would, until recently, have been met with a degree of scepticism. The first (rodent) mAbs were potent immunogens provoking strong endogenous antibody responses. They were also unable to trigger effector functions, had a short half-life (1–2 days) and proved to be extremely expensive to manufacture. Consequently, several promising mAb-based cancer therapies were relegated to the realm of pre-clinical development.

By contrast, the situation in 2002 sees mAbs undergoing what can best be described as a renaissance with sales of mAbs expected to reach $4 billion in 2002, and $15 billion by 2010. The major factors underpinning this shift are advances in both antibody engineering and discovery technologies, to the extent that it is now possible to rapidly derive high-affinity humanized/human mAbs. To date, 12 mAbs have been approved by the regulatory authorities and of the >450 currently in clinical trials around 70 have progressed beyond phase II. In this review we discuss the design and clinical application of recombinant mAbs in cancer therapy with reference to several precedents.

Key words: antibody, cancer, discovery, monoclonal, therapeutic.

Abbreviations used: EGF, epidermal growth factor; mAb, monoclonal antibody; scFv, single-chain variable fragment.

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Antibodies by design: reducing immunogenicity

One of the fundamental problems with mouse mAbs is that administration of murine Ig induces a human anti-mouse response in about 50% of patients after a single dose and >90% after repeated administrations. This response leads to rapid clearance, allergic reactions and complications relating to hypersensitivity [1]. Three general strategies have now been developed to reduce the immunogenicity of mAbs. Firstly, advances in molecular biology have made it possible to substitute human sequences for their rodent counterparts. Early attempts produced chimaeric mAbs with 75% homology with human mAbs by retaining the rodent V genes linked to sequences encoding human constant regions. This homology was then improved to around 95% by Winter and colleagues by a process called V-region humanization where only the rodent complementarity-determining region is retained combined with human V-region frameworks and constant regions of the heavy and light chain [2]. Examples of chimaeric and humanized mAbs are Rituxan (anti-CD20 mAb; approved for the treatment of B-cell malignancies) and Synagis (approved for the treatment of Rous sarcoma virus infection), respectively. The next technology, phage display, involved selecting high-affinity recombinant antibody fragments using libraries ($>10^9$ members) of human antibody V regions presented on the surface of bacteriophage [3], thereby bypassing immunization strategies. Human V-region antibody fragments can be selected by several rounds of selection on antigen and then screened for activity, generating fully human single-chain variable fragment (scFv) or Fab [4,5]. A large number of mAbs

currently in clinical development have been generated using this approach; one example is an anti-(transforming growth factor $\beta 2$) mAb for the treatment of fibrotic disorders [6]. Finally, transgenic animals have been generated [7,8] in which natural Ig genes are deleted and replaced with the human loci necessary for the production of IgG/IgM. This *in vivo* approach has been used to generate several mAbs, including ABX-EGF (Abgenix Corp.), a high-affinity anti-[epidermal growth factor (EGF) receptor] antibody for use in the treatment of EGF-responsive tumours [9]. In summary, these techniques have reduced the immunogenicity of mAbs to an extent where repeated doses can be administered without impaired efficacy.

Antibodies by design: the right tools for the job

A sound understanding of the biochemical pathway being targeted is central to the 'wish list' for a mAb therapeutic. In many applications the recruitment of host effector functions through Fc receptors (including complement fixation and antibody-dependent cell-mediated cytotoxicity) is essential. In humans the IgG1 isotype is the preferred therapeutic choice for triggering effector cascades. An approved antibody with the potential to trigger effector function is Herceptin, an anti-HER2/neu antibody used in the treatment of breast cancer. In addition to the well-described ability to block Her2-dependent growth, some of the clinical benefit from Herceptin treatment is thought to arise from an ability to promote antibody-dependent cell-mediated cytotoxicity [10]. Conversely, when target neutralization is the only goal, it may be preferable to use the IgG4 isotype that is incapable of triggering these cascades [11]. Irrespective of isotype there are several additional benefits to the IgG format. Divalent IgG molecules have the potential to cross-link cell-surface antigen in the absence of macrophage/natural killer cell Fc receptors. For example, in some CD20-positive cell lines cross-linking has been shown to induce apoptosis [12]. An alternative to using this is to use antibody fragments such as Fab or scFv. Both of these formats are unable to trigger effector function and because of a reduced size have an increased tumour penetration. These fragments are especially suited to the delivery of 'payload' (see below). One obvious drawback of Fabs/scFvs is a reduced half-life (hours compared with 2–3 weeks with IgG). It is, however, possible to improve the serum longevity of these proteins by conjugating to inert polymers such as poly(ethylene)glycols [13]. Examples of cancer targets where neutralization is the primary goal include matrix metalloproteases ('MMPs'), urokinase plasminogen activator ('uPA') and vascular endothelial growth factor ('VEGF'), which are all secreted proteins associated with tumour progression.

Antibody conjugates: adding insult to injury

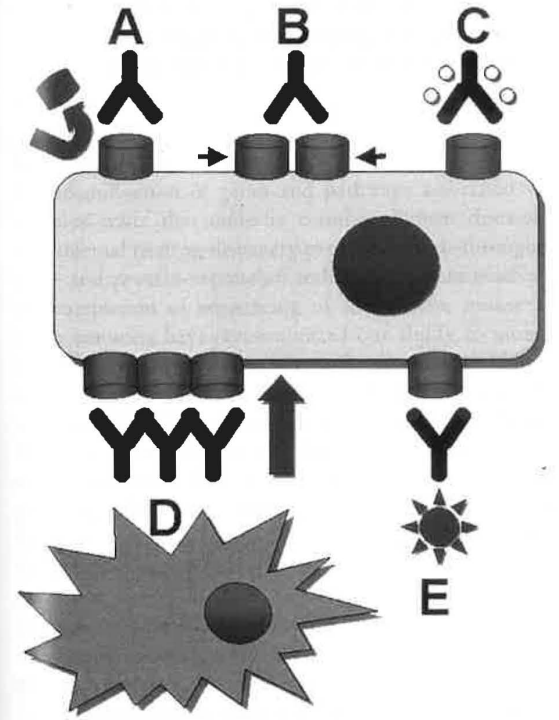
It was Paul Erlich in 1900 who first coined the phrase 'magic bullet', accurately predicting the value of antibodies as smart weapons in the delivery of destructive payload [14]. A number of approaches have been developed, which in the context of anti-cancer protocols provide measurable improvements in cell killing. The major categories are radioisotopes, protein toxin–enzyme fusions and small-molecule conjugates.

Antibodies are routinely used to concentrate doses of radiation in tissues for both therapeutic and diagnostic purposes. Common isotopes used to this end include iodine-131, yttrium-90, indium-111 and technicium-99. Tumour killing by unlabelled mAbs is limited by the degree of antigen density on the tumour cell and the ability to penetrate tumours adequately. Although radiolabelled mAbs may be less restricted by antigen density in their efficacy they can gain an advantage by a 'bystander' effect in killing antigen-negative tumour cells. Conversely, this phenomenon would also be responsible for non-specific toxicity. The two most extensively studied radiolabelled mAbs are Zevalin (^{90}Y-labelled anti-CD20) and Bexxar (^{131}I-labelled anti-CD20), the former receiving recent U.S. Food and Drug Administration (FDA) approval for the treatment of non-Hodgkins lymphoma. For therapeutic purposes ^{90}Y-labelled mAbs may be better debulking agents for larger tumours because of the increased path length of the emission compared with ^{131}I-labelled mAbs, which may be preferable for targeting post-therapy minimal disease [15].

Protein toxins are a large group derived from a variety of microbial, plant and human sources. Above all other mAb conjugates, these toxins can be engineered directly into the antibody-constant regions, significantly reducing the manufacturing costs. Examples include *Pseudomonas* exotoxin, which when conjugated to anti-CD22 has been shown to dramatically increase cell killing [16]. For plant toxins, deglycosylated ricin α-chain has the longest and most successful history. In one recent report a ricin–CD19 conjugate was shown to confer a large increase in the ability of antibody to kill malignant B-cells [17]. The major foreseeable problem with protein toxins is their potential immunogenicity, especially when considering microbial toxins to which an individual may already have been sensitized. An alternative would be to use cytotoxic human proteins. Angiogenin, a human RNase, has been shown to induce apoptosis when delivered into the cytoplasm. In one recent publication, bacterially expressed CD30L–angiogenin fusion protein was found to be capable of killing a wide range of CD30+ Hodgkin-derived cell lines [18]. This toxin may represent a clever way to avoid host immune responses.

The third payload group comprises toxic small molecules which are usually DNA-complexing agents or inhibitors of the cell cycle. In this situation the antibody conjugate is internalized and the toxic drug liberated after cleavage of a pH- or enzyme-sensitive linker. As mAbs can target chemotherapy exclusively to cancer cells, more potent chemotherapy can be used when attached to mAbs than when administered systemically, for example maytansine conjugates [19]. Small toxic drug molecules have potential advantages: in general they have a negligible immunogenicity

Figure 1 | Mechanisms of action for therapeutic antibodies
The therapeutic potential of mAbs resides in an ability to modulate several different pathways. (**A**) For a receptor involved in a growth-promoting pathway, mAb binding may prevent ligand binding and signal transduction (e.g. mAbs against EGF receptors and Her2/c-erb-B2). In a similar fashion, mAbs may target cell-surface growth factor receptors for degradation as opposed to a natural recycling process (Herceptin anti-Her2). (**B**) Cell-surface cross-linking may initiate a cascade that kills tumour cells (e.g. Rituxan-mediated cross-linking of CD20). (**C**) Several mAb formats have the capacity to activate the classical (C1q) complement pathway. (**D**) Macrophages and natural killer cells express cell-surface Fc receptors which, when bound to immobilized mAb, become activated and secrete cytotoxic mediators. (**E**) Finally, mAbs can be engineered to deliver toxic payloads including radionuclides, protein toxins and small molecules (Bexxar, ^{131}I-labelled anti-CD20; and Mylotarg Zogamicin anti-CD33).



and compared with radionuclides are relatively easy to handle. Mylotarg is a humanized anti-CD33 linked to calicheamicin. This useful therapy for acute myelogenous leukaemia ('AML') was the first toxin conjugate ever to be approved, showing that the small-molecule payload group has just as much validity as other regimes. Recruitment of effector cells using bispecific antibodies (one specificity directed towards the tumour, the other to the effector cell) has also been reported widely [20]. These reagents are expected to be difficult to manufacture in large quantities but have shown promise *in vitro*, in animal models and now also in some phase I clinical trials. Mechanisms of action for conjugated and naked antibodies are illustrated in Figure 1.

Problems, solutions and evolution

We can infer from the previous discussion that the modern antibody investigator has the technological tool kit necessary to ensure the smooth transition of a project from antigen discovery to therapeutic mAb. What then is the major bottleneck that still exists in the discovery process? The completion of the human genome combined with advances in proteomics technologies have helped to enhance our understanding of the complex interplay between genetic, transcriptional and translational alterations in human cancers. Although bioinformaticians have made strides in identifying potentially interesting novel cancer targets there is a central bottleneck at the point when cancer biologists must investigate each individual gene for therapeutic potential. These assays are time-consuming and labour-intensive. Looking at the current crop of approved antibody targets, they have all benefited from around 10–20 years of detailed academic research. The future goal will be to design high-throughput biology modes to move novel cancer targets quickly towards clinical development.

Insight into the future of antibody therapeutics can probably be glimpsed through technologies such as ribosome display. Here, antibody-fragment libraries are not displayed on bacteriophage but are produced entirely *in vitro* and there is also the potential to introduce mutagenesis steps into the antibody-encoding RNA sequences. This system has the advantages of greater library size ($>10^{12}$), potentially higher mAb affinity and improved speed compared with standard phage display [21]. If this technique tells us anything about the future of antibody discovery it suggests that soon the generation of humanized/human mAbs may become as routine as PCR is today: we await the release of the first human antibody-generation kit!

Conclusions

Kohler and Milsteins' dream of mAbs as exquisitely sensitive therapeutics has finally been realized. Two antibodies, Herceptin and Rituxan, have proved that this class of drugs can be effective (and highly lucrative) anti-cancer agents for those companies brave enough to enter this research 'graveyard'. From a stalled start, mAbs now represent around 25% of the novel biological entities entering clinical trials, indicating that the biotechnology community at large has finally recognized the speed and efficiency of the antibody platform. It is also becoming clear (and it is ironic) that the classical monoclonal antibody may yet become the commercial saviour of the high-tech proteomics and genomics revolutions.

References

1 Shawler, D.L., Bartholomew, R.M., Smith, L.M. and Dillman, R.O. (1985) J. Immunol. **135**, 1530–1535

2 Queen, C., Schneider, W.P. and Selick, H. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 10029–10033
3 Winter, G., Griffiths, A.D., Hawkins, R.E. and Hoogenboom, H.R. (1994) Annu. Rev. Immunol. **12**, 433–455
4 Marks, J.D., Hoogenboom, H.R., Bonnert, T.P., McCafferty, J., Griffiths, A.D. and Winter, G. (1991) J. Mol. Biol. **222**, 581–597
5 Griffiths, A.D. (1993) Curr. Opin. Immunol. **5**, 263–267
6 Thompson, J.E., Vaughan, T.J. and Williams, A.J. (1999) J. Immunol. Methods **227**, 17–29
7 Mendez, M.J., Green, L.L., Corvalan, J.R., Jia, X.C., Maynard-Currie, C.E., Yang, X.D., Gallo, M.L., Louie, D.M., Lee, D.V., Erickson, K.L. et al. (1997) Nat. Genet. **15**, 146–156
8 Ishida, I., Tomizuka, K., Yoshida, H., Tahara, T., Takahashi, N., Ohguma, A., Tanaka, S., Umehashi, M., Maeda, H., Nozaki, C. et al. (2002) Cloning Stem Cells **4**, 91–102
9 Yang, X.D., Jia, X.C., Corvalan, J.R., Wang, P. and Davis, C.G. (2001) Crit. Rev. Oncol. Hematol. **38**, 17–23
10 Baselga, J. and Albanell, J. (2001) Ann. Oncol. suppl. **1**, S35–S41
11 Antoniw, P., Farnsworth, A.P., Turner, A., Haines, A.M., Mountain, A., Mackintosh, J., Shochat, D., Humm, J., Welt, S., Old, L.J. et al. (1996) Br. J. Cancer **74**, 513–524
12 Shan, D., Ledbetter, J.A. and Press, O.W. (2000) Cancer Immunol. Immunother. **48**, 673–683
13 Lee, L.S., Conover, C., Shi, C., Whitlow, M. and Filpula, D. (1999) Bioconj. Chem. **10**, 973–981
14 Erlich, P. (1900) Proc. R. Soc. London. **66**, 424
15 Zelenetz, A.D. (1999) Curr. Opin. Oncol. **11**, 375–380
16 Mansfield, E., Amlot, P., Pastan, I. and FitzGerald, D.J. (1997) Blood **90**, 2020–2026
17 Conry, R.M., Khazaeli, M.B., Saleh, M.N., Ghetie, V., Vitetta, E.S., Liu, T. and LoBuglio, A.F. (1995) J. Immunother. Emphasis Tumor Immunol. **18**, 231–241
18 Huhn, M., Sasse, S., Tur, M.K., Matthey, B., Schinkothe, T., Rybak, S.M., Barth, S. and Engert, A. (2001) Cancer Res. **61**, 8737–8742
19 Smith, S. (2001) Curr. Opin. Mol. Ther. **3**, 198–203
20 Peipp, M. and Valerius, T. (2002) Biochem. Soc. Trans. **30**, 507–511
21 Hanes, J., Schaffitzel, C., Knappik, A. and Pluckthun, A. (2000) Nat. Biotechnol. **18**, 1287–1292

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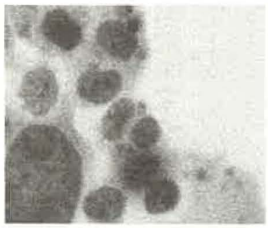

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