Renaissance of cancer therapeutic antibodies

Martin J. Glennie and Jan G.J. van de Winkel

In the past five years therapeutic monoclonal antibodies have established themselves as perhaps the most important and rapidly expanding class of therapeutic drugs. More than 25% of pharmacological agents that are currently under development are based on antibodies and the total income generated from them in 2002 exceeded $3 billion, and is predicted to rise to $10–20 billion by 2010. Many experts feel that antibodies directed at cancer targets are likely to dominate the market for the foreseeable future. In this review, we will discuss some of the factors that, after more than 25 years of development, have led to this transformation in the antibody field.

Martin J Glennie
Tenovus Research Laboratory
Cancer Sciences Division
University of Southampton
Southampton General Hospital
Southampton
UK SO16 6YD
Jan G.J. van de Winkel*
Genmab
Jenalaan 18d
3584 CK Utrecht
and the Department of Immunology
University Medical Center
Utrect
The Netherlands
*e-mail: J.vandeWinkel@nl.genmab.com

▼ Currently, there are 12 monoclonal antibodies (mAbs) approved by the FDA for human use (Table 1) [1]. With three exceptions, these molecules all contain human constant regions, which is probably an important factor in explaining their success when compared with earlier mAb reagents. Given the numbers of mAbs that have been tested in cancer indications, one might have predicted that anticancer products would completely dominate the list of approved drugs. However, only four mAb specificities are targeted for cancer therapy. The reason for their paucity probably relates to the different demands of a mAb that is designed to manage cancer, compared with those mAbs used to tackle transplantation, inflammation or infection. An anticancer antibody has to eliminate malignant host cells, often in large numbers. There are many reasons, relating mainly to the natural defences of malignant cells, why such killing is generally inefficient and that help to explain the lack of patient benefit in most clinical trials. In the case of non-cancer targets, the mAb performs a very different role, modulating a cellular response in the case of autoimmune disease and organ transplant rejection or blocking the spread or entry of an infectious agent. In these cases, mAbs are clearly effective.

A significant proportion of the on-going clinical trials involving mAbs that have been announced publicly is shown in Table 2 (supplementary material; http://archive.bmn.com/supp/ddt/glennie.pdf). Although the number and diversity of derivatives is impressive, they represent only a fraction of mAbs that are currently in preclinical development. This extensive list shows that the outlook for therapeutic mAbs is healthy and that these therapeutics are likely to dominate the market for the foreseeable future.

What makes antibodies special?

The overriding factor that determines the importance of mAbs is their specificity. The acquired immune system has evolved a unique ability to generate highly specific antibody molecules that allow recognition of almost any foreign substance, whether it is a protein, a carbohydrate or even a synthesized chemical. Antibodies are generated by a complex process in which gene segments are rearranged randomly into functional genes, thus allowing the generation of billions of different specificities. Such diversity is simply the starting point for providing the raw material (antibody) to generate a full antibody repertoire when required. Once the immune response is triggered, usually by an infection, a particular set of antibody-expressing B cells are selected and their antibody is refined to improve binding by a process called somatic mutation. This process provides the immune system with antibodies that are specifically engineered for optimal performance against the foreign invader. At the end of this process, a fully matured antibody response, of the correct class (e.g. IgG, IgA and IgE), is

1359-6446/03/$ – see front matter PII: S1359-6446(03)02714-4

Table 1. FDA approved therapeutic antibodies[a]

Product	Company	Date	Type	Target	Indication
Orthoclone OKT3® (muromonab)	Johnson and Johnson	1986	Murine IgG2a	CD3	Transplant rejection
ReoPro® (abciximab)	Centocor, Eli Lilly	1994	Chimeric Fab	GPIIb/IIIa	Blood clots
Zenapax® (daclizumab)	Roche	1997	Humanized IgG1	IL-2R (CD25)	Transplant rejection
Remicade® (infliximab)	Johnson and Johnson	1998	Chimeric IgG1	TNF-α	Crohn's disease (Rheumatoid arthritis)
Simulect® (basiliximab)	Novartis	1998	Chimeric IgG1	IL-2R (CD25)	Transplant rejection
Synagis® (palivizumab)	MedImmune	1998	Humanized IgG1	RSV	Infectious disease
Humira® (adalimumab)	Abbot	2002	Human IgG1	TNF-α	Rheumatoid arthritis
Rituxan® (rituximab)	IDEC, Genentech, Roche	1997	Chimeric IgG1	CD20	Non-Hodgkin's lymphoma
Herceptin® (trastuzumab)	Genentech	1998	Humanized IgG1	HER-2/neu	Breast cancer
Mylotarg® (gemtuzumab ozogamicin)	Wyeth Ayerst	2000	Humanized IgG4–toxin conjugate	CD33	Relapsed acute myeloid leukaemia
Campath-1H® (alemtuzumab)	Millennium	2001	Humanized IgG1	CD52	Chronic lymphocytic leukemia
Zevalin® (Ibritumomab tiuxetan)	IDEC	2002	Mouse IgG1–radio-nuclide conjugate	CD20	Rituximab-failed Non-Hodgkin's lymphoma

Abbot (http://abbott.com); Centocor (http://www.centocor.com); Eli Lilly (http://www.lilly.com); Genentech (http://www.genentech.com); IDEC (http://www.idec.com); Johnson & Johnson (http://www.jnj.com); MedImmune (http://www.medimmune.com); Millenium (http://www.mlnm.com); Novartis (http://www.novartis.com); Roche (http://www.roche.com); Wyeth Ayerst (http://www.wyeth.com).
[a]Abbreviations: IL-2R, interleukin 2 receptor; RSV, respiratory syncytial virus; TNF-α, tumour necrosis factor α.

maintained, providing a variety of different specificities and affinities, each recognizing different determinants (epitopes) on the target. Such a polyclonal response is provided by multiple clones of selected mutated B cells. In addition to binding and blocking, antibodies can also protect the body by alerting the immune system to a potential danger and recruiting a range of cytotoxic effector systems such as enzymes (complement) and protective cells [e.g. macrophages, neutrophils and natural killer (NK) cells].

For therapeutic application, the multitude of individual antibodies that make up a polyclonal mixture can be examined individually and selected to find the most useful drugs. This was achieved initially using the monoclonal antibody fusion technology developed by Kohler and Milstein [2] in the mid-1970s, in which rodent antibodies were produced by somatic cell fusion. However, it is now possible to generate fully human mAbs with equal efficiency. These reagents not only have the same precision for target specificity as provided by rodent reagents, but also interact far more effectively with the natural defences of the body and do not provoke anti-Ab responses [3].

Why has the therapeutic success of mAbs been so slow coming?

When mAbs were first generated in the mid-1970s it was expected that their exquisite specificity and unlimited supply would ensure that therapeutic products would soon follow. In fact, as is often the case with new technology, expectation far exceeded reality, and many of the difficulties of

dealing with this new class of drug were not appreciated. In most of the early clinical trials exceedingly small quantities of material, in the order of milligrams, were administered, which was inadequate to elicit therapeutic activity. Furthermore, these rodent (mainly mouse) mAbs were often highly immunogenic, resulting in human anti-mouse antibody (HAMA) responses that prevented multiple applications. In addition, the mAbs had a very short half-life, which also reduced the effective therapeutic dose. Finally, and perhaps most importantly, the significance of mAb specificity, particularly in the cancer field, was not appreciated [4]. Thus, in these early years, it was generally thought that the main role of the mAb was to distinguish between the unwanted malignant cells and normal tissue, and that once the unwanted cells were coated with mAbs, the immune system (effector cells and complement) would be able to eliminate them in much the same way as it eliminated infective organisms. Inappropriate target selection has probably contributed to several failures. It is now known that the situation is far more complex and that the fine specificity of the mAb is crucial in determining success or failure. Not only does the mAb need to seek out the malignant cells, but it must also have the capacity to use the appropriate killing mechanisms, one of the most important being the ability to directly activate an in-built cellular suicide pathway in all cells, including neoplastic cells, called apoptosis.

It was the then new technology of genetic engineering that helped to resolve many of these inherent limitations [3]. During the late 1980s and most of the 1990s, several key observations were made that allowed part, or almost all, of a rodent mAb to be replaced by human sequences (Box 1). In their most basic form, chimeric mAbs were made in which the constant regions of the rodent mAb were replaced by the equivalent human genes. These human genes were often taken from the kappa light chain and the IgG1 heavy chain. The IgG1 subclass was selected because it is arguably the most active in the immune system, being able to engage receptors on cytotoxic effector cells, called FcγR, and to activate complement (a cascade of plasma enzymes that initiate inflammation and destroy target cells) efficiently [5]. In a refinement of this strategy, humanized mAbs were made in which all of the constant regions and most of the structural elements of the variable (V)-regions (called framework regions) were replaced by human sequences. In this case, only the short sequences, called CDRs (complementarity determining regions), which form the actual antigen binding sites, were of mouse origin. Chimeric and humanized mAb now make up the bulk of the mAb derivatives approved for use in humans (Tables 1 and 2). However, the next generation of mAbs will be fully human, coming either from human antibody libraries expressed in phage or from transgenic animals that have had their endogenous antibody genes replaced by the equivalent human sequences [6–9].

The ability to generate mAbs that were almost human in structure achieved two important things: it overcame most patient anti-antibody responses, and it extended the survival of the reagent to something closer to that of normal human IgG. Despite such impressive increases in mAb survival, it is now appreciated that the vast majority of reagents only become effective when given systemically in gram quantities. Such quantities far exceed those used in most of the early investigations and this is an important factor in explaining the recent therapeutic success of mAbs.

The secret of specificity

Even with an almost human or fully human mAb, clinical success is not guaranteed. In the cancer field, most mAbs have failed to deliver clinical benefits, leaving only five with FDA approval, with two of these targeted at the same specificity and three directed at lymphoid tumours, a disease that is often thought to be more accessible to treatment than are solid tumours. Finding successful reagents, particularly against solid tumours such as breast, lung and colon cancer, has proven to be extremely difficult. This is at odds with the rapid advance in the use of mAbs in the treatment of non-cancer targets, such as transplantation, inflammation and infectious disease. Although these targets have not had the advantage of the relative easy access to patients for clinical trials that has occurred in the case of cancer targets, mAbs such as Remicade® [anti-tumour necrosis factor α (TNF-α)], and Synagis® (anti-respiratory syncytial virus) have achieved considerable success in a surprisingly short time. These contrasting situations probably relate to the task required of the mAbs *in vivo*. In the case of infection and autoimmunity, the major mechanisms of action of mAbs probably involve either the blockade of a target or the modulation of a cellular function, such as the activation of immune effector cells. For example, recent evidence suggests that Remicade®, in addition to blocking inflammation via neutralization of TNF-α, can promote the destruction of activated T cells in Crohn's disease via induction of apoptosis [10].

The significance of apoptosis

In the case of cancer, the situation is very different from that of non-cancer targets. First, the treatment needs to kill or at least control malignant cells that might have evolved over decades to escape immune detection. It is now clear

Box 1. Structural relationship between rodent IgG and genetically engineered, chimeric, humanized and fully human IgG

Antibody molecules are Y-shaped structures with two identical heavy (H) and light (L) chains (Fig. I). The variable (V)-regions of the H and L chains form a unique antigen-binding site (red and yellow regions in the rodent IgG) that engages an antigenic determinant, called an epitope (E) on the target antigen (Ag). This unique specificity is provided by loops of the V-region called complementarity determining regions (CDRs), which are held in the correct shape by a framework structure. The remainder of the H and L chains (shown in pink and blue in the rodent IgG, and in grey and orange in the chimeric IgG), are called the constant regions of the antibody. This symmetrical structure allows each antibody to engage two identical epitopes either on different or on the same Ag. Note that the hinge region of the antibody is highly flexible allowing the Ag binding arms [fragment antigen binding (Fab)] to move independent of the tail [fragment crystallizable (Fc)] region of the molecule, the region that mediates biological activity, which improves the chance of both Fab arms binding a target simultaneously.

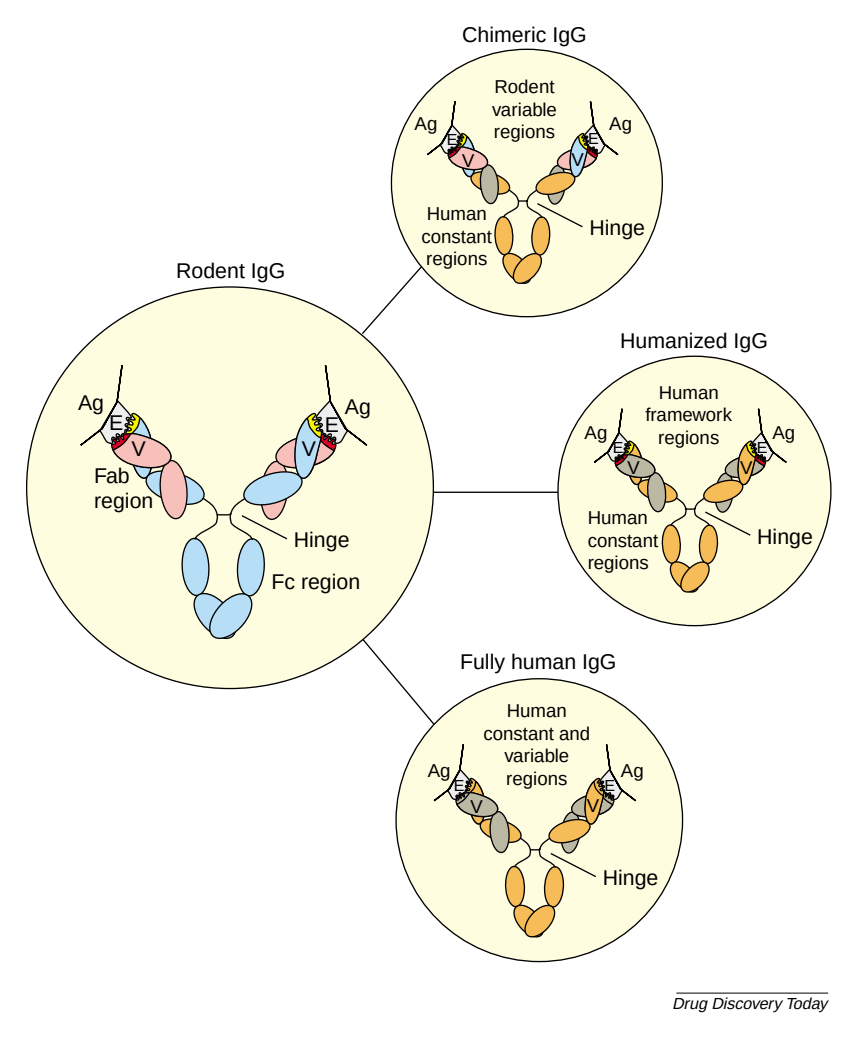

Figure I. Structural relationship between rodent IgG and genetically engineered, chimeric, humanized and fully human IgG.

Although rodent monoclonal antibodies (mAbs), as prepared in the mid-1970s, were highly specific and able to distinguish targets with great precision, they presented several drawbacks because they were foreign proteins. They were often immunogenic, they survived for a relatively short time in the circulation and they were comparatively ineffective at recruiting the human immune system. Various strategies have been adopted over the past decade to overcome such limitations. As shown in Figure I, the initial solution was to genetically engineer the genes of the rodent mAb to provide it with human constant regions (chimeric IgG). This ensured long survival and better interaction with the human immune system. However, the variable regions (H and L) were still derived from rodent genes and were therefore potentially able to provoke immune responses in patients. This final problem was partially solved by generating humanized mAbs in which only the very small CDR structures responsible for antibody specificity were taken from a rodent antibody and grafted onto a human V-region framework. These reagents contain less than 10% foreign amino acid sequence. The final solution, which left no rodent material, was to generate fully human mAbs, either from human antibody libraries expressed in phage particles, or via transgenic mice in which endogenous antibody genes (H and L) had been replaced by equivalent human genes.

that even if a cancer cell becomes coated by mAbs it does not necessarily mean it will be eliminated. It was a naïve view of the mammalian immune system and the way it uses antibodies to destroy viruses and bacteria that led to this interpretation. However, the discovery that most cells of the body are coated with a plethora of defence molecules that protect them from attack by complement and cellular effectors provides a better understanding of the

challenge that is faced in this area [11,12]. Furthermore, at least in the case of complement regulators, it is known that such defence mechanisms can be heightened in malignant cells compared with normal cells, suggesting that malignant cells have evolved to escape such attack.

Interestingly, *in vitro* assays can often be controlled so that complement and cellular effector mechanisms operate effectively. A good example of this would be B cell targets such as CD19 and CD37, which support antibody-mediated killing *in vitro*, whereas these mechanisms *in vivo* are almost always thwarted. One exception to this is the CD52 mAb Campath-1H® (Table 1) [13]. This mAb recruits the natural effectors of the body so efficiently that it appears to overcome cellular defences, efficiently eliminating malignant and normal antigen-positive cells. The reason for the success of Campath-1H® probably lies in the number of CD52 targets on the cell membrane, which approaches a million per cell, and the close proximity with which the Campath-1H® mAb is held to the plasma membrane [14], thus ensuring very close contact between the target membrane and immune effectors.

Similar success has also been observed with the CD20 mAb [15,16]. This target is also generally well expressed (>100,000 molecules per cell), it is located close to the membrane and CD20 is not shed or modulated following binding by mAbs. All these characteristics favour killing of cancer cells by mAb-recruited immune effectors. However, when engaged by mAbs in many cell lines and some fresh malignant cells, CD20 can also directly signal cell death through the process of apoptosis [4]. This ability now appears to be a key feature that distinguishes CD20 mAbs from a range of less effective anti-B cell mAbs, such as anti-CD19 and anti-CD37, and has convinced many workers in the field that induction of apoptosis is a crucial factor for the therapeutic success of cancer mAbs.

The only other B cell target that has reproducibly shown similar activity is the idiotype (Id) of the B cell receptor (BCR) for antigen. In small clinical trials, anti-Id mAbs are among the most efficacious ever used in patients, giving close to 70% overall response rates in relapsed lymphoma, including several long-term remissions [17,18]. Because anti-Id mAbs are not particularly effective at recruiting immune effectors, mainly because of their capacity to modulate and internalize when engaged by mAbs, but are unusually effective at inducing apoptosis, these results again suggest that apoptosis is a crucial factor in the *in vivo* mechanism of action of this anticancer mAb. Furthermore, such work is well supported by results *in vitro* and *in vivo* [4,19]. The only unfortunate aspect of these findings is that anti-Id mAbs have to be 'tailor-made' for each patient and, therefore, will never prove viable as a standard drug treatment. Perhaps tailor-made Id-vaccines, where patients raise their own anti-Id antibodies, will provide a practical way of moving forward with this outstanding, tumour-specific, target [20].

Disruption of signalling pathways

Another successful cancer mAb, Herceptin®, is targeted at the growth factor receptor HER-2/neu, which is over-expressed on a significant proportion of breast cancer tumours. Herceptin® modulates the signalling capacity of the target receptor. Exciting new data have recently shown that another anti-HER-2 antibody, 2C4 (pertuzumab), disrupts the association of HER-2 with other growth factor receptors and inhibits signalling in tumour cells even more effectively than does Herceptin® [21]. This resulted in a far better *in vitro* and *in vivo* killing of tumour cells, with lower HER-2 levels than observed with Herceptin®. From this, mainly circumstantial, evidence, it is generally thought that interfering (promoting or blocking) with trans-membrane signalling probably plays a crucial role in the therapeutic success of those anti-cancer mAbs that have delivered clinical benefit. However, in reality the situation is probably more complex, and single mechanisms will not explain the action of therapeutic mAbs. In Table 3, some possible modes of action are summarized for anticancer therapeutic mAbs. It is feasible that multiple mechanisms operate and that these might vary from patient to patient, depending on the status of the disease and the target cells, and could even change within one patient, with different mechanisms operating at different anatomical sites. For example, the limited availability of specific effector systems, such as NK cells, in certain tissues could restrict their activity to sites where they are present such as blood, liver and spleen. By contrast, other effectors such as complement and macrophages can have a more widespread activity.

Future directions in antibody therapy

The ability of mAbs to signal to target cells offers several exciting new prospects for immunotherapy because it is now clear that apoptosis is only one of the possible outcomes of mAb binding. The immune system is regulated by a complex network of receptor–ligand interactions that control when it should or should not respond. It now appears that mAbs that will engage these receptors can be generated and these can mimic or block (agonist or antagonist mAb) the action of the natural ligand(s) of these receptors. Thus, a range of reagents is now available that can, at least in model systems and in one clinical setting (CTLA4), control immune responses [22,23]. To date, some of the most encouraging preclinical results have come from mAbs directed at so-called co-receptors on cells of the

Table 3. Cancer therapeutic antibodies: modes of action[a,b]

Mechanism	Product	Antibody target
Blockade ligand binding	Erbitux®	EGF receptor
	HuMax-EGFr	EGF receptor
Complement dependent cytotoxicity	Rituxan®	CD20
	HuMax-CD20	CD20
	Campath-1H®	CD52
Antibody dependent cell-mediated cytotoxicity	Rituxan®	CD20
	HuMax-CD20	CD20
	Herceptin®	HER-2/neu
	HuMax-EGFr	EGF receptor
Apoptosis induction	Various	Idiotype B cell tumours
Disruption signalling	2C4 (pertuzumab)	HER-2/neu
Inhibition angiogenesis	Avastin®	VEGF
Targeted radiolysis	Zevalin®	CD20
	Bexxar®	CD20
Toxin-mediated killing	Mylotarg®	CD33
Antagonist activity	MDX-010	CTLA4
Agonist activity	Various	CD40, CD137

[a]It is probable that most cancer therapeutic antibodies use multiple mechanisms to destroy target cells. The examples in this table underline the predominant mechanisms only.
[b]Abbreviations: EGF, epidermal growth factor; VEGF, vascular endothelial growth factor.

immune system. These include molecules such as CD40, CD137, CD25, OX40 and CTLA4 [19,24–26]. In the case of CTLA4, antibodies can block the normal action of this receptor and consequently promote strong cellular immune responses against tumours. A human mAb against this target is now in clinical trials and is showing signs of anticancer activity [27]. By contrast, CD40 and CD137 trigger cells of the immune system and appear to potentiate weak anticancer responses to levels that will eliminate established tumours. A major attraction of such a system is that the target for the mAb is often not expressed by the tumour. Instead, the activated immune system (usually T cells) eliminates the unwanted cells. This has the advantage that the tumour cannot escape destruction by losing expression of the target antigen, a phenomenon that has been observed when tumours are targeted directly by mAbs. Furthermore, immunity persists once it has been established and is maintained by helper and cytotoxic T cells, thus preventing tumour reoccurrence.

There are also considerable obstacles to such an approach. It is possible that while generating the immune response, the mAbs will also promote unwanted autoimmunity and, indeed, such an outcome has been seen in mice [28,29]. However, it could be that this autoimmunity is an inevitable consequence of effective immunotherapy, in the same way that anti-CD20 mAb eliminates normal B cells for a period of months. It therefore must be ensured that the consequences of any unfortunate side effects are preferable to the cancer itself. The other major issue is whether humans carrying spontaneous malignancies will respond in the same way as mice passaged with tumour lines. This is a major question because most mouse tumours are known to be relatively immunogenic and this might not be the case in patients whose disease could have evolved over decades. However, the evidence emerging from humans is encouraging and suggests that, in the early stages of disease at least, the immune system does attempt to engage malignant cells. Unfortunately, this encounter is often won by the tumour, but with the capacity of mAbs to boost the response in favour of the immune system, perhaps this new family of reagents will reverse this trend.

Improving the cytotoxic activity of antibodies

Numerous strategies for improving the killing capacity of mAbs have been investigated. These range from simply selecting the most potent subclass of mAbs to tagging mAbs with cytotoxic modalities, such as radioisotopes or toxins. With regard to human antibodies, the IgG1 isotype is widely accepted as the most effective at recruiting the immune system. This is because the constant regions of IgG1 mAb [fragment crystallizable (Fc) region; Box 1] interact strongly with all types of FcγR on lymphoid and myeloid effectors and are strong complement activators [4,9]. By contrast, mAbs of the IgG4 isotype, which interact weakly if at all with these receptors, are generally considered more suitable as blocking reagents (e.g. when a mAb is required to prevent the interaction between a ligand and a receptor) and does not benefit from recruiting immune effectors. Unfortunately, choosing which isotype to use in any particular situation is often difficult because of the lack of understanding of the *in vivo* mechanisms involved, and this has meant that IgG1 is often selected just to cover the possibility that the immune system is required.

As an alternative to using IgG4 mAbs when interaction with FcγR is not required, Carpenter and co-workers have

shown that the Fc region of human IgG can be precisely engineered by mutating individual amino acids to inactivate binding with FcγR [30]. These reagents exhibit useful properties because they retain full antigen binding activity but can be selected for enhanced functional activity. Thus, the potential for modifying antibody isotypes by genetic engineering remains a very attractive approach for improving the usefulness of human mAbs in the future [31].

Combination therapy

It is now clear that the therapeutic activity of many, if not most, anticancer mAbs is considerably enhanced by their use in combination with either chemo- or radiotherapy [15,16]. For example, when rituximab is used alone in low-grade lymphoma, an overall response rate of ~50% with relatively few complete responses [(CRs), where no tumour cells are detected after antibody treatment] would be expected. Using such treatment in combination with a range of standard chemotherapeutic treatments not only improves the overall response rate to >90%, with high numbers of CRs and molecular CRs (where no tumours are detected by molecular technologies such as PCR after antibody treatment), but it also greatly extends the duration of the responses. The full duration of these responses has yet to be established because most of these trials are still running. Furthermore, it is now clear that combination treatment can generate significant clinical benefits even in more aggressive disease situations in which rituximab alone is of little benefit [15,16]. Similar benefits are also well documented with Herceptin® (anti-HER-2/neu) and Erbitux® [anti-epidermal growth factor (EGF) receptor] [32,33]. Furthermore, because the toxicity profiles of the mAbs and the cytotoxic modality do not usually overlap, using combination treatment in this way can improve therapeutic outcome without increased toxicity.

Considerable work is underway to understand the mechanisms of action in combination treatments. At its simplest, the mAb and the drug or radiation can work independently, attacking the cancer or other unwanted cells via different, unrelated pathways. However, there is also good evidence, particularly in cases such as Herceptin® and Erbitux®, where the mAb appears to play a role in signalling to the target cells, perhaps 'conditioning' these unwanted cells and making them more sensitive to drugs or radiation [34]. Another way in which antibodies targeted to growth factor receptors induce antitumour effects is via modulation of angiogenesis. It has been documented in animal models that anti-EGF receptor antibodies work, at least in part, via reduction in the levels of the factors that promote neo-vascularization, such as vascular endothelial growth factor (VEGF) [35].

Delivering toxic 'payloads'

An alternative method of improving the killing activity of mAbs is to tag them with a toxic modality such as a toxin, a chemotherapeutic drug or a radioisotope, thus allowing the mAb to act as a vector for a highly toxic 'payload' [36–38]. Under these circumstances, the ability of the mAb to recruit the immune system is less important and might actually be best avoided to ensure that inappropriate cells are not killed as a result of nonspecific interaction with the conjugate. There are two examples of these types of mAb conjugate that are currently approved (Table 1): gemtuzumab, directed at CD33 for the treatment of myeloid leukaemia, is linked to a small, highly toxic molecule, calicheamicin, and Zevalin®, a mouse CD20 mAb linked to yttrium-90 for the treatment of $CD20^+$ B-lymphoma. Both are effective therapies with acceptable side effects. In addition to acting as a vector for cytotoxic molecules or radiation, mAb in these applications need to bind to carefully selected targets to achieve appropriate localization at the surface or inside the unwanted cells. For toxin delivery, the ability of the mAb to be efficiently internalized once it is bound to the target is crucial, because only when the toxin is inside the cell, having been released from the antibody, and it has entered the cytoplasm is it able to poison the unwanted cell. With most toxins, only a few active molecules are required in the cytoplasm to ensure killing of the tumour cell. Relatively few surface antigens have been effective in this role. In the case of B-lymphoma malignancies, CD22 is the target of choice and a CD22 mAb linked to a modified ricin toxin is being developed for future treatment [39]. By contrast, mAbs that deliver conjugated radioisotopes are generally selected to remain at the cell surface for an extended period to allow the attached radiation to eliminate all the unwanted cells in the vicinity – even those not carrying the intended target. This latter phenomenon is called radiation 'cross-fire' and is a major benefit of using radiolabeled mAbs because it allows them to be used against populations of unwanted cells that show heterogeneous antigen expression. Internalization in this situation can actually reduce the efficacy of the mAb–isotope conjugates because it promotes the degradation and excretion of certain conjugates. For this purpose, CD20 has proved an ideal target because of its high density and stable surface expression, and this is a key factor in the success of Zevalin® and the mouse B1 mAb conjugated to iodine-131 (Bexxar®; awaiting FDA approval).

Conclusion

Although mAbs are clearly set to make a significant impact on the treatment of human disease in the next

decade, it is generally thought that their full therapeutic potential has only just started to be realized. At present, the most sophisticated mAbs in use are intact fully human antibodies, which display long half-lives and show little, if any, immunogenicity. However, these reagents are not using the wealth of knowledge of antibody effector functions and receptor signalling that is now available. For example, *in vitro* and animal models have clearly shown that by manipulating the constant regions and the carbohydrate moieties of mAbs, profound improvements in therapeutic activity can be achieved. It is now feasible to engineer antibody constant regions so that they bind more strongly to certain activating Fc receptors on effector cells, such as FcγRI and FcγRIII, and at the same time avoid interactions with inhibitory receptors, such as FcγRII. Thus, the next decade should provide exciting times as our full understanding of antibody biology is applied to human disease.

Acknowledgements

We gratefully acknowledge Ellen Broug and Ruth French for thoughtful and stimulating discussions and valuable help with Table 2.

References

1 Gura, T. (2002) Therapeutic antibodies: magic bullets hit the target. *Nature* 417, 584–586
2 Kohler, G. and Milstein, C. (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256, 495–497
3 Winter, G. and Milstein, C. (1991) Man-made antibodies. *Nature* 349, 293–299
4 Cragg, M.S. *et al.* (1999) Signaling antibodies in cancer therapy. *Curr. Opin. Immunol.* 11, 541–547
5 Deo, Y.M. *et al.* (1997) Clinical significance of IgG Fc receptors and Fc gamma-directed immunotherapies. *Immunol. Today* 18, 127–135
6 Kretzschmar, T. and von Ruden, T. (2002) Antibody discovery: phage display. *Curr. Opin. Biotechnol.* 13, 598–602
7 Kellermann, S.A. and Green, L.L. (2002) Antibody discovery: the use of transgenic mice to generate human monoclonal antibodies for therapeutics. *Curr. Opin. Biotechnol.* 13, 593–597
8 Lonberg, N. *et al.* (1994) Antigen-specific human antibodies from mice comprising four distinct genetic modifications. *Nature* 368, 856–859
9 Van Dijk, M.A. and Van de Winkel, J.G.J. (2001) Human antibodies as next generation therapeutics. *Curr. Opin. Chem. Biol.* 5, 368–374
10 Van Den Brande, J.M. *et al.* (2002) Treating Crohn's disease by inducing T lymphocyte apoptosis. *Ann. N. Y. Acad. Sci.* 973, 166–180
11 Morgan, B.P. *et al.* (1997) Role of complement in inflammation and injury in the nervous system. *Exp. Clin. Immunogenet.* 14, 19–23
12 Kirschfink, M. (2001) Targeting complement in therapy. *Immunol. Rev.* 180, 177–189
13 Hale, G. *et al.* (2002) Alemtuzumab (Campath-1H) for treatment of lymphoid malignancies in the age of non-myeloablative conditioning? *Bone Marrow Transplant.* 30, 797–804
14 Xia, M.Q. *et al.* (1993) Structure of the CAMPATH-1 antigen, a glycosylphosphatidylinositol-anchored glycoprotein which is an exceptionally good target for complement lysis. *Biochem. J.* 293, 633–640
15 Coiffier, B. (2003) Monoclonal antibodies combined to chemotherapy for the treatment of patients with lymphoma. *Blood Rev.* 17, 25–31
16 Press, O.W. *et al.* (2001) Immunotherapy of Non-Hodgkin's lymphomas. *Hematology (Am. Soc. Hematol. Educ. Program)* 1, 221–240
17 Vuist, W.M. *et al.* (1994) Lymphoma regression induced by monoclonal anti-idiotypic antibodies correlates with their ability to induce Ig signal transduction and is not prevented by tumour expression of high levels of bcl-2 protein. *Blood* 83, 899–906
18 Davis, T.A. *et al.* (1998) Anti-idiotype antibodies can induce long-term complete remissions in non-Hodgkin's lymphoma without eradicating the malignant clone. *Blood* 92, 1184–1190
19 Glennie, M.J. and Johnson, P.W. (2000) Clinical trials of antibody therapy. *Immunol. Today* 21, 403–410
20 Stevenson, F.K. *et al.* (2001) New strategies for vaccination and imunomodulation in NHL. *Ann. Hematol.* 80 (Suppl. 3), B132–B134
21 Agus, D.B. *et al.* (2002) Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumour growth. *Cancer Cell* 2, 127–137
22 Leach, D.R. *et al.* (1996) Enhancement of antitumour immunity by CTLA-4 blockade. *Science* 271, 1734–1736
23 Van Elsas, A. *et al.* (2001) Elucidating the autoimmune and antitumour effector mechanisms of a treatment based on cytotoxic T lymphocyte antigen-4 blockade in combination with a B16 melanoma vaccine: comparison of prophylaxis and therapy. *J. Exp. Med.* 194, 481–489
24 Tutt, A.L. *et al.* (2002) T cell immunity to lymphoma following treatment with anti-CD40 monoclonal antibody. *J. Immunol.* 168, 2720–2728
25 Chen, S.H. *et al.* (2000) Rejection of disseminated metastases of colon carcinoma by synergism of IL-12 gene therapy and 4-1BB costimulation. *Mol. Ther.* 2, 39–46
26 Miller, R.E. *et al.* (2002) 4-1BB-specific monoclonal antibody promotes the generation of tumour- specific immune responses by direct activation of CD8 T cells in a CD40- dependent manner. *J. Immunol.* 169, 1792–1800
27 Tchekmedyian, S. *et al.* (2002) MDX-010 (human anti-CTLA4): a phase I clinical trial in malignant melanoma. *Am. Soc. Clin. Oncol. Proc.* 1, 1
28 Bowne, W.B. *et al.* (1999) Coupling and uncoupling of tumour immunity and autoimmunity. *J. Exp. Med.* 190, 1717–1722
29 Shimizu, J. *et al.* (1999) Induction of tumour immunity by removing CD25+CD4+ T cells: a common basis between tumour immunity and autoimmunity. *J. Immunol.* 163, 5211–5218
30 Carpenter, P.A. *et al.* (2000) Non-Fc receptor-binding humanized anti-CD3 antibodies induce apoptosis of activated human T cells. *J. Immunol.* 165, 6205–6213
31 Presta, L.G. *et al.* (2002) Engineering therapeutic antibodies for improved function. *Biochem. Soc. Trans.* 30, 487–490
32 Milas, L. *et al.* (2000) *In vivo* enhancement of tumour radioresponse by C225 antiepidermal growth factor receptor antibody. *Clin. Cancer Res.* 6, 701–708
33 Prewett, M.C. *et al.* (2002) Enhanced antitumour activity of anti-epidermal growth factor receptor monoclonal antibody IMC-C225 in combination with irinotecan (CPT-11) against human colorectal tumour xenografts. *Clin. Cancer Res.* 8, 994–1003
34 Harari, P.M. and Huang, S-H. (2001) Head and neck cancer as a clinical model for molecular targeting of therapy: combining EGFR blockade with radiation. *Int. J. Radiat. Oncol. Biol. Phys.* 49, 427–433
35 Bancroft, C.C. *et al.* (2002) Effects of pharmacologic antagonists of epidermal growth factor receptor, PI3K and MEK signal kinases on NF-κB and AP-1 activation and IL-8 and VEGF expression in human head and neck squamous cell carcinoma lines. *Int. J. Cancer* 99, 538–548
36 Vose, J.M. (2000) Therapeutic uses of MAbs directed against CD20. *Cytotherapy* 2, 455–461
37 Vitetta, E.S. (2000) Immunotoxins and vascular leak syndrome. *Cancer J.* 6 (Suppl. 3), S218–S224
38 Dillman, R.O. (2002) Radiolabeled anti-CD20 monoclonal antibodies for the treatment of B- cell lymphoma. *J. Clin. Oncol.* 20, 3545–3557
39 Ghetie, M.A. *et al.* (1997) Immunotoxins for the treatment of B-cell lymphomas. *Mol. Med.* 3, 420–427