Therapeutic application of monoclonal antibodies in cancer: advances and challenges

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Introduction: Monoclonal antibody (mAb)-based products are highly specific for a particular antigen. This characteristic feature of the molecules makes them an ideal tool for many applications including cancer diagnosis and therapy.

Sources of data: We performed comprehensive searches of PubMed, Medline and the Food and Drug Administration website using keywords such as ‘therapeutic antibodies’ and ‘anti-cancer antibodies’.

Areas of agreement: Treatment of cancer patients with antibodies when used alone or in combination with chemotherapy and radiotherapy, or conjugated to drugs or radioisotopes, prolongs overall survival in cancer patients. Currently, there are 14 mAb-based drugs that have been approved for the treatment of cancer patients.

Areas of controversy: The response of cancer patients to antibody therapy can be of short duration. Therapeutic antibodies are expensive and may have side effects. There are no reliable predictive biomarkers for sensitivity or resistance to certain therapeutic antibodies.

Future focus: There should be additional studies to discover novel therapeutic targets, to develop more effective antibody-based drugs with fewer side effects, to identify more reliable predictive biomarker(s) for response to therapy with antibody-based drugs and to develop alternative strategies (e.g. transgenic plants, transgenic farm animals) for production of large quantities and more affordable batches of therapeutic antibodies.

Areas timely for developing research: A better understanding of cancer biology, the hallmarks of human cancers and the immune system would lead to identification of additional cell surface biomarkers. These in turn would facilitate the development of novel and biosimilar antibody-based drugs and their routine use as ‘magic bullets’ for the targeted therapy of human cancers.

Keywords: monoclonal antibodies/targeted therapy/cancer

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Despite major advances in our understanding of cancer biology and technological advances in cancer diagnosis and therapy over past three decades, cancer is a global health problem and a major cause of death worldwide. In the UK, there were over 320,500 new cancer cases and cancer was responsible for 157,250 deaths in 2010. Worldwide, there were an estimated 12.7 million new cancer cases and 7.6 million cancer deaths in 2008. In addition, the worldwide cost of cancer due to premature death and disability, excluding direct medical costs, was estimated to be around US$895 billion in 2008. In the next 50 years, the majority of the cancer burden cost will fall on low–middle income countries.

At present, there are three major approaches for reversing the worldwide increase in cancer incidence and mortality. The first and simplest approach is preventive measures such as reducing exposure to known carcinogenic agents (e.g. smoking, chemicals, infectious agents), healthier diets and the development of prophylactic cancer vaccines (e.g. HPV vaccines Gardasil and Cervarix). The second approach is detecting cancer at an earlier stage of the disease. This in turn would require the identification of more reliable tumour biomarkers and simpler screening methods. The third, and most expensive, approach is by developing more effective, tumour specific and consequently less toxic and more affordable anti-cancer drugs. In this review, we discuss and highlight some of the advances, current challenges and future opportunities for targeted therapy of human cancers using monoclonal antibody (mAb)-based products.

The advent of hybridoma technology by Kohler and Milstein in 1975 for which they were awarded the Nobel prize in medicine in 1984 revolutionized many areas of biological and medical research. Prior to hybridoma technology, antibodies were generated by the repeated immunization of animals with the antigen of interest and then sera from these animals were used for many applications, including therapy. Unfortunately, the administration of crude preparations of sera, which contained other animal proteins and a mixture of antibodies, produced allergic reactions and no clinical benefit in many patients. In contrast, the advent of hybridoma technology has allowed scientists, for the first time, to produce unlimited quantities of a specific type of antibody.
(i.e. mAb) against a particular antigen by immortalizing the antibody-producing B lymphocytes from the spleen of immunized mice.\textsuperscript{14}

mAbs have become essential tools in unravelling the role of many genes and their protein products in tumour pathogenesis and other pathological conditions; in the discovery of novel and overexpressed cell surface antigens and in the diagnosis and therapy of many diseases including human cancers.\textsuperscript{10,12} However, most mouse mAbs, produced

Fig. 1 Structure of mouse (first generation, 1970s), chimeric (second generation, 1980s), humanized, fully human and bispecific mAbs (third generation, 1990s and 2000s) and antibody fragments developed by genetic engineering for tumour imaging and therapeutic applications. (A) An intact mouse antibody consists of two identical heavy chains and two identical light chains connected by disulphide (−S−S−) bonds. Both heavy (larger) chains and light (smaller) chains contain a constant portion (CH and CL, respectively) and a variable portion (VH and VL, respectively). The antigen-binding site of an antibody is located at the variable VH and VL domains (i.e. complementary-determining regions, CDRs) of the antibody and the antibody’s immunological effect is mediated by the constant (Fc) portion of an antibody. (B) The chimeric versions of the mouse antibodies are generated by replacing the constant region of the mouse antibody with the constant region of human IgG antibody. (C) Humanized antibodies contain \textasciitilde 90% of human sequences and are formed by fusion of DNA for three CDRs from the mouse variable domain into a human IgG framework. (D) Fully human antibodies are generated using transgenic mice containing human immunoglobulin or phage display technology and contain 100% human and therefore are less immunogenic than their normal mouse counterparts, chimeric and humanized antibodies. (E) Bispecific antibodies contain two distinct antigen-binding domains and are capable of binding to two distinct antigens simultaneously. To increase tumour penetration, smaller fragments of antibodies, such as (F) monovalent scFv (30 kDa) and (G) divalent (scFv)\textsubscript{2} (60 kDa), have been generated which retain the antigen-binding specificity of the intact antibody.
using the traditional hybridoma technology, are highly immunogenic in cancer patients leading to the generation of human anti-mouse antibody (HAMA) response, resulting in their rapid clearance from the patients' serum. Following technological advances in genetic engineering in the 1980s and 1990s, it has been possible to reduce the immunogenicity and to increase the serum half-life of some rodent antibodies in humans by producing chimeric (Fig. 1B) and humanized (Fig. 1C) versions of such antibodies. Alternatively, using either transgenic mice containing human immunoglobulin gene or phage display technology, it has been possible to develop fully human mAbs against human

Fig. 2 The mechanisms by which mAb-based drugs can induce their therapeutic effects. Unconjugated antibodies can induce their therapeutic effect by (A) blocking the binding of growth factors to growth factor receptor and subsequent cell signalling pathways essential for cell proliferation such as anti-EGFR mAb cetuximab, (B) blocking and trapping an angiogenic factor such as anti-VEGF mAb Avastin, (C) preventing growth factor receptor–receptor dimerization and subsequent signal transduction pathways such as anti-HER mAb pertuzumab, (D) by blocking a key negative regulator of immune activity on T cells such as anti-CTLA-4 mAb ipilimumab, (E) binding to Fc receptors on effector cells (e.g. NK cells, macrophages, dendritic cells) and inducing ADCC such as anti-CD20 rituximab, (F) activating the complement system and inducing complement-mediated cytotoxicity CDC, (G) inducing apoptosis via upregulation of pro-apoptotic factors and downregulation of anti-apoptotic factors. (H) Blocking a key suppressor of the immune system expressed on tumour cells such as anti-programmed cell death 1 ligand 1 (PD-L1) antibody. In addition, mAbs can be conjugated to a therapeutic radio-isotope, drug or toxin for delivering a lethal dose of such agents to cancer cells (I).
Further biomedical and technological advances have facilitated the development of bispecific mAbs (Fig. 1D), antibody–drug conjugates as well as smaller antibody fragments (e.g. Fig. 1F and G) for use in therapeutic applications and cancer imaging (Fig. 1).

mAbs can induce their anti-tumour activities by several mechanisms. These are dependent on (i) the subclass of the antibodies and whether they are capable of binding to, and activating, the Fc receptors on host immune effector cells by inducing antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), (ii) the target antigen and (iii) whether the antibodies are conjugated to a lethal drug, toxin or therapeutic radioisotope (Fig. 2). In general, an ideal antigen for mAb-based targeted therapy of human cancers should

<table>
<thead>
<tr>
<th>Antibody name generic/trade</th>
<th>Antibody format</th>
<th>Target antigen</th>
<th>Therapeutic area</th>
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<tr>
<td>Rituximab/Rituxan</td>
<td>Chimeric IgG1</td>
<td>CD20</td>
<td>B-cell lymphoma, NHL</td>
<td>1997</td>
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<td>HER2</td>
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have the following characteristics. First, the antigen should be overex-
pressed on the tumour cell surface and therefore be accessible to the
therapeutic antibody for inducing ADCC and CDC, with no or a low
level of expression in normal cells. Secondly, there should be homogen-
ous expression of such antigens on tumour cells, but limited, or no
shedding of antigens (e.g. growth factor receptor extracellular domains
such as EGFR, HER-2) or other factors in patients’ sera, which could
trap or compete with the administered therapeutic antibody for
binding to the target antigen on tumour cells. Thirdly, the antigen
should also play an important role in cancer progression and contribute
to the hallmarks of cancer such as sustained proliferating signals,
increased angiogenesis, migration and invasion, resistance to apoptosis
or induction of immunosuppression. Because of their specificity and
high affinity towards particular antigens, mAb-based products and
antibody fragments account for around 30% of all new biotechnology
drugs in development and hundreds of these antibodies are currently at
different stages of clinical trials.

There are currently several mAb-based products which have been
approved by the US Food and Drug Administration (FDA) and/or the
European Union Health Authorities for the treatment of a wide range
of diseases including autoimmunity, organ transplant rejection, inflam-
mation, infection as well as human cancers. Of these, 14
antibody-based products have been approved for the treatment of
patients with haematological malignancies and a wide range of solid
cancers (Table 1). In the following sections, the characteristic features
of some of the antibodies and their target antigens are discussed.

Unconjugated and conjugated mAbs for the treatment of
haematological cancers

Anti-CD20 mAbs

The first mAb that was approved by the US FDA for the treatment of
cancer was rituximab (Rituxan) (Table 1). Rituximab is a chimeric
mAb directed against B-lymphocyte-restricted differentiation antigen
CD20. CD20 antigen is expressed on the surface of >90% of B cell
non-Hodgkin’s lymphomas (NHLs), on pre-B lymphocytes and on
mature lymphocytes. However, it is not expressed on stem cells,
plasma cells and other normal tissues. B-cell lymphoma accounts for
95% of all lymphomas and the binding of rituximab to CD20 antigen
in NHL patients causes B cell death by inducing ADCC, CDC and
apoptosis (Fig. 2 E and F). In 1997, rituximab was approved for short-
course outpatient treatment of relapsed or refractory CD20-positive,
low-grade or follicular B-cell NHLs in combination with chemotherapy. The addition of rituximab to chemotherapy prolongs overall survival in many of these patients.\textsuperscript{27,28} In addition to its use in the treatment of NHL, rituximab has also been approved for the treatment of rheumatoid arthritis symptoms and disease progression in 2006 and 2008, chronic lymphocytic leukaemia in 2010; Wegener’s granulomatosis and microscopic polyangiitis in 2011 and it was the best selling anti-cancer drug ($3 billion) in the USA in 2011.\textsuperscript{29}

Despite the success with rituximab, about half of the patients with NHLs do not respond to treatment or acquire resistance to therapy and this may be reduced by the usage of other anti-CD20 mAbs.\textsuperscript{30} In addition to rituximab, three other anti-CD20 mAbs have been approved by the FDA for the treatment of patients with haematological malignancies.\textsuperscript{27,28} Of these, ibritumomab tiuxetan was the first radioimmunotherapy (RIT) agent to gain the FDA approval for the treatment of cancer (NHL) in 2002. Ibritumomab tiuxetan is a mouse anti-CD20 mAb conjugated to \textsuperscript{90}Y radioisotope (Table 1). Tosituzumab was the second mouse anti-CD20 mAb conjugated to \textsuperscript{131}I which gained FDA approval for the treatment of patients with refractory NHL in 2003.\textsuperscript{27,28,31} The goal of RIT is to deliver cytotoxic radiation from therapeutic radioisotopes to tumours using the antibody molecule as a ‘guided missile’. While RIT has the advantage of killing adjacent antigen-negative tumour cells and therefore increasing the overall percentage of cell kill, this cross-fire effect can cause toxicity to normal host tissues due to the killing of normal cells.\textsuperscript{27} To reduce the total body irradiation and to facilitate the rapid clearance of radiolabelled mAbs, both ibritumomab tiuxetan and Tosituzumab are of mouse origin, and require pre-infusion with unconjugated rituximab and murine tositumomab to ‘de-bulk’ the body of normal B cells that would otherwise compete for localization to the tumour.\textsuperscript{31} Such treatments prolong survival rates in rituximab-refractory NHL patients.

The fourth anti-CD20 mAb to gain FDA approval is Arzerra. Unlike the other three anti-CD20 mAbs, Arzerra is a fully human anti-CD20 mAb and binds to a different distinct epitopes on CD20 from the previously discussed mAbs. In 2009, it was approved for the treatment of patients with chronic lymphocytic leukaemia who were refractory to Fludarabine and anti-CD52 mAb Aemtuzumab (Table 1).\textsuperscript{28}

**Anti-CD33, CD52 and CD30 mAbs**

Gemtuzumab ozogamicin (Mylotarg) was the first toxin-linked antibody to be approved for therapy. In May 2000 under the FDA’s accelerated approval programme, it was approved to treat patients with
acute myelogenous leukaemia (AML). Gemtuzumab ozogamicin is a humanized anti-CD33 mAb attached to the cytotoxic anti-tumour antibiotic, calicheamicin. The binding of this immunotoxin to the CD33 antigen on AML cells results in the internalization of the immunotoxin and dissociation of calicheamicin, its transport into the nucleus, and the degradation of the DNA leads ultimately to cell death (Fig. 2I). It has been approved by the US FDA as a single agent for the treatment of patients with CD33-positive acute myeloid leukaemia at the first relapse, who are over 60 years and not suitable for therapy with conventional cytotoxic drugs. However, a confirmatory, post-approval clinical trial was stopped early when there was no improvement in clinical benefit and greater toxicity in the group of patients who received Mylotarg compared with those receiving chemotherapy alone. The drug was voluntarily withdrawn from the market in June 2010.

Alemtuzumab (Camplt-1H) is a humanized mAb directed against another cluster of differentiation antigens named CD52. While CD52 is absent on haematopoietic stem cells, it is present on normal T and B lymphocytes and a high proportion of lymphoid cancers. The original rat anti-CD52 mAb was developed in 1980 and was the first antibody to be humanized. The humanized version of this antibody was approved for the treatment of patients with B-cell chronic lymphocytic leukaemia in 2001. Recent studies also suggest its potential in the management of patients with T-cell prolymphocytic leukaemia (T-PLL), multiple sclerosis and graft-versus-host disease. This antibody can induce its anti-tumour activity by inducing ADCC, activating complement and CDC. However, as a result of depletion of normal B- and T-lymphocytes, almetuzumab can cause immunosuppression and there is an increased risk of opportunistic infections in such patients.

Another important therapeutic target in patients with haematological malignancies is CD30, a member of the tumour necrosis factor receptor superfamily. CD30 was found to be overexpressed on the surface of tumour cells in patients with Hodgkin’s lymphoma (HL) and systemic anaplastic large cell lymphoma which is an aggressive, but rare, type of NHL. Brentuximab Vedotin (Adcetris) is a chimeric anti-CD30 antibody IgG1 antibody (i.e. mAb cAC10) conjugated to four molecules of the microtubule-disrupting agent monomethyl auristatin E by a protease cleavable covalent linker. Under the FDA’s accelerated approval programme, this antibody–drug conjugate has been approved for the treatment of patients with HL who have failed autologous stem cell transplant (ASCT) or who are not candidates for ASCT and who had had failed at least two prior combination chemotherapy (FDA labelling information, d cetris, 2011). In a single trial involving 102 HL patients, 73% of the patients had either a complete or partial response and on average the response to therapy lasted for 6.7 months. This drug has
also been approved for the treatment of patients with systemic anaplastic large cell lymphoma (ALCL) who failed at least one prior multi-agent chemotherapy. In a single clinical trial involving 58 patients, 86% of the patients receiving this drug had a complete or partial response and the median response duration was 12.6 months (FDA labelling information, Adcetris, 2011). The most common side effects associated with this drug were neutropenia, peripheral sensory neuropathy, fatigue, nausea, upper respiratory tract infections, diarrhoea and thrombocytopenia.

mAbs for the treatment of solid tumours

Anti-HER antibodies

The HER [also called erbB or epidermal growth factor receptor (EGFR)] family of receptors is one of the best characterized growth factor receptor family with tyrosine kinase activity. It consists of four family members namely, EGFR (HER-1), ErbB2 (HER-2), ErbB3 (HER-3) and ErbB4 (HER-4). Since the early 1980s, aberrant expression and activation of the HER family members, in particular EGFR and HER-2, have been reported in a wide range of epithelial cancers and in some cases have been associated with a poor prognosis. The biological consequences of EGFR and HER-2 activation in human malignancies include increased cell proliferation, reduced apoptosis, increased angiogenesis, increased motility, invasion and metastasis which are some of the hallmarks of human cancers. These observations have led to the strategic development of several mAbs, four of which have already been approved by the FDA for the treatment of cancer patients in combination with chemotherapy or radiotherapy (Table 1).

Of the HER inhibitors, Herceptin (trastuzumab) was the first humanized anti-HER-2 mAb approved by the FDA in 1998 for the treatment of HER-2 over-expressing metastatic breast cancer. Subsequently in 2006, it was approved by the FDA for the treatment of early stage HER-2 over-expressing breast cancer patients and in 2010 for the treatment of HER-2 positive stomach or gastro-oesophageal junction cancer. Herceptin was the third best-selling anti-cancer drug with sales of $1.66 billion in 2011. Although treatment with Herceptin can induce clinical benefit in 30% of HER-2 positive breast cancer patients, the duration of response can be limited and many patients acquire Herceptin resistance and disease progression within 1 year of treatment. It is therefore very important to identify molecular markers that are responsible for the poor response or development of resistance to therapy with HER-2 inhibitors. In some studies, however, the expression
of other members of the EGFR family (e.g. EGFR and HER-3) or other growth factor receptors (e.g. IGF-IR), production of EGFR ligands and expression of truncated forms of HER-2 have been associated with the development of Herceptin-resistant breast cancer.41,42 At present, several clinical trials are underway examining the therapeutic advantage of trastuzumab when used in combination with other therapeutic strategies (www.clinicaltrials.gov) to circumvent this resistance for longer. Interestingly, on 8 June 2012, the FDA approved another anti-HER-2 mAb pertuzumab for use in combination with trastuzumab and Docetaxel for the treatment of HER-2 positive metastatic breast cancer patients who have not received prior anti-HER2 therapy or chemotherapy for metastatic disease, based on the results of the CLEOPATRA study. Like Herceptin, pertuzumab is a humanized antibody but binds to a different distinct epitopes on the extracellular domain of HER-2, thereby blocking the heterodimerization of HER-2 with other members of the HER family. Indeed, this pivotal study showed that the combination of two mAbs which are directed against two distinct epitopes on a single target can prolong median progression free survival in cancer patients.43 In addition, the efficacy and safety of neoadjuvant with pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer in a randomized multicentre, open-label, phase II trial (NeoSphere) has been reported recently. The combination of pertuzumab and trastuzumab, without chemotherapy, had a favourable safety profile and eradicated tumours in a proportion of these patients.44

In addition to the two anti-HER-2 mAbs, two antibodies against the EGFR have been approved for the treatment of cancer patients.38 Of these, cetuximab is a chimeric (IgG1) antibody which has gained FDA approval for the treatment of metastatic colorectal cancer in combination with fluoropyrimidine-based chemotherapy. It has also been approved for the treatment of head and neck cancer patients in combination with radiotherapy. The second anti-EGFR mAb panitumumab is the first fully human mAb (IgG2) which has gained FDA approval for the treatment of patients with metastatic colorectal cancer (Table 1).

Three challenges with the routine use of anti-EGFR mAbs in the clinic are the lack of reliable markers for the identification of patients who gain benefit from therapy with anti-EGFR mAbs, the short duration of response in many patients and the high cost of the antibodies. At present, there are conflicting data in the literature on the importance of EGFR as a predictive biomarker for response to therapy with anti-EGFR mAbs.38,45 The results of ongoing clinical studies should unravel the importance of relative expression levels of various forms of the EGFR (e.g. membranous, cytoplasmic, nuclear, wild type, mutated forms) as predictive markers for response to therapy with anti-EGFR mAbs as well as...
their prognostic significance. On 6 July 2012, the FDA approved cetuximab for use in combination with folinic acid, fluorouracil and irinotecan (FOLFIRI) for first-line treatment of patients with K-ras mutation-negative (wild-type), EGFR-expressing metastatic colorectal cancer as determined by FDA-approved tests. They also included a new limitation for its use stating that it is not indicated for the treatment of colorectal cancer which carries the K-ras mutation. Concurrent with this, the FDA also approved the companion Therascreen® KRAS RGQ PCR Kit (Qiagen Manchester Ltd) for analysis of Kras status of colorectal tumour.

**Anti-VEGF mAb Avastin**

One of the key hallmarks of human cancer is angiogenesis, which is the formation of new blood vessels, essential for the local growth of tumour cells. Bevacizumab (Avastin) was the first anti-angiogenic factor approved in 2004 as a first-line treatment for patients with metastatic colorectal cancer in combination with chemotherapy. Since angiogenesis also plays an important role in the growth and spread of other solid tumours, the anti-tumour activity of bevacizumab has also been investigated in other types of cancers. In 2006, bevacizumab gained the FDA approval for its use as a first-line treatment for patients with advanced non-squamous non-small cell lung cancer in combination with carboplatin and paclitaxel. In February 2008, the FDA also granted accelerated approval for bevacizumab, subject to further studies, for its use in combination with paclitaxel for the first-line treatment of HER-2 negative, locally recurrent or metastatic breast cancer, based on statistically significant progression-free survival, but not overall survival, in patients receiving this combination. However, in December 2011, the FDA rescinded this decision as additional clinical studies showed no increase in overall survival or improvement in patients’ quality of life. In 2011, bevacizumab in combination with capecitabine was approved by the European Medicines Agency (EMA) for the treatment of metastatic breast cancer when other chemotherapy treatments are unsuitable. In the UK, National Institute for Health and Clinical Excellence (NICE) indicated that the average cost of treating breast cancer patients with bevacizumab is around £3689 per month; NICE rejected the use of bevacizumab in combination with a taxane or capecitabine as it did not meet its cost-effectiveness criteria.

In 2009, bevacizumab gained FDA approval for the treatment of patients with metastatic renal cancer. Bevacizumab also improved progression-free survival in glioblastoma multiforme (GBM) and remains as the only FDA-approved molecular-targeted drug in patients with GBM (Table 1).
In 2011, bevacizumab in combination with carboplatin and paclitaxel chemotherapy was approved by the EMA as a first-line treatment for women with newly diagnosed advanced ovarian cancer based on the results of two-phase III clinical trials (i.e. GOG218 and ICON7). This is the first breakthrough treatment that has shown a survival advantage in advanced ovarian cancer patients, for over 20 years. However, it has not yet been approved by FDA for the treatment of ovarian cancer patients in the USA; this may be due to side effects in some patients. The main side effects reported with bevacizumab are hypertension, risk of bleeding and bowel perforation, but the latter two side effects are rare.

**Anti-CTLA-4 and other promising immune-stimulating antibodies**

Cytotoxic T-lymphocyte antigen-4, also known as CD152, is a member of the immunoglobulin superfamily and when expressed on the surface of cytotoxic T lymphocytes (CTLs) and regulatory T suppressor cells can result in downregulation of the immune response. In contrast to CD28, CTLA-4 has a higher affinity for binding to B7-1 (CD80)/B7-2(CD86) expressed on the surface of antigen-presenting cells resulting in T cell anergy and immunosupression. Ipilimumab is a fully human anti-CTLA-4 mAb (IgG1). CTLA-4 blockade by ipilimumab stimulates T cell activation and proliferation of cancer specific T cells, leading to stronger anti-tumour responses in several preclinical and clinical studies. Indeed, it was the first agent to show improved overall survival in patients with advanced or metastatic melanoma. In March 2011, it gained FDA approval for the treatment of patients with advanced melanoma. Adverse effects included autoimmune-related toxicities and further clinical trials are currently underway in other tumour types. In addition, encouraging results have been reported recently with antibodies against other inhibitory receptors expressed by T cells such as mAb BMS-936558, which is directed against programmed death 1 (PD-1) protein and mAb against PD-1 ligand (PD-L1) expressed on tumour cells. The results of ongoing clinical trials should unravel the full potential of this class of antigens (i.e. the negative regulators of the immune system) as therapeutic targets for mAb-based directed therapy.

**Tri-functional anti-EpCAM X anti-CD3 mAb Catumaxomab**

Catumaxomab is another type of antibody-based product that was approved by the EMA in April 2009 for the intraperitoneal treatment of malignant ascites in patients whose tumours are epithelial cell adhesion molecule (EpCAM, also called CD326) positive and for whom the
standard therapy in not feasible or available. EpCAM is a transmembrane glycoprotein that plays an important role in preventing cell–cell adhesion, tumour cell migration and proliferation. Catumaxomab is a bispecific rat/mouse hybrid antibody containing two different antigen-binding specificities with the mouse Fab binding to EpCAM antigen on tumour cells and the rat Fab binding to CD3 antigen on T cells. In addition, the Fc hybrid region of Catumaxomab binds to the Fcγ receptors on effector cells such as NK cells, macrophages and dendritic cells (Fig. 2). Consequently, catumaxomab is a tri-functional antibody as it can induce its anti-tumour activity via T-cell mediated tumour lysis and induction of ADCC and phagocytosis via the activation of FcR-positive effector cells. In a Phase II/III trial involving 258 patients with malignant ascites, catumaxomab showed a clear clinical benefit compared with paracentesis and had an acceptable safety profile. Since catumaxomab is a mouse/rat hybrid antibody, recent studies suggest that the development of HAMAs, 8 days after the fourth antibody infusion, could be a useful predictive biomarker for response to therapy with this antibody. In patients with malignant ascites, a greater benefit is seen with catumaxomab therapy in those patients who developed HAMAs sooner. This could be due to the production of anti-idiotypic antibody (Ab2) against the administered therapeutic antibody (Ab1). Catumaxomab is the first tri-functional antibody and also the first drug to be approved for the treatment of malignant ascites, and clinical trials are currently underway for other indications including ovarian and gastric cancer as well as an open label, dose-escalating study to determine the safety and tolerability of ascending intravenous doses of this antibody in patients with epithelial cancers (http://clinicaltrials.gov). In addition, the approval of catumaxomab as well as the two mouse antibodies described earlier (i.e. ibritumomab tiuxetan, tositumomab) suggest that humanization of all rodent antibodies is not essential and that therapeutic benefits can also be gained by cancer patients using various forms (i.e. both conjugated and unconjugated) of rodent mAbs.

**Challenges and future opportunities in targeted therapy of cancer using mAbs**

One major limitation of cytotoxic drugs and radiation in the treatment of cancer patients is their inability to discriminate between malignant and normal tissues. This in turn prevents the delivery of the optimal (therapeutic) dose of such agents to malignant tissues for their eradication. Thanks to the advent of hybridoma technology, and subsequent
advances in genetic engineering and our understanding of cancer biology, mAb-based products are a well-established treatment modality for a wide variety of solid tumours and haematological malignancies, when used alone or in combination with chemotherapy or radiotherapy. However, there are several outstanding challenges with the routine application of mAb-based products for use in oncology. First, there is currently no reliable biomarker for the identification of patients who are most likely to benefit from the antibody therapy for some of the antibodies. Secondly, it is clear that acquired resistance is not unique to cytotoxic drugs and does also occur following treatment with repeated doses of antibody-based drugs. Downregulation of the target antigen and the heterogeneous nature of human malignancies could be some of the contributing factors for the short duration of response (i.e. less than a year) to antibody-based drugs in some patients. Thirdly, none of the currently approved mAbs are directed against a cancer specific antigen. These antigens are often expressed at lower levels on normal cells in the epithelial tissue, stroma or on normal white blood cells. As a result they would contribute to some common, but not life threatening, side effects, that occur with some of these antibodies (e.g. allergic reactions, diarrhoea, skin rashes, flu-like symptoms). However, in comparison with chemotherapy, serious side effects are not common with mAb-based products. Side effects, such as allergic reactions, can often be prevented by a slow infusion rate and prophylactic premedication with intravenous antihistamine therapy prior to the antibody infusion. Specific side-effects such as the facial rash associated with cetuximab are often effectively controlled by the concurrent use of doxycycline and skin emollients.

Further studies of tumour cells, the tumour microenvironment (e.g. normal cells, cancer stem cells) and the heterogeneous molecular pathways which are involved in the development of human cancers could help in the identification of additional cell surface antigens (overexpressed or tumour specific) in cancer cells, and provide additional therapeutic targets for the development of novel and more effective mAb-based products for use in cancer therapy. This is currently an area of active research in many academic laboratories, including our laboratory, and many pharmaceutical companies. Finally, the treatment of cancer patients with mAb-based drugs is very expensive, with 1 year’s treatment with some antibodies costing up to $100 000. This together with the increasing demand for various forms of antibodies for use in the diagnosis and treatment of cancer and other human diseases would dictate the development of alternative strategies and cheaper manufacturing facilities for the production of therapeutic antibodies. Two alternative strategies are the usage of transgenic plants and transgenic farm animals (e.g. cow, goat, chicken).
genetically engineered hens have the potential to lay eggs containing high levels of therapeutic antibodies. Further technological advances and optimization of transgenic plants and transgenic animals (e.g. for the proper folding, glycosylation and stability of antibody, antibody secretion, purification, expression yield) would help to meet the increasing demand for the antibody-based products and ultimately to reduce the antibody production cost. These together with better companion diagnostic and predictive tests to exclude patients who will not benefit from these treatments, and more definitive selection of patients who should benefit, will in the future, lead to the production of more effective and affordable batches of mAb-based products for therapeutic application in oncology and other pathological conditions. These would also help to prevent the exclusion of all patients from receiving these new therapies, based on the argument of high cost alone.

Concluding remark

The advent of hybridoma technology and subsequent advances in genetic engineering and our understanding of cancer biology have helped to establish mAb-based products as a therapeutic modality for patients with both haematological malignancies and a wide range of solid tumours, when used alone or in combination with chemotherapy and radiotherapy. Indeed, Rituxan, Avastin and Herceptin were the top three best-selling anti-cancer drugs in the USA, with total sales of $7.32 billion in 2011. mAb-based therapeutics are expected to be in the 6 out of the 10 best-selling drugs in 2012. Currently, there are several hundred mAb-based products at different stages of preclinical studies and clinical trials for use in oncology. Undoubtedly, in the coming months and years, additional mAb-based products will be approved for the treatment of cancer patients by health authorities worldwide. A good candidate is anti-HER-2 mAb trastuzumab conjugated to the cytotoxic drug emtansine which showed encouraging results in a phase-three EMILIA trial for the treatment of patients with HER-2 positive metastatic breast cancer. Further advances in our understanding of cancer biology, tumour microenvironment and the immune system should lead to the identification of novel and more relevant tumour biomarkers (i.e. tumour drivers) as therapeutic targets. These, together with further technological advances, should facilitate the development, and more effective usage, of antibody-based products as ‘magic bullets’ for targeted therapy of human cancers, when used alone or in combination with other therapeutic interventions and ultimately reverse the worldwide increase in cancer mortality.
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