Monoclonal Antibodies as Therapeutic Agents: Advances and Challenges

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ABSTRACT

Despite the major advances in conventional forms of treatment (i.e. surgical techniques, radiotherapy and chemotherapy) and improved survival rates, cancer is still the second leading cause of death in developing countries. 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. With the advent of hybridoma technology in 1975, it has been possible for the first time to produce large amounts of an antibody (i.e. monoclonal antibody) against any antigens of interest. Since each antibody is highly specific for a particular antigen, this typical feature of the antibodies has resulted in their widespread use in diagnostic kits, medical research (e.g. to unravel the function of the antigen in physiological and pathological conditions), and more recently, for the management of a wide range of human diseases such as autoimmune disease and human cancers. Thanks to recent advances in genetic engineering, the immunogenicity of rodent antibodies was reduced by producing the chimeric or humanized version of such antibodies or by developing the fully human antibodies. In other instances, as intact antibodies are too large for rapid penetration into solid tumours, it has been possible to develop a smaller fragment of such antibodies (e.g. Fab, scFv, VHH) with greater potential for use in cancer imaging and therapy. Depending on the target antigens and the antibody format, monoclonal antibodies can induce their anti-tumour activities by several mechanisms including activation of the host effector cells. To date, several mAbs have been approved for management of human cancers including: anti-EGFR antibody cetuximab and anti-VEGF antibody bevacizumab for treatment of metastatic colorectal cancer, anti-HER-2 antibody trastuzumab for metastatic breast cancer, anti-CD20 antibodies rituximab and ibritumomab tituxetan for non-Hodgkin lymphoma, anti-CD52 antibody alemeutumab for chronic lymphocytic leukaemia, and anti-CD33 antibody gemutuzumab ozogamicin for the treatment of acute myeloid leukaemia patients. Monoclonal antibodies currently account for about 30% of all new drugs in development, with more than 500 antibodies at different stages of clinical trials worldwide. In this review, the characteristic features of some of the therapeutic antibodies and the antigens recognised by such antibodies will be discussed as well as several challenges that need to be addressed in order to facilitate their widespread use as “magic bullets” in the management of human diseases and in particular human cancers.

Keywords: Cancer, Monoclonal antibodies, Therapy

INTRODUCTION

Cancer is a global problem and despite the major advances in surgical techniques, radiotherapy and chemotherapy and improved survival rates in certain types of cancer, it is still the second leading cause of death in Western countries. In the year 2000, there were 10 million new cases, 6 million deaths, and 22 million people living with cancer worldwide (1). While the high incidence and mortality of cancer may be reduced by several approaches, the first and easiest approach is through preventive measures such as reduced exposure to known carcinogenic agents (e.g. smoking, chemicals, infectious agents, radiation). Indeed, of the 10 million cancer cases in 1995, 75% was related to one of the three factors as smoking, dietary factors and infectious agents (2). The next best approach in winning our battle against cancer is by detection of the disease at an earlier stage. This in turn would require the identification of reliable tumour markers for screening purposes and simple screening methods (3). A third approach and, currently the most expensive one, is by the development of more effective and specific therapeutic strategies (4,5). As the majority of patients with solid tumours are diagnosed with advanced stage disease, such tumours have often a poor response to treatment with cytotoxic drugs. In addition, cytotoxic drugs are not specific for tumour cells and there is often a wide range of toxicity associated with the use of such drugs in cancer patients. This in turn results in the delivery of suboptimal doses of such drugs for treatment of cancer patients. It is; therefore, of prime importance to identify tumour antigens of biological and clinical importance that can be used not only in the early detection of human cancers but also those markers that can predict the response to cancer therapy or form ideal targets for the development of cancer specific therapeutic strategies.

MONOCLONAL ANTIBODY TECHNOLOGY AND THE ANTIBODIES' MECHANISM OF ACTION

With the discovery of a procedure called hybridoma technology by Kohler and Milstein in 1975 for which they received the Noble Prize in Medicine in 1984, it became possible to produce large quantities of a specific type of antibody (i.e. monoclonal antibody) against any virtual target antigen (6). Since each antibody is highly specific for a particular antigen, this characteristic feature of antibodies has led to their routine use in diagnostic kits and in uncovering the function of such antigens in a number of physiological and pathological conditions. Using hybridoma technology, monoclonal antibodies have been prepared against a wide range of antigens including growth factors, growth factor receptors, mutated (i.e. tumour specific) antigens, viruses, bacterial products, hormones, drugs, enzymes, and differentiated antigens. Such antibodies are used routinely in the identification of the antigens in human tumour biopsies and sera, and in investigating their role in tumour progression. In addition, following the recent success in the mapping of the human genome, monoclonal antibody technology is becoming an essential tool in the discovery of novel human tumour antigens which are overexpressed in human malignancies and in the identification of antigens, which are differentially expressed between the primary and

metastatic tumours, and for the management of a wide range of diseases (7-13).
With the exception of naturally occurring antibodies in camels, llamas and sharks that sometimes lack the light chains of antibodies (e.g. IgG2 subclass), all conventional antibodies have the same basic structure and consist of two identical heavy (H) and two identical (L) chains that are further divided into variable (V) or constant (C) regions (Fig. 1). The variable portion of both the heavy (VH) and light (VL) chains forms the two antigen binding fragments (Fab) of the antibody. On the variable domain of the VH and VL chains, there are three hypervariable sequences called complementarity-determining regions (CDRs) that are responsible for the specificity of the antibodies to their target antigens. The constant portions of heavy chains, called crystallisable fragment (Fc), are responsible for mediating the effector functions of the antibodies by binding to Fc receptors (FcgRs) on host immune cells (e.g. macrophages, natural killer cells and neutrophils) and inducing antibody-dependent cellular cytotoxicity (ADCC) and complement activation and mediating complement-

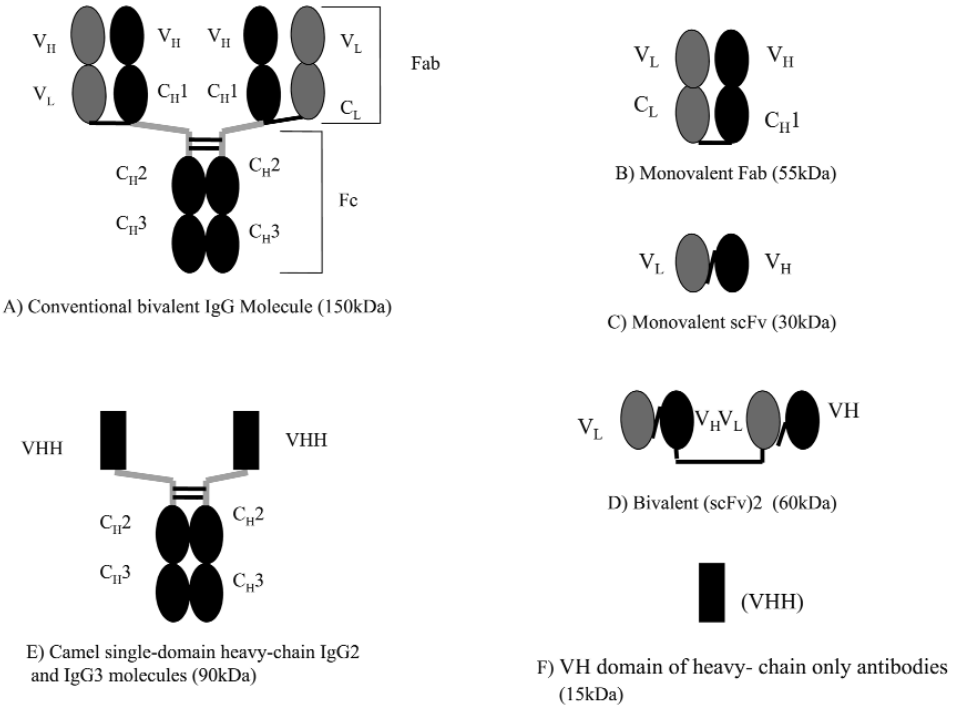

Figure 1. Structure of an intact antibody (immunoglobulin) and antibody fragment developed by genetic engineering for tumour imaging and therapeutic applications. An intact antibody consists of two identical heavy chains and two identical light chains connected together by disulphide (-S-S-) bonds (A). The antigen binding site of an antibody is located at the variable domain of the antibody (i.e. VH and VL), the immunological effect of antibody is mediated by the constant (Fc) portion of an antibody. In order to increase tumour penetration, smaller fragments of antibodies such as monovalent Fab (55kDa), scFv (30kDa), or bivalent scFv2 (60kDa) have been generated that retain the antigen binding specificity of intact antibody (B, C and D respectively). Certain subclasses (e.g. IgG2) of camel and shark antibodies are single-domain heavy chain antibodies (i.e. lack light chains) (E). The variable domain of heavy-chain antibodies are the smallest antigen recognition unit (15kDa) developed by genetic engineering (F)

dependent cytotoxicity (CDC) (14-16). Of all human antibodies, IgG1 antibodies are found to be the most effective ones in mediating effector function via ADCC and CDC (17).

As the first panel of monoclonal antibodies was developed against the human antigens in mice, treatment of some patients with chronic (repeated) doses of such antibodies had resulted in the development of human anti-mouse antibody (HAMA) response. The HAMA response in turn can result in the rapid clearance of such antibodies from a patient's blood, before reaching the target antigen, and therefore reducing their therapeutic benefit. Following advances in genetic engineering, the immunogenicity of mouse antibodies was reduced by producing various human recombinant forms of such antibodies. In some cases, chimeric or humanized versions of mouse antibodies have been developed by transferring the VL and VL regions or three stretches of amino acids in the variable region of mouse antibodies (i.e. CDR grafting) into the human IgG framework, respectively (18-22). As the chimeric and humanized forms of such antibodies contain more than 70% and 90% human sequences, such antibodies may be less likely to trigger an immunogenic response than the parent mouse antibodies. All chimeric and humanized antibodies that have been approved for the management of human cancers are IgG1 antibodies due to their superiority in mediating ADCC and CDC (Table 1). In other cases, it has been possible to develop fully human antibodies against human tumour antigens, using transgenic mice and phage display technology (23-26). In addition, as intact antibodies such as whole IgG (160kDa) are too large for rapid penetration of solid tumours and such antibodies have slow blood clearance, smaller fragments of the antibodies such as Fab (55kDa), scFv (30kDa) have been developed containing a single antigen binding fragment (i.e. monovalent) of the intact bivalent antibody (Fig. 1B-D, 27). More recently, the immune systems of camels and sharks have been shown to produce naturally occurring heavy-chain antibodies that are devoid of light chain antibodies (Fig. 1E; 28-32). The variable domain of the heavy-chain IgGs, called (VHH), is the smallest antigen recognition unit (Fig. 1F), which can be attached to radioisotopes or toxins for use in cancer imaging and therapy (12,13). Finally, as each antibody molecule has two identical antigen binding domains, in order to enhance the effector function of an antibody, bispecific monoclonal antibodies have been generated that are directed against two different target antigens, with one arm binding to a tumour antigen and the other to either an antigen on host immune wells (e.g. CD3 antigen on T cells or CD16 on NK cells, neutrophils and macrophages) or a different antigen (33-37). The results of several Phase II/III clinical trials with different bispecific monoclonal antibodies should unravel their full potential in retargeting the host immune effector functions at tumour sites and the possible side effects associated with such approaches.

CURRENT USE OF MONOCLONAL ANTIBODIES IN THE TREATMENT OF HUMAN DISEASES

Since 1986, the United States Food and Drug Administration (FDA) has approved several monoclonal antibodies for the management of a wide range of human diseases including the prevention of graft rejection, infectious agents, treatment of autoimmune

Table 1. Monoclonal Antibodies which have been approved by the United States Food and Drug Administration for clinical use in cancer and non-oncological conditions

Antibody name	Target antigen	Antibody format	Therapeutic area	Year
Muromonab-CD3 (Orthoclone)	CD3	Mouse	Transplant rejection	1986
Abciximab (ReoPro)	GPIIb &IIIa	Chimeric Fab	Cardiovascular disease	1994
Daclizumab (Zenapax)	CD25	Humanized	Transplant rejection	1997
Rituximab (Rituxan)	CD20	Chimeric (mouse-human IgG1)	Non-Hodgkinlymphoma	1997
Infliximab (Remicale)	TNF alpha	Chimeric	Crohn disease, Rheumatoid arthritis	1998 1999
Baslixamab (Simulect)	CD25	Chimeric	Transplant rejection	1998
Synagis	RSV	Humanized	Infectious disease	1998
Trastuzumab (Herceptin)	HER-2	Humanized (IgG1)	Breast cancer	1998
Gemtuzumab ozogamicin (Mylotarg)	CD33	Humanized (IgG1) (attached to toxin)	Acute myeloid Leukaemia	2000
Alemzutumab (Campath 1H)	CD52	Humanized (IgG1)	Chronic lymphocytic leukaemia	2001
Adalimumab (Humira)	TNF alpha	fully human IgG1	Rheumatoid arthritis	2002
Ibritumomab tiuxetan (Zevalin)	CD20	Mouse (attached to yettrium 90)	Non-Hodgkin lymphoma	2002
Tosituzumab (Bexxar)	CD20	mouse (attached to Iodine-131)	Non-Hodgkin lymphoma	2003
Erbitux (Cetuximab)	EGFR	Chimeric (mouse-human IgG1)	Metastatic colorectal cancer	2004
Bevacizumab (Avastin)	VEGF	Humanized (mouse-human IgG1)	Metastatic colorectal cancer	2004

disease and cancer (Table 1, 12). Of these, there are eight monoclonal antibodies that have been approved for the treatment of human cancers. The characteristic features of these antibodies, in particular the three monoclonal antibodies that have been approved for the treatment of solid tumours, together with the antigens recognised by such antibodies will be discussed here.

1) Anti-EGFR mAb Erbitux (Cetuximab, C225, Imclone System\Bristol Myer Squibb, USA) for treatment of metastatic colorectal cancer

The human epidermal growth factor receptor (EGFR) is a 170 kDa transmembrane glycoprotein with tyrosine kinase ac tivity, and the prototype of the type I growth factor receptor family, which transmits the biological effects of the EGF family of ligands such as EGF, TGFa, amphiregulin, HB-EGF, betacelluin, epiregulin (38). The binding of ligands to the external domain of the EGFR results in autophosphorylation of several tyrosine residues in its intracellular domain and subsequent phosphorylation of several downstream intracellular substrates associated with cell proliferation, apoptosis, angiogenesis, and invasion and metastasis (39,40). Overexpression of the EGFR accompanied by production of one or more of its ligands has been reported in a wide range of human malignancies including cancers of bladder, brain, breast, head and neck, oesophagus, lung, cervix and colon (41,42). In addition, in several studies, high levels of expression of this receptor have been associated with resistance to chemotherapy, hormonal therapy and radiotherapy, high-grade tumours and a poor prognosis (43-45). Overexpression of the EGFR has also been reported in Iranian patients with cancers of breast, head and neck and gastric cancer (46,47).

Since the early 1980s, a series of mouse (225, 528, 425), rat (our ICR16, ICR62, ICR64), chimeric (IMC-225, also called Erbitux or cetuximab), humanized (EMD7200, H-R3) or fully human anti-EGFR antibodies (ABX-EGF, IMC-11F8) have been developed against the external (i.e. ligand binding) domain of human EGFR (48-53). Preclinical studies with these antibodies have indicated that they are very effective in 1) blocking the binding of EGF family of ligands to the EGFR, 2) preventing the ligand-induced phosphorylation of the EGFR and 3) inhibiting the growth of human EGFR overexpressing tumours both in culture and as xenografts in athymic mice as a single agent or in combination with cytotoxic drugs or radiotherapy (40,48-56). The antitumour activities of these antibodies have been shown to be mediated via several mechanisms including down regulation of the EGFR from the cell surface, induction of G1 cell cycle arrest, promotion of apoptosis, inhibition of angiogenesis, and immune destruction via ADCC and CDC (40,41,44-55). Clinical trials with several of these anti-EGFR antibodies are currently underway in patients with a wide range of epithelial tumors (50,52-65).

As the mouse anti-EGFR mAb 225, developed by Mendelsohn and colleagues in 1983, was found to be highly immunogenic in cancer patients, a chimeric form of mAb 225 (IMC-225 also called cetuximab or Erbitux) was developed using genetic engineering (50,51). In addition, as Erbitux contains the antigen binding domain of mouse anti-EGFR mAb 225 and a human IgG1 constant region, it can interact efficiently with the effector arm of the patient's immune system to induce tumour killing via ADCC (50,51). In November 2004, the USA Food and Drug Administration approved Erbitux for the treatment of metastatic colorectal cancer patients whose tumours overexpress the EGFR (60,61). Erbitux has been approved for use in combination with irinotecan, in the treatment of EGFR overexpressing metastatic colorectal cancer patients who are unresponsive to irinotecan-based chemotherapy, or as a single agent in patients who are intolerant to irinotecan-based chemotherapy (66). The FDA approval of Erbitux was based on the results of a Phase II clinical trial in which the effect of Erbitux was investigated as a single agent or in combination with irinotecan in 329 patients with EGFR expressing metastatic colorectal cancer refractory to irinotecan-based chemotherapy. The combination treatment of Erbitux and Irinotecan (n=218) resulted in an objective response rate of 23% and a median time to progression of 4.1 months. When used as a single agent, Erbitux showed a tumour response rate of 11%, a median duration of response of 4.2 months and a median time to disease progression of 1.5 months (60,61,66). Clinical trials with Erbitux are currently underway in patients with other types of epithelial tumours including those with lung, head and neck or pancreatic cancer (56,62). Despite an improved response rate and increased survival of several months, treatment of colorectal cancer patients with Erbitus is currently very expensive. An estimated cost of treatment with Erbirtux with a loading dose of 450 mg/m2 in the first week and followed by weekly dose of 250 mg/m2 per patient for an eight week duration is around $20,300 (4). In addition, no clear association has so far been found between the expression of EGFR and the response to therapy with anti-EGFR antibody. This is in contrast to the correlation between the expression of HER-2 antigen and response to therapy with anti-HER-2 antibody Herceptin (see below, 55,60). In several experimental studies, coexpression of other growth factor receptors (e.g. IGF-IR) or mutated forms of the EGFR (e.g.

EGFRvIII) has been associated with a poor response to therapy with the EGFR inhibitors (50,67-70). Indeed, in our most recent study, we have found that coexpression of EGFR, EGFRvIII, IGF-IR and HER-2 occurs in a high proportion of colorectal cancer patients (71). Therefore, a major challenge for the routine use of anti-EGFR antibodies in the treatment of cancer patients is the identification of more specific molecular markers that can be used not only in the selection of a more specific subpopulation of EGFR positive cancer patients who benefit from therapy with the anti-EGFR antibodies but also those factors that are responsible for the poor response or the development of resistance to therapy with the anti-EGFR antibodies (45,55,67,68). The results of such investigation may in turn lead to the use of anti-EGFR antibody in combination with other growth inhibitory antibodies for the treatment of cancer patients and such combinational therapy may also help to overcome the low response rates or delay the development of resistance to therapy with anti-EGFR antibody (55,67-72).

2) Anti-HER-2 mAb Herceptin (Trastuzumab, Genentech, USA) for treatment of metastatic breast cancer

HER-2/neu protooncogene is another member of the type I growth factor receptor (i.e. EGFR) subfamily with tyrosine kinase activity. In contrast to the EGFR and other members of the EGFR family (i.e. HER-3 and HER-4), HER-2 is an orphan (i.e. ligand-less) receptor and its activation is mediated via the formation of homodimers or heterodimers with other members of the EGFR family (73,74). Overexpression of HER-2 has been reported in 20-30% of patients with breast cancer and this in turn is often associated with a more aggressive disease and a poor response to the conventional form of therapy, increased risk of metastasis and poorer prognosis (75-78). High levels of HER-2 expression have also been reported in patients with cancer of ovary, colon and rectum, prostate, stomach and bladder (47,75,79,80).

In the past twenty years, several monoclonal antibodies have been developed against the external domain of HER-2 (81,82). Antibody blockade of HER-2 extracellular domain has been shown to inhibit the proliferation of HER-2 overexpressing tumour cell lines by several mechanisms including: down-regulation of HER-2 from cell surface, induction of cell cycle arrest, promotion of apoptosis, inhibition of angiogenesis, and activation of host immune effector cells (e.g. ADCC) (16,83). Of these, Herceptin is the first humanized anti-HER-2 mAb, containing human IgG1 domain, which has been approved by the FDA for the treatment of HER-2 overexpressing metastatic breast cancer patients in 1988 (20,84-86). This antibody when used as both a single agent or in combination with cytotoxic drugs such as paclitaxel, has been shown to improve survival in HER-2 overexpressing metastatic breast cancer patients, in particular those patients whose tumours express the highest level of HER-2 (i.e. 3+ transmembrane expression of HER-2) (86-88).

Although treatment with Herceptin can induce clinical benefit in 25% of HER-2 positive breast cancer patients, cardiac toxicity is seen in a minority of patients treated with Herceptin alone (about 2%) and this was greater in patients who received Herceptin in combination with an anthracycline regimen. In addition, the duration of such response is limited, many patients acquire resistance and the disease progresses

during Herceptin treatment (89). While there is currently no reliable biological marker that can be used for the selection of patients who gain benefit from Herceptin therapy, recent studies suggest that co-expression of other growth factor receptors such as the EGFR and its ligands, EGFRvIII or IGF-IR in HER-2 positive tumours might be important factors in promoting resistance to Herceptin therapy (71,90,91). For example, the IGF-I receptor (IGF-IR) is another important growth factor receptor with tyrosine kinase activity and the activated IGF-IR is mitogenic, plays an important role in tumourigenesis, and protects tumour cells from programmed cell death (35). In addition to tissue overexpression of HER-2, the extracellular domain (ECD) can be shed by protolytic cleavage into the serum of normal individual and cancer patients and high levels of circulatory HER-2 ECD have been associated with resistance to chemotherapy and increased risk of metastasis in patients with breast cancer (92,93). High levels of circulatory HER-2 ECD have also been associated with an increased risk of metastasis in Iranian patients with breast cancer (Doroudchi et al., Submitted). In addition, as Herceptin is directed against HER-2 ECD, high levels of serum HER-2 ECD can bind to Herceptin and reduce the effective dose reaching the HER-2 over-expressing tissues (92). It is therefore possible that high levels of circulating HER-2 in breast cancer patients may be responsible for a poor response, lack of response or the development of resistance to Herceptin. Also a reduction of serum HER-2 ECD level was found recently to be a significant predictor of response to Herceptin-based therapy (95,96).

In several preclinical studies, the response rate of HER-2 overexpressing tumours to Herceptin has been increased by the simultaneous targeting of HER-2 and other growth factor receptors (e.g. EGFR, IGF-IR) or angiogenic factors using a combination of monoclonal antibodies or small molecules tyrosine kinase against such antigens (97-98). In other instances, treatment with a combination of two anti-HER-2 mAbs (e.g. Herceptin and Pertuzumab) that target different regions of HER-2 extracellualr domain have been shown to be more effective in inhibiting the growth of HER-2 overexpressing breast carcinoma cell lines than treatment with a single anti-HER-2 antibody (99). The conjugation of Herceptin to a-emitting particle (actinium-225) has also resulted in an increase in the therapeutic effect of Herceptin against human breast tumour cell lines that express intermediate to high levels of HER-2 (100). The results of future clinical trials, using a combination of these approaches, should help unravel the therapeutic advantages of such strategies in increasing the response rate or decreasing resistance to therapy with anti-HER-2 antibody Herceptin.

3) Anti-VEGF mAb Bevacizumab (Avastin, Genentech, USA) for treatment of metastatic colorectal cancer

Angiogenesis, the formation of new blood vessels, has been shown to be fundamental for the local growth of solid tumours (i.e. beyond the size of a few millimetres), and tumour metastasis (101,102). High levels of angiogenesis have been associated with a poorer prognosis in many patients with a wide range of solid tumours (103). The angiogenic switch in the tumours (i.e. growth from the avascular phase to vascular phase) is believed to be simulated by an increase in the expression of proangiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast factor, interlekin-8 (IL-8), and the EGFR ligands such as EGF and TGFa, or following a

decrease in the expression of anti-angiogenic proteins (e.g. thrombospondin, IFN-a). Of these, the most potent proangiogenic factor is VEGF (also referred to as VEGF-A) that acts as both a potent mitogen and survival factor for endothelial cells. In 1971, the inhibition of angiogenesis was proposed as a new form of cancer therapy by Judah Folkman (104). Several preclinical and clinical studies are currently underway to determine the therapeutic potential of a new generation of angiogenesis inhibitors in the treatment of solid tumours (105).

On 26th of February 2004, the first angiogenesis inhibitor (i.e. bevacizumab, Avastin) was approved by the FDA as the first line treatment for patients with metastatic colorectal cancer in combination with standard cytotoxic drugs [(i.e. irinotecan, 5-flurorouracil (5FU) and leucovorin) (106,107). Bevacizumab is a humanized version of a mouse anti-human VEGF antibody (i.e. mAb A.4.6.1). As mAb A.4.6.1 was found to be a potent inhibitor of angiogenesis and the growth of several human tumour cell lines in athymic nude mice, the humanized version of this antibody was developed using site-directed mutagenesis (108). Bevacizumab binds to all isoforms of VEGF-A but not other members of the VEGF family (i.e. VEGF-B, VEGF-C, VEGF-D). When used in combination with cytotoxic drugs, bevacizumab increased overall survival by 5 months and median progression-free survival by 4 months in colorectal cancer patients compared to patients receiving the cytotoxic drugs. Clinical trials with bevacizumab in combination with cytotoxic drugs, interferon-alpha, EGFR inhibitors, or radiotherapy are currently underway in patients with pancreatic cancer, non-small cell lung cancer, renal cancer, melanoma and ovarian cancer (105,107,109).

Similar to therapy with anti-EGFR antibodies, there are currently no reliable molecular markers for response to therapy and for the selection of patients who benefit from therapy with anti-VEGF antibody (107). In addition, the average cost of treating a 75-kg patient for a two-week period with bevacizumab at 5mg/kg is currently high at about $ 2271 in the USA (5). It is therefore of utmost importance to identify reliable markers for response to therapy with anti-VEGF antibody and other angiogenesis inhibitors in order to prolong the survival and reduce the cost. Studies investigating the potential of bevacizumab for the management of other conditions such as rheumatoid arthritis and psoriasis are currently underway (107). In addition to anti-VEGF antibodies, monoclonal antibodies have also been developed against other angiogenic factors including the VEGF receptors (VEGFR) for use in cancer therapy (105,106). Imclone Systems Inc. has recently reported the development of a fully human recombinant bispecific antibody to VEGFR2 and VEGFR3 (110). This bispecific antibody blocks the interaction between different family members of VEGF and their receptors (i.e. VEGF/VEGFR2, VEGF-C/VEGFR2, and VEGF-C/VEGFR3) and inhibits the VEGF-induced activation of EGFR2 and VEGFR3 and migration of endothelia cells (110). The results of ongoing clinical trials using a combination of bevacizumab and radiotherapy, cytotoxic drugs and/or monoclonal antibodies or small molecules specific for other cell surface antigens (e.g. EGFR, HER-2) should provide new opportunities for improving the response rate to therapy with anti-angiogenesis monoclonal antibodies such as bevacizumab (111).

4) Monoclonal antibodies for treatment of haematological cancers

Since 1997, several monoclonal antibodies against different CD antigens (i.e.CD20, CD33, CD52) have also gained the FDA approval for the treatment of haematological cancer. Of these, rituximab *(Rituxan,, Biogen-IDEC, Cambridge, MA)* was the first human recombinant monoclonal antibody to be approved by the FDA for the treatment of cancer in 1997 (112). This antibody is directed against B-lymphocyte restricted differentiation antigen CD20 which is expressed on the surface of more than 90% of B-cell NHL, on pre-B lymphocytes and mature lymphocytes but not on stem cells, plasma cells and other normal tissues (113). Rituxan , is jointly marketed by two American companies (IDEC Pharmaceutical and Genentech, California) for short-course outpatient treatment of relapsed or refractory CD20 positive low-grade or follicular B-cell non-Hodgkin's lymphoma (NHL). As a single agent, rituximab has been shown to produce a response rate of 50% in patients with relapsed low-grade and follicular NHL (112,114). It is a less toxic alternative to chemotherapy and can induce anti-cancer activity by binding to CD20 positive cells by promoting apoptosis and recruiting immune effector functions (i.e. mediating ADCC) and activating the complement (112-116). Recent studies have also indicated that rituximab may have therapeutic benefit in autoimmune diseases such as dermatomyositis, an inflammatory disease of skin and muscle, and systemic lupus erthematosus (117,118).

Alemtuzumab (Campath 1, Millenium/ILEX, USA) is a humanised monoclonal antibody, which is directed against the CD52 antigen (119). The original rat monoclonal antibody against CD52 was generated in Cambridge in 1980. The humanised version of this antibody, containing human IgG1 (i.e. Campath 1), has been approved by the FDA for the treatment of patients with chronic lymphocytic leukaemia (CLL) in 2001 (119). The CD52 antigen is present on the surface of normal T- lymphocytes, B-lymphocytes, and on a high proportion of lymphoid cancers, but is absent on haemopoietic stem cells. This antibody is able to kill CD52 positive target cells by activating the complement and by inducing ADCC and induces remission in about one third of patients with fludarabine refractory B-CLL (113,114,119). However, as this antibody induces immunosuppression, due to depletion of normal B- and T– lymphocytes, there is often an increased risk of opportunistic infections in patients treated with this antibody (120,121). Monoclonal antibodies Epratuzumab and Apolizumab, which are directed against two different antigens CD22 and HLD-DR, respectively are also under clinical investigation for use in NHL (115,122,123). Further clinical trials in patients with NHL with a combination Rituxan, Epratuzumab and Apolizumab should enable the full exploitation of such strategies in the management of haematological cancer (22,115).

In several studies monoclonal antibodies have been attached to radioisotopes (e.g. Iodine 131, or yettrium 90) or toxins in order to deliver lethal doses of such molecules to tumours cell (13,124-126). The success of radioimmunotherapy depends on several factors, including the choice of the target antigens, antibody molecules (guided missiles) and the therapeutic radioisotopes (127,128). Currently, two radiolabelled monoclonal antibodies directed against the CD20 antigen have gained FDA approval for treatment of NHL. Of these, *Ibritumomab tiuxetan (Zevalin, IDEC)* is an yttrium-90 labelled anti-CD20 antibody (IDEC Pharmaceuticals, USA) and *tosiumomab* is

iodine-131 labelled anti-CD20 antibody (Bexxar, Corixa Corp). To facilitate their rapid clearance and to reduce the prolonged total body irradiation, both radiolabelled antibodies are of mouse origin (126,127,129). The advantage of radioimmunotherapy over unconjugated antibody in cancer therapy is that the former has a longer path which allows further deeper penetration and kills tumour cells (i.e. both antigen positive and antigen negative tumours) without direct binding of antibody to such tumours. Therefore, patients who are not responsive to or those who relapse following chemotherapy or treatment with unconjugated antibodies (e.g. rituximab) may be suitable candidates for radioimmunotherapeutical approaches (114,126,127).

Gemtuzumab ozogamicin (Mylotarg, Wyeth-Ayerst) is the first toxin-linked antibody to be approved for the treatment of human cancer. It is a humanised IgG4 anti-CD33 monoclonal antibody, which is attached to the cytotoxic drug calicheamicin (130). It has been approved by the FDA, as a single agent for the treatment of patients over 60 years of age with CD33-positive acute myeloid leukaemia (AML) in the first relapse who are not suitable for therapy with conventional cytotoxic drugs (113,130,131). AML is the most common type of acute leukaemia in adults and is characterised by accumulation and proliferation of myeloblasts in the bone marrow. The CD33 antigen is not expressed on stem cells or nonhaemopoietic normal cells but has been shown to be expressed on myeloblasts in 80-90% of patients with acute myeloid leukaemia. The binding of this immunotoxin to CD33 antigen on AML cells results in the internalisation of the immunotoxin, dissociation of calicheamicin and its transport into the nucleus and degradation of the DNA leads ultimately to cell death. Clinical studies with gemtuzumab ozogamicin (GO), as a single agent in patients with CD33-positive AML, produced a complete response rate of 15-20% (130). The results of in vitro and in vivo studies with GO have indicated that this immunotoxin may also have potential in the treatment of patients with CD33 positive acute lymohoblastic lekaemias (132).

5) Monoclonal antibodies for management of other human diseases

In addition to their use in the treatment of human cancers, monoclonal antibody based products have also been used for the management of non-oncological conditions such as prevention of grafts rejections, cardiovascular conditions, allergy, and the prevention and treatment of infectious agents and autoimmune diseases (12,27,133-135, Table 1). For example, the mouse monoclonal antibody orthoclone OK3 (muromonab, Johnson and Johnson/Ortho Biotec), which is directed against the CD3 antigen, was approved by the FDA for prevention of graft rejection following transplantation in 1986. A chimeric Fab form of mouse anti-glycoprotein IIb/IIa antigen (i.e. Abcimimab/ReoPro, Centocor, USA) was approved by the FDA for prevention of platelet aggregation during surgery, angioplasty and other cardiovascular conditions in 1994. Both daclizumab (Zenpax, Hoff-LaRoche), a humanized anti-CD25 mAb, and basiliximab (Simulect, Norvartis), a chimeric anti-CD25 antibody, gained FDA approval for prevention of graft rejections following renal and liver transplantation in 1997 and 1998, respectively. A chimeric monoclonal antibody directed against TNFa (i.e, infliximab, Remicade) was also approved for the treatment of Crohn disease in 1998 and rheumatoid arthritis in 1999. The first fully human monoclonal antibody adalimumab (Humira, Abbott Laboratories) that was developed by phage display

technology against human TNF alpha gained FDA approval for the treatment of rheumatoid arthritis in 2002. Finally, a humanized anti-IgE monoclonal antibody omazlizumab (Xolair, Genentech/Norvartis) was approved for the treatment of asthmatic patients in 2003 (136). The full details of antibody-based products that have been approved by the FDA for clinical use can be found by visiting their website: http://www.fda.gov/.

CURRENT CHALLENGES AND FUTURE CONSIDERATIONS

Since the discovery of hybridoma technology and subsequent advances in genetic engineering, monoclonal antibodies have been generated against a wide range of human tumour antigens. Due to encouraging clinical results (i.e. prolonged survival with acceptable levels of toxicity), several antibody-based products have now been approved for the clinical management of human diseases (Table 1). The market value of therapeutic antibody products for use in oncology and autoimmune disease is estimated to reach $16.7 billion by 2008 (135). However, there are currently several challenges associated with the routine use of antibody-based products for therapeutic application. The chronic use of certain antibodies for treatment of patients (e.g. cetuximab, bevacizumab, omalizumab) is currently very expensive (4,5,135). To facilitate the routine use of such drugs worldwide, the costs of drugs should be reduced substantially. The use of conjugated forms of antibody (i.e. attached to toxins, radionuclides, enzymes) should reduce the need for frequent administration of the antibody and the subsequent cost. In addition, there are currently no reliable markers for response to therapy with some of the antibodies (e.g. anti-EGFR and anti-VEGF antibodies) (137). It is imperative to identify molecular markers of prognostic significance and predictive value for response to therapy with monoclonal antibody-based products. In addition, while antibody-based products are very useful in the treatment of cancer patients by increasing overall survival, the duration of response in some patients can be short (less than 4 month) due to acquired resistance to antibody treatment. It is necessary to identify those factors/markers that are responsible for the low response or the development of a phenotype resistance to therapy with monoclonal antibody based products (137-140). The identification of cell surface antigens of biological and clinical importance and simultaneous targeting of such antigens with a combination of monoclonal antibodies (i.e. poly monoclonal antibodies) and other therapeutic strategies may increase response rate and improve overall survival rates in the great majority of such patients. The recent advances in monoclonal antibody technology, in the ability to generate various forms of antigen specific antibody (e.g. human antibody, immunotoxin, radiolabelled antibodies, bispsecific antibodies), together with our better understanding of the biology of cancer, tumour immunology and genetic engineering have generated a new wave of excitement among scientists in both academia and industry regarding therapeutic use of monoclonal antibody based products for the management of a wide range of human diseases and in particular cancers (13,23,27,30,141-143). The results of ongoing clinical trials with hundreds of monoclonal antibodies, together with determination of molecular signature of a patient tumour, will illustrate the full potential of monoclonal antibody-based products as magic bullets in the treatment of human cancer.

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