MONOCLONAL ANTIBODIES FOR CANCER THERAPY

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Abstract

A large number of monoclonal antibodies (mAbs) are currently under investigation for the treatment of malignant diseases. To date, a significant amount of experimental and clinical research had been conducted which has furthered our understanding of the potential clinical applications of mAbs. At present, five monoclonal antibodies have been approved by the US Food and Drug Administration (FDA) for cancer therapy. Further advances will occur in key areas such as identification of optimal treatment regimens, and the development of new mAb therapeutic agents that produce the best clinical outcomes with the fewest possible side effects.

Introduction

The development of monoclonal antibody technology by Köhler and Milstein 27 years ago has provided the potential for the diagnosis and treatment of a range of disorders[1]. However, until recently, little progress had been achieved in the area of immunotherapy of human disease, since induction of strong human anti-mouse antibody (HAMA) responses in patients to murine mAbs has limited the use of murine mAbs to very few injections. Advances in molecular biology and protein engineering technologies for creating and producing better-tolerated antibodies have led to a renewed interest in the development of mAbs as therapeutic products. Numerous clinical trials have shown that antibodies can deliver therapeutic activity without long-term toxicity. There are now five monoclonal antibodies approved by the FDA for the treatment of cancer, and a growing number of mAbs are being developed (Table 1). This turnaround has resulted from two major developments:

■ recognition of the critical importance of the choice of appropriate tumour-associated antigens as targets, and the development of genetic engineering techniques that have allowed production of human/rodent chimeric and humanised antibodies. Antibodies have several potential advantages over traditional therapies, including fewer unwanted side effects as a result of high specificity for the disease target; and

■ an ability to deliver various payloads, including drugs, radiation and toxins, to specific disease sites, as well as an ability to elicit an immune response, principally through Fc function.

Tumour antigens recognised by monoclonal antibodies

Significant progress has been made in the last few decades in the detection and classification of defined tumour-associated antigens (TAA) recognised by monoclonal antibodies. Most of these antigens are expressed not only by malignant cells, but also by at least one subset of normal adult cells. However, tumours often express these antigens at higher levels than normal tissues (often up to 100-fold). In addition, accessibility of antigen on tumour to circulating mAbs may be greater than on normal tissue. Therefore, these antigens are not tumour-specific, but often referred to as tumour-associated.

Identification and characterisation of TAA reactive to mAbs involves comparative serological, immunohistochemical and proteomic-based approaches to analysis of normal tissues and tumours[2]. It is often the case that molecular nature and function of the tumour-associated antigens (especially cell surface antigens in solid tumours) are poorly defined. They represent a diverse group of molecules, including proteins, glycoproteins and glycolipids. The expression of TAA is often heterogeneous, and loss of expression may be observed in anaplastic transformation and as a result of immune escape.

Importantly, tumour-associated antigens recognised by antibodies are potential targets for antigen-specific cancer immunotherapy, they can be used for diagnostic purposes, and often serve as prognostic markers in cancer. Different categories of tumour antigens have been identified in a variety of malignancies, and include:

■ hematopoietic differentiation antigens – glycoproteins usually associated with cluster differentiation (CD) groupings (eg CD5, CD19, CD20, CD33, CD45, CD52);

■ cell surface differentiation antigens, including glycoproteins [such as carcinoembryonic antigen (CEA), sialyl Tn antigen (TAG-72), polymorphic epithelial mucin (PEM), epithelial cell adhesion molecule (Ep-CAM), A33, G250, prostate-specific membrane antigen (PSMA) and prostate-specific antigen (PSA)], glycolipids (such as gangliosides, eg GD2, GD3, GM2) and carbohydrates (such as blood group-related antigens, eg Le^y and Le^b);

■ growth factor receptors, including epidermal growth factor receptor (EGFR) and its mutant form EGFRvIII, HER-2/neu and IL-2 receptor; and

■ angiogenesis and stromal antigens, including fibroblast activation protein (FAP), vascular endothelial growth factor receptor (VEGFR), tenascin and integrin $\alpha_V\beta_3$.

Chimerisation and humanisation of antibodies

The development of genetic engineering has been central to the clinical use of antibodies. This technology allowed construction of chimeric mAbs (constructed from variable regions derived from murine Ab, and constant regions derived from human Ab) and humanised antibodies (mAbs constructed with only the complementarity-determining regions, or CDRs, derived from a mouse, and the remainder of the variable and constant regions derived from a human source). In addition, primatised mAbs, which are constructed from variable regions derived from Cynomolgus macaques and constant regions derived from a human source, have also been described. Production of fully human antibodies and antibody fragments in vitro has been made possible with the advent of phage display

technology, originally described in 1985[3]. Phage display allows selection of proteins, such as antibodies and antibody fragments, with specific or novel functions. Most antibody display libraries are constructed by cloning the antibody gene's repertoire either from immune or naïve sources into phage display vectors, resulting in antibody fragments being expressed as fusions with coat proteins of bacteriophage, while the DNA encoding the fusion resides within the virion. Important advances in phage display technology include development of libraries with synthetic complementarity-determining regions, and affinity maturation of conventional antibodies. Production of fully human mAb in immunised transgenic mice has also emerged as an important technology that is currently in early phase clinical trials. Engineered bispecific antibodies (bsAbs) carry dual specificity because of their two binding regions – one specific for tumour-associated antigen, and the other to immunological anti-tumour effector cells. The chimerisation and humanisation technologies have had a key impact upon the reduction of immunogenicity of the mAbs, their capacity to recruit cytotoxic cells and complement, and stability in circulation. Despite these advances, chimeric and humanised mAb have the potential to stimulate HACA (human anti-chimeric antibody) or HAHA (human anti-human antibody) immune responses directed to the variable regions of the mAb, although the incidence of such responses is generally low[4].

Mechanisms of action of unconjugated antibodies

Unconjugated mAbs may induce therapeutic effect by a variety of different mechanisms, including blocking receptor–ligand interactions; initiating cellular ablation via recruitment of effector cells and/or complement; and cross-linking target molecules and delivering transmembrane signals that control cell cycle progression and/or induce apoptosis/cell death.

Receptor binding and signalling inhibition

The binding of mAb to growth factor receptors expressed on tumour cells may result in blocking of ligand binding to the receptor, and subsequent inhibition of receptor protein tyrosine-kinase phosphorylation. Alternatively, receptor binding by mAb may prevent receptor homodimerisation or heterodimerisation, and result in inhibition of downstream signalling events. Important examples of this mechanism of action of mAbs include ErbB2 (eg Herceptin), EGFR (eg C225), and VEGFR (eg IMC-IC11). The binding of mAb against these receptors induces measurable changes in the phosphorylation status of the receptor and key signalling molecules, and inhibition of cellular functions that can be linked to signalling events including apoptosis, therapy resistance, and angiogenesis[5]. Herceptin has been approved for the treatment of patients with advanced breast cancer, and a number of antibodies against the EGFR[5] are being developed (Table 1). Anti-angiogenesis has been proposed as a potential strategy for the treatment of cancer[6-8], and mAbs are also being studied in clinical trials to prevent the interaction between VEGF and its receptors[9-12]. The cross linking of tumour cell surface receptors and induction of signalling inhibition by mAb has also been reported with other antigen systems, including CD20.

Tumour cell killing via Fc function

A key component of mAb-based therapy of cancer is dependent upon Fc function. This mechanism of cell killing may be through activation of the classical pathway of complement; the recruitment of cellular effectors against target tumour cells; and the induction of apoptosis and phagocytosis. The relative contribution of such mAb-effector mechanisms to cancer cell killing in vivo is difficult to assess, however in experimental settings the Fc regions of antibodies have been shown to make major contributions to mAb biologic activity[13]. In mice lacking activation Fc receptors FcγRI and FcγRII, the anti-tumour effects of trastuzumab (Herceptin) and rituximab (Rituxan) were reduced, while mice deficient in inhibitory FcγRIII receptors showed inhibition of tumour growth and enhanced antibody-dependent cellular cytotoxicity (ADCC). There are a number of examples of mAb with highly potent Fc function that have demonstrated biologic efficacy in the clinic, including mAb against GD2, GD3 and Lewis[y] antigens[14,15]. Campath1H, a humanised version of rat anti-CD52 mAb, which is licensed for treatment of refractory chronic lymphatic leukaemia in the USA, had been shown to be a potent recruiter of effector cells in vitro, and is thought to operate via this mechanism in cancer patients[16,17].

Anti-idiotype network cascade

Monoclonal antibodies can also serve as immunogens for cancer vaccines through the anti-idiotype-network cascade. Anti-idiotypic antibodies bind to the antigen-binding sites of antibodies, thus effectively mimicking the three-dimensional structures of antigens. Hence, anti-idiotypic antibodies can serve as surrogate antigens for active specific immunotherapy. Numerous studies in animal models have demonstrated the efficacy of the anti-idiotypic antibodies as vaccines for triggering specific anti-tumour responses, and clinical trials are underway in a number of tumours (eg ovarian, melanoma) with this approach.

Recombinant antibodies as targeting systems

Conjugated monoclonal antibodies

Some antibodies, which target neoplastic cells, do not induce cell death by themselves. Instead, they deliver natural toxins, radioisotopes, chemotherapy drugs or cytokines that require cell entry or close proximity to tumour cells to be effective[18-20]. Conjugated mAbs and antibody fragments have been developed for solid tumours and hematological malignancies, and intensively characterised for their biological activity in vitro, as well as in in vivo animal models.

Radioisotopes can be chemically linked to anti-tumour mAbs and administered to patients to deliver radiation selectively to tumour sites. Radioimmunoconjugates are constructed either by covalently binding the radioisotope directly to the antibody, or by crosslinking through a chelating agent or chemical linker. The cytotoxic efficacy of a given radioimmunoconjugate depends on the kinetics of antibody localisation and retention of the radionuclide. Lymphoma cells are particularly sensitive to radiation. The anti-CD20 mAb radiolabelled with yttrium ^{90}Y (Zevalin) has been shown to increase delivery of radiation to neoplastic versus normal tissue by nearly 1000-fold[21,22], and is now approved for treatment of B-cell lymphomas. Another anti-CD20 mAb conjugated to ^{131}I (Bexxar) has completed phase III trials and is awaiting FDA approval[23]. There are no clinically approved immunoconjugates for the treatment of solid tumours as yet, although numerous trials are underway using this approach. The selection of appropriate isotopes, eg alpha versus beta emitters, is a key component of the optimisation of this approach.

Biological toxins, such as ricin or diphtheria toxins, and cytotoxic drugs, such as doxorubicin and calicheamicin, can also be attached directly to anti-tumour mAbs. Discovery of the in vitro and in vivo potential of recombinant immunotoxins has lead to their preclinical development, and to the initiation of clinical trial protocols in patients with cancer refractory to

traditional ways of treatment. The linking of calicheamicin to an anti-CD33 mAb has achieved success in the treatment of elderly patients with AML, and Mylotarg has been approved for this indication. Another strategy for mAb-mediated drug delivery involves a two-step approach known as ADEPT, or antibody directed enzyme-prodrug therapy[24], where mAbs are used to localise enzymes to tumour cell surface antigens. Once the mAb-enzyme conjugate binds neoplastic cells and excess conjugate is cleared from circulation, anti-tumour prodrugs are administered and are converted to active drugs by the targeted enzyme. This type of therapy approach is currently under investigation in clinical trials.

Engineered antibody fragments

Smaller forms of antibodies have been made in an attempt to improve penetrability into avascular tumours. Engineered antibody fragments such as Fv (non-covalently associated heavy and light chain variable domains), single chain Fv (scFv;

Table 1: Selected examples of monoclonal antibodies currently in clinical use and development

Antigen Target	Cancer	Antibody	Antibody Type	Company / Institute	Trial Status
CD33	AML	Zamyl Mylotarg (Gemtuzumab ozogamicin)	Humanised Humanised	Protein Design Labs Wyeth-Ayerst	Phase III FDA approval 2000
CD20	NHL	Rituxan (Rituximab) Zevalin (Ibritumomab Tiuxetan) Bexxar (Tostitumomab)	Chimeric Murine, radiolabelled (^{90}Y) Murine, radiolabelled (^{131}I)	IDEC Pharm/Genetech IDEC-Pharm/Schering AG Corixa Corporation	FDA approval 1997 FDA approval Feb 2002 Phase III
CD22	NHL	LymphoCide (Epratuzumab) LymphoCide Y-90	Humanised Humanised, radiolabelled (^{90}Y)	Immunomedics, Inc Immunomedics, Inc	Phase III Phase I
HLA-DR	NHL	Smart1D10 (Remitogen)	Humanised	Protein Design Labs	Phase II
CD52	B-CLL	Campath1H (Alemtuzumab)	Humanised	Millenium Pharm/ ILEX Oncology Inc	FDA approval 2001
HER2/neu (ErbB-2)	Breast, Colon, NSCLC	Herceptin (Trastuzumab)	Humanised	Genentech	FDA approval 1998 (breast cancer)
CEA	Solid Tumours	CEA-cide Y-90	Humanised, radiolabelled (^{90}Y)	Immunomedics, Inc	Phase I/II
EpCam	Colorectal	Panorex	Murine	Glaxo-Smith Kline	Approved in Germany in 1995
EGFR	Head and Neck, NSCLC, Colon	IMC-C225 (Erbitux) ABX-EGF h-R3	Chimeric Human Humanised	Imclone Sys Abgenix CMI	Phase I/II/III Phase I/II Phase I/II
VEGF	Solid Tumours	Bevacizumab	Humanised	Genentech	Phase II/III
VEGFR-2	Solid Tumours	IMC-1C11	Chimeric	Imclone Sys	Phase II and III
A33	Colorectal	huA33	Humanised	LICR	Phase I/II
Lewis^y	Solid Tumours	SGN-15 hu3S193	Chimeric Humanised	Seattle Genetics LICR	Phase I/II Phase I
GD3	Melanoma	KW-2871	Chimeric	Kyowa Hakko Kogyo / LICR	Phase I
G250/MN	Renal, Biliary	cG250	Chimeric	LICR / Wilex	Phase I/II
PSMA	Prostate	MLN591	Humanised	Millenium Pharm	Phase I/II
CTLA-4	Prostate, Melanoma	MDX-010	Human	Medarex	Phase I/II

Abbreviations: AML – acute myeloid leukaemia; B-CLL – B-cell chronic lymphocytic leukaemia; CEA – carcinoembryonic antigen; CMI – Centre for Molecular Immunology, Cuba; CTLA-4 – cytotoxic T lymphocyte-associated antigen 4; EGFR – epidermal growth factor receptor; FDA – US Food and Drug Administration; HLA – human leukocyte antigen; LICR – Ludwig Institute for Cancer Research; NHL – Non-Hodgkin's lymphoma; NSCLC – non-small cell lung carcinoma; PSMA – prostate-specific membrane antigen; VEGFR-2 – vascular endothelial cell growth factor receptor-2.

heavy and light chain variable domains covalently linked by short peptide linkers), Fab (non-covalently associated heavy and light chain antigen-binding domains) and diabodies/triabodies (linked recombinant scFv fragments) maintain the parental antibodies' specificity and penetrate tumours more easily[25]. They are often used as vehicles to deliver toxins and drug conjugates. One drawback, however, is that these smaller molecules tend to have short half-lives, and are rapidly eliminated from circulation. Some of the small therapeutic Ab fragments have been modified by linking to various agents, such as polyethylene glycol and albumin, to block reabsorbtion in kidneys[26-28].

Bispecific antibodies

Bispecific antibodies (bsAbs), F(ab)'$_2$ fragments and diabodies can override the natural specificity of an effector cell for its target, and re-direct the lysis towards a cell population it would otherwise ignore. Immunological effector cells that can potentially be recruited by bispecific agents include granulocytes, macrophages, natural killer and T cells. For example, cytotoxic T lymphocytes can be re-directed against a tumour by bsAbs that have specificities for a constant component of the T cell antigen receptor complex (TCR) such as CD3, and a molecule expressed on the neoplastic cells surface. This technique was pioneered in the 1980s and confirmed in many recent animal studies and in clinical trials[29-35]. The combining of tumour cell specificity with cytokine effector mechanisms (eg IL-2, TNF) is another potential approach that has shown encouraging preclinical results. Recent clinical trials of bsAbs using both TCR and FcRs as triggering molecules have also been reported.

Monoclonal antibodies in combination with other treatment modalities

The combination of monoclonal antibody therapy with other treatments, particularly chemotherapy and radiotherapy, has been shown in in vivo models and in clinical trials to have potential additive or synergistic effects. The mechanisms of this effect are complex, and related to the interactions between conventional therapy mechanisms of action, and the effect of Fc function or signalling inhibition on tumour cell proliferation and repair mechanisms. The majority of data exists from combining mAb-based therapy with chemotherapy[5,36-41]. The combining of radiotherapy with EGFR-targeted mAbs, and chemotherapy with radioimmunotherapy, has also been shown in in vivo models – and in early phase clinical trials – to have synergistic effects. This approach of combination therapy will have increasing importance in the development of mAbs as therapeutics, particularly in solid tumours.

Summary and future directions

It is now apparent that the choice of target antigen, immunogenicity of antibodies, extent of antibody half-life, potential of antibodies to recruit immune effectors, decisions on conjugation partners and mAbs manufacturing processes are critical in the development of monoclonal antibodies for cancer therapy. Advances in hybridoma technology, and more recently developments in antibody engineering, have been essential for progress in targeted immunotherapies. Optimisation of monoclonal antibody therapies will be directed towards design of better antibodies and immunoconjugtes, enhancement of tumour-specific cytotoxicity, and the development of more effective combination therapy approaches. Clearly, the full potential of mAb-based immunotherapy is yet to be reached.

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