Therapeutic monoclonal antibodies in oncology

Adam P Levene MB BSc¹ Guminder Singh BSc² Carlo Palmieri PhD MRCP³

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The mainstay of systemic therapy for solid and haematological malignancies in the 20th century was chemotherapy. This approach has the drawbacks of toxicity to normal tissue, drug resistance and lack of efficacy. The demand for more effective and tolerable treatments has led to the development of novel therapeutic agents that specifically target the malignant cell.

The hallmarks of malignant disease are self sufficiency in growth signals, insensitivity to growth inhibition, evasion of apoptosis, acquisition of limitless replicative potential, induction of angiogenesis and invasion and metastasis.¹ All these processes are ultimately due to genetic defects which subsequently lead to the abrogation of normal cellular processes. Key to the development of targeted therapies is the ability to define the growth factors, transcription factors or receptors that phenotypically distinguish, in some way, the tumour from its normal counterpart. One class of novel agents that can specifically target and disrupt molecular pathways underlying tumorigenesis are the therapeutic monoclonal antibodies.

Monoclonal antibodies are produced by a single clone of B-cells, and are monospecific and homogeneous. Since the original report on production of such antibodies, by Kohler and Milstein² in 1975, a vast number have become available. Early developments in the cancer sphere were made in the academic sector, with the identification of tumour-associated antigens and immunization with tumours to produce novel monoclonal antibodies. Initially, the antibodies were created by fusing B cells from immunized mice with human lymphoma cells, thus creating murine monoclonal antibodies. A big disadvantage of these preparations was that human recipients developed antimouse antibodies, which led to allergic reactions and reduced the efficacy.³ However, application of recombinant DNA technology led to the development first of chimeric antibodies, then of partially humanized antibodies,^{4,5} and ultimately of fully humanized antibodies.⁶ Box 1 outlines the features of the different types in this progression. Radiochemistry and antibody engineering research were initially driven by the

Box 1 Types of monoclonal antibody that have been developed

Type of antibody

Chimeric

Chimeric antibodies are 65–90% human and consist of the constant or effector domain of the human antibody molecule combined with the murine variable regions (which bring about antigen recognition) by transgenic fusion of the immunoglobulin gene

Partially humanized

Partially humanized antibodies are about 95% human and consist of the complementarity determining regions of the murine antibody (which determine antibody specificity) and a limited number of structural aminoacids grafted onto a CDR-depleted human antibody backbone by recombinant technology

Deimmunized

Deimmunized antibodies have the immunogenic epitopes in the murine variable domains replaced with benign aminoacid sequences, resulting in a deimmunized variable domain. The deimmunized variable domains are linked genetically to human antibody constant domains

Primatized

Primatized antibodies have a chimeric antibody structure of human and monkey that is close to an exact copy of a human antibody

Fully humanized

Fully human antibodies have been developed by use of genetically engineered transgenic mice and advances in the generation of synthetic human antibody libraries

academic sector, followed by start-up biotech companies and subsequently larger pharmaceutical conglomerates. Whilst many of the antibodies were tested in the clinical setting—either unconjugated or more commonly as radio-immunoconjugates—very few (anti-CD20 being the first major exception) went on to be commercially developed.

At present, therapeutic monoclonal antibodies are being used in haematological and solid malignancies including non-Hodgkin's lymphoma, breast cancer and colorectal cancer. The mechanism of their antitumour effect is not precisely known but is thought to include complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity and blocking or steric hindrance of the function of the target antigen. This review focuses on current use in

¹Department of Cardiology, Leeds General Infirmary, Leeds LS1 3EX; ²Guy's, King's & St Thomas' School of Medicine, Guy's Campus, London SE1 9RT;

³Department of Medical Oncology, Charing Cross Hospital, London W6 8RF, UK

Correspondence to: Dr Carlo Palmieri, Department of Medical Oncology, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, UK

E-mail: c.palmieri@imperial.ac.uk

oncology but Table 1 lists some of the antibodies in clinical development.

RITUXIMAB

Low-grade non-Hodgkin's lymphoma

Rituximab targets CD20, a cell surface protein present on healthy B-lymphocytes and on 95% of B-cell lymphomas. It was the first therapeutic antitumour monoclonal antibody to be licensed in the USA (in 1997), the indication being treatment of recurrent or refractory low-grade B-cell lymphoma.

In the pivotal phase II trial, heavily pretreated patients with relapsed low-grade non-Hodgkin's lymphoma (NHL) were given single agent rituximab intravenously once a week for 4 weeks; 48% of patients responded, with a median response of 12 months.⁷ When previous responders were re-treated, 40% had a second response (11% complete response, 30% partial response) with a median

duration of response of 16.3 months (range 3.7 to 25.1).⁸ The antibody has also been found safe and effective when combined with standard-dose chemotherapy as first-line treatment: in a phase III trial in CD20 positive follicular NHL the response rate was 81% in the combination group (rituximab plus cyclophosphamide, vincristine and prednisolone) compared with 57% in the patients given chemotherapy alone ($P < 0.001$). Also, the median time to treatment failure was longer in the combination group—27 months versus 7 months, $P < 0.001$. This benefit was not associated with a significant increase in toxic effects and represents an advance in the treatment of patients with follicular NHL.⁹

High-grade non-Hodgkin's lymphoma

The activity of rituximab in high-grade NHL was revealed in a randomized phase III study of cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP)

Table 1 A summary of monoclonal antibodies used in cancer medicine or in development

Cancer	Target antigen	Monoclonal antibody (Ref)	Clinical status
Non-Hodgkin lymphoma	CD20	Rituximab (7–20)	In clinical use
		⁹⁰ Y-ibritumomab tiuxetan	In clinical use
		¹³¹ I-tositumomab	In clinical use
	CD22	Epratuzumab (56)	In clinical trials
		⁹⁰ Y-epratuzumab	In clinical trials
	HLA-DR	Remitogen (57)	In clinical trials
Acute myeloid leukaemia	CD33	Gemtuzumab ozogamicin (52–55)	In clinical use
B-cell chronic lymphocytic leukaemia	CD52	Alemtuzumab (58)	In clinical use
Colorectal cancer	Epidermal growth factor receptor (also in trials in head and neck and non-small-cell lung carcinoma)	Cetuximab (26–35)	In clinical use
		h-R3 (59)	In clinical trials
		ABX-EGF (60)	In clinical trials
	A33	huA33 (61)	In clinical trials
Renal cell carcinoma	G250	cG250 (62)	In clinical trials
Melanoma	GD3	KW-2189 (63)	In clinical trials
Breast cancer	HER2 (C-erb2)	Trastuzumab (36–45)	In clinical use
Prostate cancer	Prostate-specific membrane antigen	HuJ591 (64)	In clinical trials
Small-cell lung cancer	CD56	BB-10901 (65)	In clinical trials
Solid tumours	Vascular endothelial growth factor (VEGF)	Bevacizumab (47–51)	In clinical use for metastatic colorectal cancer and in clinical trials in other tumour types
	VEGF2	IMC-1C11 (66)	In clinical trials
Epithelial cancers	Carcinoembryonic antigen	⁹⁰ Y-CEA-cide (67)	In clinical trials
	Lewis Y antigen	Hu3S193 (68)	In clinical trials

chemotherapy with or without rituximab in 399 previously untreated patients aged 60–80 years with stage II–IV diffuse large-B-cell lymphoma.¹⁰ At median follow-up of 3 years, the combination group had a higher event-free survival (53% versus 35%, $P=0.00008$) and better overall survival (62% versus 51%, $P=0.008$), without an increase in toxicities.¹¹ The clinical benefit in the combination arm seemed to depend on tumour expression of BCL2.¹²

The Mabthera International Trial (MinT study) was a phase III study which investigated patients aged 18–60 years and compared CHOP or similar chemotherapy plus rituximab with chemotherapy alone as first-line treatment for good-prognosis diffuse large-B-cell lymphoma. The study was closed early when an interim analysis showed a significantly longer time to treatment failure in the combination group. If the findings are borne out by further work, this combined strategy could mark a step forward in management of younger patients with this tumour.

Why should rituximab plus chemotherapy give better results than chemotherapy alone? One suggestion, emerging from *in vitro* studies, is that monoclonal antibodies sensitize lymphoma cells to the effects of chemotherapy.^{13,14}

Chronic lymphocytic leukaemia and small-cell lymphocytic leukaemia

Response rates of 12% were initially reported in patients with previously treated small-cell lymphocytic leukaemia who were given rituximab;¹⁵ subsequently, however, better results were achieved with more frequent use of rituximab at standard doses and in dose-escalation studies.^{16,17} As first-line treatment in a phase II trial rituximab monotherapy gave a response rate of 51%.¹⁸ In a further phase II trial by the Cancer and Leukemia Group B, rituximab plus fludarabine-based chemotherapy in previously untreated patients gave a higher response rate and more complete remissions than chemotherapy alone or the sequential use of rituximab after chemotherapy (47% versus 28%, $P=0.0049$).¹⁹

Rituximab is well tolerated. The most common side-effects are infusion related and include fever, rigors, rash, bronchospasm and hypotension. Myelosuppression has also been reported.

ANTI-CD20 MONOCLONAL ANTIBODIES CONJUGATED TO RADIOISOTOPES

In the hope of improving their therapeutic efficacy, CD20 antibodies have been combined with yttrium and iodine as ⁹⁰Y-ibritumomab tiuxetan and ¹³¹I-tositumomab. These molecules are administered as a single course treatment with the aim of eradicating not only the antibody-coated cells but also, by radiation, the antigen-negative cells in close proximity.

⁹⁰Y-ibritumomab tiuxetan

⁹⁰Y-ibritumomab tiuxetan consists of an anti-CD20 antibody that is covalently linked to MD-diethylenetriamine penta-acetic acid, allowing the binding of yttrium 90, a pure beta emitter. Multicentre studies of ⁹⁰Y-ibritumomab tiuxetan for relapsed low-grade or intermediate-grade NHL have shown a response rate of 67% (26% complete response), and median time to progression was more than 12.9 months.²⁰ In follicular NHL that was refractory to rituximab treatment, 74% of patients responded (15% complete response), with an estimated time to progression of 6.8 months.²¹ In a phase III trial that compared ⁹⁰Y-ibritumomab tiuxetan with rituximab in relapsed or refractory low-grade NHL, or transformed CD20-positive NHL with less than 25% bone-marrow involvement, patients responded significantly better to ⁹⁰Y-ibritumomab tiuxetan (80% versus 56%, $P=0.002$; complete response 30% versus 16%, $P=0.04$).²²

¹³¹I-tositumomab

¹³¹I-tositumomab consists of an anti-CD20 antibody conjugated with iodine-131, a gamma emitter. Of patients with refractory low-grade or transformed low-grade lymphoma treated with ¹³¹I-tositumomab, 65% responded (20% complete response) with a median response duration of 6.5 months—results that compared favourably with patients' responses to their last chemotherapy.²³ In untreated low-grade lymphoma a response rate of 100% (56% complete response) has been reported with ¹³¹I-tositumomab.²⁴ So far there has been no randomized trial directly comparing ⁹⁰Y-ibritumomab tiuxetan with ¹³¹I-tositumomab.

The adverse effects associated with these radioisotopes include infusion-related reactions and myelosuppression with resultant neutropenic sepsis. Myelodysplasia and acute leukaemia have also been reported in treated patients, but interpretation is complicated by the fact that previous treatment has usually included alkylating agents.²⁵

CETUXIMAB

Epidermal growth factor receptor (EGFR or HER1) is a tyrosine-kinase receptor and a member of the EGFR family. It is overexpressed in epithelial tumours such as lung, breast and colon, and its overexpression is associated with a poor prognosis.^{26–28} Cetuximab is a chimeric monoclonal antibody that binds to EGFR, blocking ligand binding and thus preventing receptor activation and downstream signalling.

Phase II trials have shown evidence of the activity of cetuximab, alone or in combination with irinotecan, in patients with EGFR-positive irinotecan-refractory metastatic colorectal cancer.^{29,31} A randomized controlled trial

confirmed these results and suggested that re-treatment with cetuximab and irinotecan gave better results than cetuximab alone. The combination therapy group had a higher response rate (22.9% versus 10.8%, $P=0.007$) and longer median time to progression (4.1 versus 1.5 months, $P<0.001$). Median overall survival was also somewhat higher (8.6 months versus 6.9 months, $P=0.48$).³¹ On this evidence cetuximab has been licensed for use in the treatment of EGFR-positive metastatic colorectal cancer in combination with irinotecan for patients who are refractory to irinotecan. Studies on the use of cetuximab in head and neck, pancreatic and non-small-cell lung cancer have yielded promising results.^{32–35}

The main adverse effect of cetuximab is an acne-like rash that occurs in up to 75% of patients. Development of this rash is a predictor of increased survival.³⁵

TRASTUZUMAB

Trastuzumab is a humanized monoclonal antibody that targets HER2 (also known as C-erbB2), another member of the EGFR family. It is overexpressed in 30% of breast cancers and this overexpression in early-stage breast cancer is associated with adverse prognostic factors such as higher histological tumour grade,³⁶ axillary lymph node involvement,³⁷ increased mitotic rate,³⁸ DNA ploidy,³⁹ and lack of oestrogen and progesterone receptor expression;⁴⁰ it is also an independent adverse prognostic factor.⁴¹

In the pivotal phase III trial, patients with metastatic breast cancer overexpressing HER2 who had not previously received chemotherapy for metastatic disease were randomized to receive either standard chemotherapy alone or standard chemotherapy plus trastuzumab. Those who received trastuzumab plus chemotherapy had a longer median time to disease progression (7.4 months versus 4.6 months, $P<0.001$), a higher rate of objective response (50% versus 32%, $P<0.001$), a longer median duration of response (9.1 months versus 6.1 months, $P<0.001$), longer median survival (25.1 months versus 20.3 months, $P=0.046$) and a 20% lower risk of death than patients who received chemotherapy alone.⁴² Those receiving combination treatment also had better gains in quality of life than those receiving chemotherapy alone.⁴³ Currently this agent is licensed for the treatment of metastatic HER2-overexpressing breast cancer. Several randomized multicentre trials are now underway to investigate the benefits of adjuvant treatment with trastuzumab in HER2-positive primary breast cancer.

The main concern with trastuzumab is treatment-related cardiac dysfunction,⁴⁴ which occurs with monotherapy but seems particularly troublesome with combined therapy in patients who have previously received anthracycline-based chemotherapy. The cardiac effects are probably explained

by the expression of HER2 by cardiac myocytes,⁴⁵ so cardiac function needs to be checked at baseline and monitored during treatment.

As with other monoclonal antibodies, trastuzumab in combination with chemotherapeutic agents has given higher response rates than either single agent alone. This has been particularly noteworthy with cisplatin, possibly because trastuzumab interferes with DNA repair induced by cisplatin and, as a result, promotes cytotoxicity in HER-2/neu-overexpressing tumour target cells in a synergistic fashion. This effect of trastuzumab, termed receptor-enhanced chemosensitivity, is specific for HER-2/neu-overexpressing cells, having no effect on cells without overexpression.⁴⁶

BEVACIZUMAB

Bevacizumab is a humanized murine monoclonal antibody that targets vascular endothelial growth factor-A (VEGF-A) isoform. VEGF is an important endothelial cell-specific mitogen that regulates vascular proliferation and permeability and functions as an antiapoptotic factor for newly formed blood vessels.⁴⁷ This therapeutic antibody targets the process of angiogenesis and the acquisition of new blood vessels by a tumour—a key process if a tumour is to grow and metastasize.

A phase III study in metastatic colorectal cancer showed better results with bevacizumab plus chemotherapy than with chemotherapy alone in terms of response (45% versus 35%, $P=0.0029$), median progression-free survival (10.6 versus 6.2 months, $P<0.00001$), and median overall survival (20.3 versus 15.6 months, $P=0.00003$).⁴⁸ In metastatic renal cell carcinoma high-dose bevacizumab has increased the time to progression compared with placebo.⁴⁹ In a phase II study high-dose bevacizumab plus carboplatin and paclitaxel gave a higher response rate (31.5% versus 18.8%) and longer median time to progression (7.4 versus 4.2 months) than chemotherapy alone.⁵⁰ A phase III trial in taxane-resistant metastatic breast cancer showed a better response rate with bevacizumab and capecitabine than with capecitabine alone (19.8% versus 9.1%) but there was no difference in median time to progression.⁵¹

Adverse effects reported with bevacizumab have included grade 3 hypertension, proteinuria, gastrointestinal perforation, pulmonary haemorrhage, epistaxis and thrombosis.

GEMTUZUMAB OZOGAMICIN

Gemtuzumab ozogamicin is a combination of cytotoxic agent (calicheamicin) and anti-CD33 monoclonal antibody. CD33 is expressed on myeloid blasts in 80% of acute myeloid leukaemia as well as on maturing haemopoietic-progenitor cells but is not present on healthy stem cells.⁵² On binding to CD33, the molecule is internalized into the

cell, and the active drug is subsequently released, resulting in cleavage of double-stranded DNA.⁵³

In phase II studies in acute myeloid leukaemia at first relapse, 30% of patients achieved complete remission, with a median relapse-free survival for these patients of 7.2 months.⁵⁴ Adverse effects include infusion reactions, thrombocytopenia, neutropenic sepsis and reversible hepatotoxicity. Currently this agent is indicated for patients with AML who are older than 60 years of age.⁵⁵ Studies are underway on the effects of gemtuzumab ozogamicin in combination with chemotherapy for high-risk myelodysplastic syndromes and as first-line treatment of acute myeloid leukaemia.

CONCLUSION

The development of therapeutic monoclonal antibodies has already improved the outlook for a large number of patients. In most studies to date their efficacy seems greatest when they are combined with standard cytotoxic agents. The challenge now facing oncologists is to learn how to use these agents to their maximum benefit—the optimal timing with regard to chemotherapy, the optimal duration of use, what to do at disease progression, their value in the adjuvant setting and their value in combination with other novel agents such as tyrosine kinase inhibitors.

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