

Monoclonal Antibody-Directed Cytotoxic Therapy

Potential in Malignant Diseases of Aging

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Abstract

The advent of monoclonal antibodies has allowed the development of tumour directed therapies utilising antibody-dependent effector mechanisms and immunoconjugates (e.g. drug, isotope and toxin coupled antibodies) against human malignancies. Preclinical studies in mouse tumour models have been most impressive and have led to numerous clinical trials. Whereas the majority of these phase I/II trials have been less impressive, a few trials have shown efficacy in highly pre-treated refractory patients and have led to phase III trials. The therapeutic monoclonal antibodies examined in these trials will become clinically available in the near future.

In this review, various methods of utilising antibody-directed anticancer strategies are presented, with emphasis on recent advances in the field. The advantages and disadvantages of these methods together with the role of antibody-directed therapeutics in cancer management are discussed.

Cancer now affects a large percentage of the population^[1] and although it can occur at any stage in the life of an individual, most patients are middle-aged or older. In the US alone, the National Cancer Institute estimates that this year approximately 1 228 600 new patients will be diagnosed with cancer [excluding carcinomas *in situ* (other than urinary bladder) or basal and squamous cell skin cancers], and about 564 800 people are expected to die of the disease.^[2]

Despite considerable advances in the diagnosis and therapy of cancer over the last 30 years, the aforementioned statistics indicate that current cancer therapies only provide a 40% probability that a patient diagnosed with cancer today will be alive in 5 years time. Surgery, radiation and chemotherapy fail to cure many of the most prevalent cancers, and often have serious adverse effects associated with their

use. There are numerous reasons for the failure of these treatments; tumours that cannot be resected because of their location or metastatic spread, and poor sensitivity to radiation and/or chemotherapy. The severe morbidity that is often associated with the use of chemotherapy and radiotherapy often makes them less than an ideal form of therapy. The relatively low therapeutic index of many drugs^[3] results in severe toxic effects on many normal tissues when used at dosages necessary to kill tumour cells.

Therefore, new cancer therapies are being investigated which aim to specifically target tumour cells for therapy, sparing normal cells exposure to the cytotoxic agent. To enable cytotoxic agents to be specifically targeted to tumour cells, monoclonal antibodies recognising only the cancer cells for which they are specific for are being utilised.

The immunological targeting of tumours dates

Table I. Agents linked to monoclonal antibodies

Agents	Examples
Toxins	Pseudomonas exotoxin, ricin, saporin, diphtheria toxin
Isotopes	¹³¹ Iodine, ⁹⁹ Tcnetium, ⁹⁰ Yttrium, ¹¹¹ Indium, ¹⁸⁶ Rhenium
Drugs	Anthracyclines: Doxorubicin Daunorubicin Idarubicin Nemorubicin Antimetabolites: Aminopterin Methotrexate Fluorouracil Floxuridine Cytarabine Alkylating agents: Melphalan Chlorambucil Mitomycin Cisplatin Trenimon T-2 toxin Antimitotic drugs: Vinca alkaloids Podophyllotoxin Colchicine DNA intercalators: Ethidium dimer Neocarcinostatin Nitroacridine C921 Tyrosine kinase inhibitors: Genistein
Enzymes	Carboxypeptidase Alkaline phosphatase β-lactamase Glucose oxidase Cytidine deaminase
Lymphokines	TNFα IFN IL-2
Sensitisers	Radiosensitisers Photosensitisers
Other	Chemotactic factors Fluorochromes NMR contrast agents

IFN = interferon; **IL-2** = interleukin-2; **NMR** = nuclear magnetic resonance; **TNF** = tumour necrosis factor.

back to the 1890s with the attempted treatment of cancer patients with antisera from dogs and in the original ‘magic bullet’ concept of Paul Ehrlich,^[4] in which antibodies are used to specifically target toxins to tumour cells. Over the past 20 years considerable research efforts have focused on developing

targeting systems for the delivery of cytotoxic drugs, radioisotopes and toxins to tumour cells as treatment modalities (table I).

Monoclonal antibody-targeted therapy is based on the presumption that differences in cell-surface protein expression between cancerous and normal tissues can facilitate the selective targeting of tumours based on the unique specificity of monoclonal antibodies for tumour associated antigens. Recent advances in the identification of tumour associated antigens and oncoproteins have provided some ways of distinguishing between normal and cancerous cells, and with the advent of hybridoma technology, these molecules are an ideal target for the production of antibodies to be used in antibody based therapies.

Preclinical studies have shown numerous advantages in the using monoclonal antibody-based drug therapies compared with conventional chemotherapy, including:

- the selective delivery of drugs to the tumour cell
- preferential uptake of drug immunoconjugate by the tumour cell
- plasma clearance of the drug is greatly slowed which results in higher levels of the drug remaining in the circulation and, therefore, prolonged exposure of the tumour cells to the drug
- greater drug localisation at the tumour site.

Whereas preclinical studies have shown promising results in the use of many drug immunoconjugates, clinical trials have revealed a number of problems and limitations associated with this form of therapy.

1. Monoclonal Antibodies as Carrier Molecules

Since the initial ‘magic bullet’ concept of Paul Ehrlich, many studies have been conducted in the hope of utilising this approach to treat cancer.^[5,6] The earliest studies used heteroantisera raised in goats, mice, sheep, rabbits and horses immunised with cancer cells or their subcellular fractions.^[7] Heteroantisera were rendered relatively ‘tumour specific’ by removing antibodies reactive with normal tissue antigens through their adsorption by normal tissue

homogenates. The lack of tumour antigen specificity displayed by these reagents rendered these methods ineffective. The problems with tumour antigen specificity were overcome through the advent of hybridoma technology.^[8] As a result of this technology, monoclonal antibodies possessing a unique specificity for their target antigen could be readily obtained, and greatly extended the possibilities of targeting cytotoxic agents to tumours.

Monoclonal antibodies display numerous characteristics which make them ideal candidates as carriers of cytotoxic agents to tumour cells and as single-agent therapeutic molecules. They are easily produced, display a unique target specificity and their affinity towards antigenic epitopes can be readily selected for. These characteristics resulted in monoclonal antibodies rapidly surpassing the use of polyclonal preparations as carriers for the targeted delivery of cytotoxic agents to tumour cells. The development of monoclonal antibodies displaying some degree of specificity for tumours allowed the concepts of immunological targeting of tumours to be readily explored, with studies demonstrating that xenogenic monoclonal antibodies could not only be safely administered to patients and localise on tumours,^[9] but could also have a therapeutic effect of their own in xenograft models^[10,11] and in patients with leukaemia and lymphoma.^[12,13]

Although therapeutic effects against cancers have been detected using monoclonal antibodies alone, and these responses have involved complement-mediated effects or modulation of effector macrophages and natural killer cells,^[14] initial clinical responses to serotherapy have been variable,^[15,16] with animal studies indicating limitations associated with this approach.^[17] However, as a greater understanding of the recruitment of effector functions by naked antibodies is gained, the use of monoclonal antibodies alone is increasingly contributing to cancer therapeutics. The variable tumour effects of monoclonal antibodies alone may be improved by conjugation to cytotoxic agents, given that the cytotoxic potential and mode of action of many drugs and toxins are well understood as many have already been utilised in the clinic.

2. Antibodies Alone

Since antibodies are clearly able to function *in vivo* after passive or active immunisation, particularly against infectious disease, the question may be asked as to the therapeutic efficacy of passively administered monoclonal antibodies against cancer. In the past, the use of antibodies alone has rarely been successful because of factors including: (i) amount of antibody bound; (ii) poor mobilisation of effector mechanisms by mouse antibodies; and (iii) the development of immune responses to the foreign immunoglobulin, referred to as human antimouse antibodies (HAMA). However, the development of strategies to overcome the main factors involved in the poor results has resulted in renewed interest in their use in naked form. Recombinant monoclonal antibodies consisting of murine variable sequences and human constant domains are now available and some have been tested in clinical trials.^[18-20]

These recombinant 'humanised' antibodies bind complement and have antibody-dependent cellular cytotoxicity (ADCC) activity and, therefore, have the potential in humans to activate effector functions against the tumours that they are specific for. This humanisation of murine antibodies has significantly reduced HAMA responses associated with their use. Alternatively, antibody constant domains have been modified (e.g., altered hinged region) to improve various functional activities for improved therapy in humans.^[21] More recently, additional approaches to increase the antitumour activity of monoclonal antibodies *in vivo* by administering biological response modifiers such as interferons,^[22] interleukins^[23-25] and colony stimulating factors have been studied.^[26]

The use of naked monoclonal antibodies for the immunotherapy of cancer has involved the utilisation of various strategies including: (i) recruiting of effector functions for the destruction of cancer cells; (ii) anti-idiotypic antibodies; and (iii) interference with the growth and differentiation of malignant cells. Although the use of anti-idiotypic antibodies has shown favourable responses, particularly against non-Hodgkin's lymphoma, this approach is rarely used today because of the financial and logistic

limitations associated with the production of anti-idiotypic molecules and hence will not be discussed further in this review.

2.1 Recruiting of Effector Functions

Effective treatment of malignant disease with monoclonal antibodies alone depends on the recruitment of host effector mechanisms such as complement, ADCC and phagocytosis/cytostasis of monoclonal antibody coated tumour cells.^[27] The anticancer effect of naked monoclonal antibodies has been shown to be dependent on the Fc portion of the antibody molecule and its isotype. Human effector cells which express the Fc receptor are capable of mediating ADCC^[28] and it has been shown that interleukin (IL)-2 can also activate these cells to facilitate ADCC against target cells.^[29] Several studies in animals^[30] and patients^[25,31,32] have combined IL-2 with monoclonal antibodies and demonstrated antitumour effects.

In patients with stage III colorectal cancer, antibody treatment has been shown to reduce the overall death rate by 30% and decrease the recurrence rate following potentially curative surgery by 27%.^[33] Preclinical studies into the effect of monoclonal antibodies against renal cell carcinomas have been promising.^[34] Renal cell carcinoma is relatively resistant to chemotherapy and radiotherapy, and most monoclonal antibodies raised against human renal cell carcinoma induce little complement-dependent cytotoxicity or ADCC. In this study,^[34] a chimeric antibody (ch-G250) comprising a murine variable region which recognises a tumour associated antigen expressed on 95% of primary and 80% of metastatic renal cell carcinomas, was engineered onto the constant region of human immunoglobulin (Ig)G1 isotype. This antibody facilitated potent ADCC against several renal cell carcinoma cell lines and clinical radio-localisation studies demonstrated the relative tumour-targeting potential of this antibody.

The most promising recent results on the use of a naked monoclonal antibody against human cancer comes from recent phase III results of an anti-CD20 antibody, rituximab (Rituxan[®], IDEC Pharmaceuticals/Genentech). Of 151 evaluable patients

with relapsed low grade or follicular non-Hodgkin's lymphoma, 76 responded to treatment with rituximab as a single agent for an overall objective response rate of 58 to 50%, including 6 to 10% complete responses. In combination with chemotherapy, phase II results indicate an overall 100% response rate, with 63% complete responses.^[35] Although these results are undoubtedly impressive, the use of monoclonal antibodies alone on most other cancers, particularly solid tumours, have failed to produce such results.

2.2 Blocking Antibodies

Monoclonal antibodies that interfere with the growth and regulation of cancer cells, by 'blocking the accessibility of these tumours to stimulating molecules', have shown some encouraging results in recent phase I clinical trials and preclinical studies. In a recent phase I trial,^[36] in 13 patients with previously treated small cell lung cancer (SCLC), the use of a murine monoclonal antibody (2A11) produced 1 complete reduction of radiographically detectable tumour lasting 4 months, whilst 4 patients (33%) had stable disease. 2A11 is a monoclonal antibody which binds to gastrin-releasing peptide. Since SCLC cells express and secrete gastrin-releasing peptide which binds to receptors and stimulates growth of these cancer cells, the high affinity binding of 2A11 to gastrin-releasing peptide impairs this interaction. Anti-epidermal growth factor receptor (EGFR) blocking antibodies have also been evaluated in recent phase I trials.

In 1 study,^[37] the first rat monoclonal antibody (Mab ICR-62) to EGFR was administered to 11 patients with squamous cell carcinoma of the head and neck, and 9 patients with squamous cell carcinoma of the lung with all tumours expressing EGFR. No serious toxicities were observed with the use of the rat antibody and only 4 of 20 patients developed human anti-rat antibodies (HARA). Localisation of the antibody to tumour membranes was seen. Anti-EGFR antibodies have also been evaluated in a recent preclinical study against human prostate carcinoma.^[38] In prostate cancer, a correlation exists between overexpression of EGFR and poor clinical

prognosis. Since late-stage metastatic disease is characterised by a change from paracrine to autocrine expression for transforming growth factor (TGF)- α (ligand for EGFR), the blockage of this interaction using a monoclonal antibody [anti-EGFR monoclonal antibody 225 (C-225)] may offer a means of therapeutic intervention of this disease. C-225, either alone or in combination with doxorubicin, significantly inhibited tumour progression of tumour xenografts in nude mice.

Although blocking monoclonal antibodies to gastrin-releasing peptide and EGFR are providing encouraging results in preclinical studies and phase I trials (gastrin-releasing peptide), the therapeutic efficacy of these treatment strategies in more advanced trials remains to be seen. Nonetheless, the potential of utilising the unique specificity of monoclonal antibodies to block important ligand-receptor interactions required for the maintenance of the malignant phenotype by certain tumour types should not be understated and warrants further evaluation.

3. Immunoconjugates

Four different agents can be used to arm antibodies against cancerous cells – isotopes, drugs, toxins and enzymes. Many examples of each of these moieties have been used for preclinical studies (table I), but few have progressed beyond phase I/II studies. At present, it is debatable which is the ideal entity to use – each have their advantages and disadvantages (table II). While enzyme-antibody conjugates have been studied widely for use in prodrug activa-

tion and the use of antibody pre-targeting strategies are appearing in the literature, in this review, we will focus on the salient features of the 3 direct therapeutic moieties: isotope, drug and toxin-immunoconjugates.

3.1 Isotopes

The use of isotopes in the formation of anticancer immunoconjugates has been a popular choice as they are readily available, in general, are easily conjugated to antibodies and, as they are radiolabelled, the *in vivo* distribution, half life and tumour accumulation can be easily detected. They are mostly non-immunogenic and their small size, relative to proteins and drugs, aids tumour penetration. These characteristics have resulted in more clinical studies being undertaken with radiolabelled antibodies than with any other form of conjugate, in particular with ^{131}I iodine, although more recently ^{90}Y yttrium has been used.^[39]

Radionuclides potentially useful as immunoconjugates can be divided into 3 major groups: halogens, technetium/rhenium and other metals (table III). The main disadvantages associated with radionuclide use lies in the need to handle large amounts of radioactive material and that isotopes, particularly yttrium, can irradiate normal tissues (such as bone marrow) as well as cancerous tissue because of their strong emission energies. This last problem can be overcome by storing autologous marrow prior to treatment for post-therapy reinfusion. Whereas in this case the irradiation of bone marrow is an unwanted adverse effect associated with the use of radionuclide, compared with drug-

Table II. Comparison of immunoconjugates

Parameter	Isotope	Drug	Toxin/enzyme
Chemistry	Easy	Difficult	Easy
Yields	High	Medium	Low
Stability/half-life	Variable	Good	Good
Handling	Difficult	No problem	No problem
Tumour heterogeneity	Good	Not good	Not good
Damage to surrounding tissues	Yes	No	No
Damage elsewhere	Yes	No	Yes
Anti-conjugate antibodies	No	Usually no	Yes (toxins)
Access to tumour	Good	Good	Depending on size

or toxin-labelled antibodies, at the cellular level the irradiation of surrounding tissues may in fact irradiate and destroy tumour cells that do not bind the immunoconjugates (as they lack the antigen or have a low density of antigen expression). In comparison, toxin and drug-based immunoconjugates would not be able to effect these cells as tumour cell eradication can only be facilitated following the binding of the immunoconjugate to antigenic epitopes on the surface of the cell. In practice this has yet to be conclusively proven.

Some of the best results obtained yet with immunoconjugates in patients have been against lymphoma's with ¹³¹iodine labelled anti-CD20 antibodies and non-myeloblastic doses of ¹³¹iodine-tositumomab (Bexxar®, Coulter Pharmaceuticals).^[40,41] Myeloblastic doses of ¹³¹iodine-tositumomab have produced even better therapeutic results. In another lymphoma trial, the use of ¹³¹iodine-lym-1 (anti-HLA-DR) antibodies provided some promising results,^[42] and the use of ⁹⁰yttrium-BrE-3 antibodies in breast cancer were promising.^[43] For the other

radioisotopes listed, preclinical studies have been performed, but few clinical trials.

3.2 Cytotoxic Drugs

The conjugation of anticancer drugs to monoclonal antibodies is chemically difficult as most of the drugs in common use are hydrophobic and are conjugated with difficulty to hydrophilic antibodies. A number of methods have been developed and most rely on conjugation of the drug through modification of the drug moiety. Carboxylic acid group-containing drugs are activated by forming *N*-hydroxysuccinimide esters which can then react with antibody amino groups to form amide linkages between the drug and antibody.^[44] Hydroxyl group-containing drugs are reacted firstly with succinic anhydride to introduce a carboxylic acid group and then coupled to the antibody utilising methods similar to other carboxyl group containing drugs.^[45] These and other methods have been extensively reviewed elsewhere^[46] and result in 4 to 10 drug molecules being conjugated per molecule of antibody.

Table III. Radionuclides for monoclonal antibody radiolabelling

Isotope	Half-life	Energy (KeV)	Decay	Comments
Halogens				
¹³¹ Iodine	8 days	182	γ, β	High energy; good local toxicity; γ emissions for imaging
¹²³ Iodine	13h	159	Auger	Low energy radiation capable of specific cell kill if near nucleus
¹²⁵ Iodine	60 days	27-35	Auger	Low energy radiation; commonly used for preclinical characterisation
²¹¹ Astatine	7.2h	5900	α	High energy radiation capable of cell killing with few emissions
Technetium/rhenium				
^{99m} Technetium	6.0h	140	γ	Inexpensive; ideal for imaging
¹⁸⁶ Rhenium	3.7 days	1100β, 137γ	γ, β	Can be used in both imaging and therapy
¹⁸⁸ Rhenium	17h	2110β, 155γ	γ, β	Carrier free
Other				
⁹⁰ Yttrium	2.5 days	2200	β	High energy β emissions for cell killing
¹¹¹ Indium	2.8 days	274	γ	Suitable for imaging
²¹² Bismuth	1h	6100	α	Some β emissions
²¹³ Bismuth	47 min	8400	α	Some β emissions
³² Phosphorus	14.3 days	1700	β	Pure high energy β emitter

KeV = kiloelectron volts.

In general, the conjugation procedure can result in up to a 10-fold decrease in drug activity and a loss in antibody activity. The deleterious effects of conjugation on antibody and drug activities tend to increase as the number of drug molecules conjugated per molecule of antibody increases. Therefore, a compromise must be reached where enough drug is conjugated to permit the delivery of a therapeutic dose to the tumour cells, while not compromising the conjugate through losses in activity.

An interesting exception to this rule has been observed when using alkylating drugs such as chlorambucil or melphalan wherein an increase in drug activity has been observed.^[47] Following conjugation, the drug-antibody conjugates are generally stable and display specific anti-tumour effects with minimal adverse effects *in vivo*. As noted in table II, although neither isotope nor drug-antibody conjugates are likely to be immunogenic, it is possible for these entities coupled to antibodies to act as haptens and there has been a report of the vinca alkaloid used in a vinca alkaloid-antibody conjugate being immunogenic.^[48]

3.3 Toxins

Toxins are usually of plant or animal origin and most work has been done with either whole ricin or ricin A chain. Most of these toxic molecules consist of 2 chains – one which binds to carbohydrate residues present on virtually all cells (B chain), and a second, the A chain, which is toxic intracellularly. Ricin is extremely toxic, with 1 molecule of ricin able to inhibit the protein synthesis machinery and lead to cell death. Since intact ricin molecules can bind to almost all cells through the B chain, isolated A chains have been conjugated to antibodies for therapy.^[49] Because intact ricin is generally more potent than A chain derivatives, methods have been developed which can mask the carbohydrate binding site on the B chain.^[50,51]

Chemical linkage of protein toxins to antibodies is generally straight forward and relies on the use of disulphide bridges. However, this procedure is not very efficient and immunoconjugate yields are generally low. Although extremely toxic to cancer

cells, this toxicity can also lead to nonspecific toxicities, especially against liver tissue. Ricin A-chain immunoconjugates have been reported to bind to the liver. However, deglycosylation of these compounds has partly overcome this disadvantage. Further disadvantages include the relatively large size of toxin molecules (e.g. Mwt = 65 000 for ricin) and the development of antibodies to the toxins as they are immunogenic.

3.4 Which of These Agents to Choose?

As indicated in table II, all of these immunoconjugates have advantages and disadvantages associated with their use. Although at this time it is important that preclinical and phase I/II trials are conducted so that value of existing and emerging immunoconjugates can be compared, ongoing and planned phase III clinical trials, together with acceptance and continued use by the medical community, will ultimately dictate the overall usefulness of immunoconjugates against cancer.

4. Clinical Examples of Immunoconjugates

As mentioned in section 3.1, by far the most commonly used moiety for clinical studies into immunoconjugates against cancer have been isotope-antibody conjugates, rather than drugs or toxins. In this section we will highlight illustrative examples of the use of these immunoconjugates.

4.1 Isotopes

The potential of monoclonal antibodies in the radioimmunotherapy of cancers has been recognised for some time but, to date, clinical success with radioimmunotherapy has been limited. Most radioimmunotherapy studies are conducted with the medium energy β emitter ¹³¹iodine [0.6 MeV (million electron volts)], as labelling antibodies with this radionuclide is relatively straight forward and the gamma emissions of this isotope allow for tumour imaging and quantitative biodistributive studies. Clinical studies in this area have been extensively reviewed and only recent studies are

Table IV. Clinical studies with isotope-antibody conjugates

Disease	No. of patients	Antibody (isotope)	Toxicity	Response	References
B-cell lymphoma and B-cell leukemia	20	Lym-1 (¹³¹ I)	Moderate	10% CR, 85% PR, 5% PD	42
B-cell lymphoma	25	anti-CD20 tositumomab (¹³¹ I)	Myelosuppression (stem cell reinfusion)	18/21 objective responses, 16 CR	40,41
Ovarian cancer	12	B72.3 (¹³¹ I)		2 CR, 4 PD, 1 SD, 5 PR	52
Ovarian cancer	25	HMFG (⁹⁰ Y)	Myelosuppression	Tumour regression in 1 patient, ascites decrease in 1 patient	53
Ovarian cancer	27	CC49 (¹⁷⁷ Lu)	Arthralgia, myelosuppression	1/13 patients with gross disease had >50% tumour reduction, 7/9 patients (<1cm nodules) progressed and 2/9 disease free at 4-5mo. Microscopic disease – 4/5 disease free at >6 to 35mo	54
Non-Hodgkin's lymphoma	16	LL2 (¹³¹ I)	Myelotoxicity	2 PR, 2 mixed responses, 1 NR in 5 evaluable patients	55
Hodgkin's disease	45	Anti-ferritin (⁹⁰ Y)	Haematological	62% response in 29 assessable patients, 9/18 CR (2-26mo)	56
Colorectal cancer with liver metastases	10	Anti-CEA F6 (¹³¹ I)	Haematological	1 PR, 2 SD, 6 PD	57
CEA expressing tumours	13	Anti-CEA NP-4, HAMA (¹³¹ I)	Haematological	6 SD (3.5-7mo)	58

CEA = carcinoembryonic antigen; **CR** = complete response; **HAMA** = human antimouse antibodies; **HMFG** = human milk fat globule; **NR** = no response; **PD** = progressive disease; **PR** = partial response; **SD** = stable disease.

summarised in table IV. The radionuclide ⁹⁰yttrium is an attractive alternative to ¹³¹iodine owing to its high energy pure β emissions (2.3 MeV) and relatively short half-life. With ⁹⁰yttrium, dose-limiting toxicity frequently results in myelosuppression as ⁹⁰yttrium, when dissociated from antibody, accumulates in bone and causes marrow toxicity. The advent of new macrocyclic bifunctional chelating agents consisting of a moiety for antibody attachment and a metal chelator,^[59,60] has allowed the production of more stable ⁹⁰yttrium immunoconjugates with favourable biodistribution characteristics.^[61-63]

To date, most clinical studies with ¹³¹iodine-immunoconjugates have been conducted against lymphomas. It is within this group of haematological malignancies that the most impressive clinical results have been obtained to date and this form of therapy is crossing the threshold to becoming the standard mode of treatment. Initial studies using ¹³¹iodine-lym-1 (anti-HLA-DR) indicated that B-cell lymphomas and leukaemias appeared highly radiosensitive^[42] and impressive results have been obtained using ¹³¹iodine-labelled antibodies in

these diseases. In recent studies myeloblastic doses of ¹³¹iodine-tositumomab with autologous stem cell rescue have yielded response rates in excess of 90% with 79% complete remissions, and remission duration over 3 years.^[41]

Whereas radioimmunotherapy is most successful against lymphomas, in solid tumours the initial preclinical studies which showed radiotherapy to be superior to chemotherapy, have not lived up to expectations when subjected to clinical trial. In 1 study, a complete clinical remission was noted in a patient with refractive ovarian cancer treated with a murine anti-carcinoembryonic antigen (CEA) antibody, anti-CEA-monoclonal-antibody-¹³¹I-MN-14.^[64] Grade 1 thrombocytopenia and grade 2 leucopenia were reported following the second of 3 injections. However, all adverse effects were transient with both reversing within 6 weeks. In a recent phase I/II study using ¹³¹iodine-labelled anti-CEA antibody F6 F(ab')₂ in 10 patients with non-resectable liver metastases from colorectal cancer, 9 patients with radiological evidence of liver metastases showed uptake of the radiolabelled antibody.^[57]

The only toxicity observed was haematological and tumour response was evaluated in 9 out of 10 patients with 1 patient showing a partial response of 1 metastasis and a stable evolution of the metastases, 2 patients had stable disease and 6 showed tumour progression. In a trial examining radioimmunotherapy in breast cancer using ^{131}I -labelled ChL6 antibodies, 6 of 10 patients treated had clinically measurable responses to treatment, and 5 had responses that lasted greater than 1 month.

Although lymphoma is the form of cancer best studied and responsive to radioimmunotherapy, solid tumours refractory to other forms of therapy have displayed some responses in phase I/II trials. It is believed that this form of therapy against solid tumours is best targeted to minimal residual disease and in the future this should be the prime target for the use of immunoconjugates.

4.2 Drug-Antibody Immunoconjugates

Impressive preclinical data on the use of drug-antibody immunoconjugates in the treatment of cancer has led to their use in clinical trials (table V).

Whereas most are phase I trials carried out in patients with advanced disease have less than impressive results, the results of recent phase II trials on the use of a new investigational immunoconjugate (CMA-676) in patients with acute myeloid leukaemia have been most promising.^[74] Of 39 patients treated in a study determining appropriate dosing for patients with leukaemia, 2 patients achieved complete remission of the disease for approximately 6 months. In addition, 7 patients achieved 'morphological remission' – the temporary removal of all leukaemia cells from the blood. The immunoconjugate used in this study has utilised an anti-CD33 monoclonal antibody coupled to the extremely potent anticancer drug calicheamicin. Calicheamicin is known to be approximately 1000-fold more active than doxorubicin against murine tumours, and its ability to cause double-stranded DNA breaks at very low concentrations is believed to be responsible for its potent antitumour activity.^[75] CMA-676 is now in a pivotal clinical study in 13 centres in North America. Supporting European studies have begun.

Table V. Clinical studies with drug-antibody conjugates

Disease	No. of patients	Drug	Toxicity	Response	Reference
Colorectal and ovarian cancer	4	Vindesine	None	None	65
Neuroblastoma	7	Daunorubicin, chlorambucil	Marrow depression	3/7 NED, 1/7 PR, 2/7 MR	66
Colon cancer	41	Neocarzinostatin	Leucocytosis fever	3/8 tumour decrease, 3 pain relief	67
Various	23	Doxorubicin	Rash, chills, fever, haemopoetic depression	5/23 MR	68
Colon cancer	10	N-acetyl melphalan	Serum sickness	3/9 MR	69
Various	19	Mitomycin	Thrombocytopenia, diarrhoea, GI bleeding	1 SD	70
Colon cancer	13	Vinca alkaloid	GI tract toxicity, abdominal pain	None	71
Various	43	Doxorubicin, mitomycin	Doxorubicin: fever chills, rash haemopoetic depression alopecia. Mitomycin: fever chills, rash, thrombocytopenia	5/43 (doxorubicin)	72
Non-small cell lung cancer	5	Methotrexate	Fever, chills, vomiting, diarrhoea	None	73
Acute myeloid leukaemia	39	Calicheamicin	Fever, chills, some degree of liver toxicity in 3/39	2 CR, 7/39 'morphological remission'	74

CR = complete response; **GI** = gastrointestinal; **MR** = minor response; **NED** = no evidence of disease; **PR** = partial response; **SD** = stable disease.

It is clear from the effectiveness of CMA-676 that the need for extremely potent drugs in the development of drug-antibody conjugates is paramount. Since the high specificity of monoclonal antibody allows for the delivery of high levels of the drug to the tumour with minimal systemic toxicity, and tumour imaging in patients has shown that the proportion of monoclonal antibody localising to the tumour is usually less than 0.01%,^[76] only immunconjugates carrying potent anticancer drugs can be expected to produce significant positive clinical results.

4.3 Toxins

There have been several clinical trials with toxin antibody conjugates. These have been with ricin, ricin chain A and pseudomonas exotoxin (table VI). Dose-limiting toxicity for whole ricin antibody conju-

gates is mainly hepatotoxicity, whereas ricin A chain conjugates can give rise to capillary leak syndrome. In a recent phase I study on the use of an anti-CD25 ricin chain A immunotoxin in patients with refractory Hodgkin's lymphoma,^[84] responses included 2 partial remissions, 1 minor response, 3 stable disease and 9 progressive diseases. Given these patients were heavily pretreated, these results indicate clinical efficacy of this new immunotoxin. The first report of immunotoxin activity against epithelial tumours is a phase 1 trial on the use of immunotoxin LMB-1 [anti-Le(Y) monoclonal coupled to pseudomonas exotoxin]^[85] in 38 patients who had failed conventional therapy and whose tumours expressed Le(Y) antigen. Objective antitumour activity was observed in 5 patients, 18 had stable disease and 15 progressed. A complete remission was observed in a patient with metastatic breast cancer to supraclavicular nodes.^[85]

Table VI. Clinical studies with toxin-antibody conjugates

Disease	No. of patients	Conjugate	Toxicity	Response	Reference
Metastatic colon cancer	17	79IT/36-Ricin A	Decrease serum albumin, fever, proteinuria, mental status change	5/17 mixed responses	77
Metastatic breast cancer	4	260F9-r-Ricin A	Bodyweight gain, oedema, dyspnea, hypoalbuminaemia	None	78
Metastatic breast cancer	5	260F9-r-Ricin A	Fluid overload	None	79
Metastatic melanoma	20	XMME-001 RTA + cyclophosphamide	Capillary leak syndrome	None	80
Cutaneous T-cell lymphoma	14	H65-Ricin A (α CD5-Ricin A)	Myalgia, mild hypoalbuminaemia with bodyweight gain, oedema, fevers	4 PR (3-8mo)	81
B-cell lymphoma	25	α -B4-blocked Ricin	Hypoalbuminaemia and thrombocytopenia	1 CR, 2 PR and 8 MR	82
Metastatic melanoma	20	Xomazyme-Mel	Oedema, bodyweight gain, hypoalbuminaemia	1 CR (12mo), 1 MR (3mo)	83
Refractory Hodgkin's lymphoma	15	RFT5-SMPT-dgA (α CD25-Ricin A)	Vascular leak syndrome	2 PR, 1 MR, 3 SD	84
Solid tumours	38	LMB-1-P.exotoxin	Vascular leak syndrome	1 CR, 5 objective responses, 18 SD	85
Leptomeningeal neoplasia	8	454A12-rRA (α TFR-Ricin A)	CSF inflammatory response at high doses	4/8 >50% reduction of tumour cell counts in CSF	86
Non-Hodgkin's lymphoma	23	IgG-HD37-SMPT-dgA	Acrocyanosis	1 PR, 1 CR	87
Metastatic colorectal cancer	12	XMMCO-791/RTA	Non-cardiac pulmonary oedema	None	88

CR = complete response; **CSF** = cerebrospinal fluid; **IgG** = immunoglobulin G; **MR** = minor response; **P** = pseudomonas; **PR** = partial response; **SD** = stable disease; **TFR** = transferrin receptor.

The major dose-limiting toxicity was found to be vascular leak syndrome.

5. Summary and Future Directions

At this time, the use of naked antibodies and immunoconjugates in cancer therapy is viewed pessimistically by many. However, such views are inappropriate given the results from clinical trials reported over the 5 years and results yet to be formally reported. In the case of lymphomas, radioimmunotherapy is crossing the threshold of becoming a standard mode of treatment. Thus far, mostly phase I/II trials have been conducted with any toxic moiety of isotope, drug or toxin conjugated to antibody and, therefore, it is too early to determine the efficacy of these agents.

Traditionally, these studies are performed in patients with advanced disease who are unlikely to display major responses to these new forms of therapy. For the most part, most agents are potent, specific, relatively non-toxic and successfully target tumours. Preclinical studies have been impressive and positive responses noted *in vivo*. Although problems with HAMA are a major impediment to the use of murine monoclonal antibodies, the further implementation of chimeric, humanised or fully human antibodies as immunoconjugates will solve this problem. The relative non-toxicity of the treatment should encourage the development of new anticancer agents which on their own are far too toxic to be utilised, but when coupled to antibodies can be used in humans.

Although outside the scope of this present review, genetic engineering techniques are being utilised to manufacture recombinant antibodies with better tumour targeting characteristics. It can be envisaged that in the future most or all patients with stage I/II disease will receive immunoconjugate therapy, together with more traditional forms of therapy, after surgery, irrespective of whether microscopic disease is detected or not. The unique specificity of monoclonal antibodies of being able to reach microscopic tumour deposits, outside the reach of surgical intervention, makes them ideal reagents in this regard.

References

1. Hoel DG, Davis DL, Miller AB, et al. Trends in cancer mortality in 15 industrialised countries 1969-1986. *J Natl Cancer Inst* 1992; 84: 313-20
2. Ries LAG, Kosary CL, Hanky BF, et al. (editors). SEER cancer statistic review, 1973-1995. Bethesda (MD): National Cancer Institute, 1998
3. Double JA, Bibby MC. Therapeutic index: a vital component in selection of anticancer agents for clinical trial. *J Natl Cancer Inst* 1989; 81: 988-99
4. Erlich P. Collected studies on immunity. Vol. II. New York: John Wiley, 1906
5. Pietersz GA, McKenzie IFC. Antibody conjugates for the treatment of cancer. *Immunol Rev* 1992; 129: 57-80
6. Poznansky MJ, Juliano RL. Biological approaches to the controlled delivery of drugs: a critical review. *Pharmacol Rev* 1984; 36: 277-336
7. Mathe G, Loc TB, Bernard JC. Effect sur la leucemie 1210 del la souris d'un combinaison par diazotation d'amethopterine et de gamma globulines de hamsters porteurs de cette leucemie par hetrogreffe. *C R Seances Acad Sci D* 1958; 246: 1626-8
8. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975; 256: 495-7
9. Schroff RW, Woodhouse CS, Foon KA, et al. Intratumour localization of monoclonal antibody in patients with melanoma treated with antibody to a 250,000 dalton melanoma associated antigen. *J Natl Cancer Inst* 1985; 74: 299-306
10. Herlyn DM, Steplewski Z, Herlyn MF, et al. Inhibition of growth of colorectal carcinoma in nude mice by monoclonal antibody. *Cancer Res* 1980; 40: 717-21
11. Katano M, Irie RF. Human monoclonal antibody to tumour associated ganglioside GD2: suppressed growth of human melanoma in nude mice. *Immunol Lett* 1984; 8: 169-74
12. Dillman RO, Shawler DL, Sobol RE, et al. Murine monoclonal antibody therapy in two patients with chronic lymphocytic leukaemia. *Blood* 1989; 59: 1036-45
13. Miller RA, Levy R. Response of cutaneous T cell lymphoma to therapy with hybridoma monoclonal antibody. *Lancet* 1981; II: 226-30
14. Ritz J, Pesando JM, Sallan SE, et al. Serotherapy of acute lymphoblastic leukaemia with monoclonal antibody. *Blood* 1981; 58: 141-52
15. Adams DO, Hall T, Steplewski Z, et al. Tumours undergoing rejection induced by monoclonal antibodies of the IgG2a isotype contain increased numbers of macrophages activated for a distinctive form of antibody-dependent cytotoxicity. *Proc Natl Acad Sci* 1984; 81: 3506-10
16. Goodman GE, Beaumier P, Hellstrom I, et al. Pilot trial of murine monoclonal antibodies in patients with advanced melanoma. *J Clin Oncol* 1985; 3: 340-52
17. Sears HG, Atkinson B, Mattis J, et al. Phase-I clinical trial of monoclonal antibody in treatment of gastrointestinal tumours. *Lancet* 1982; I: 762-5
18. Adair AJ. Engineered antibodies for therapy. *Immunol Rev* 1992; 130: 5-40
19. Meredith RF, Khazaeli MB, Plott WE, et al. Comparison of two mouse/human chimeric antibodies in patients with metastatic colon cancer. *Antibody Immunoconjugates Radiopharm* 1992; 5 (1): 75-80
20. LoBuglio A, Wheeler R, Trang J, et al. Human/mouse chimeric monoclonal antibodies in man: kinetics and immune response. *Proc Natl Acad Sci U S A* 1989; 86: 4220-4

21. Michaelson TE, Aase A, Norderdaug L, et al. Antibody dependent cell-mediated cytotoxicity induced by chimeric mouse/human IgG subclasses and IgG3 antibodies with altered hinge regions. *Mol Immunol* 1992; 29: 319-26
22. Guadagni F, Schlom J, Pothien S, et al. Parameters involved in the enhancement of monoclonal antibody targeting in vivo with recombinant interferon. *Cancer Immunol Immunother* 1988; 26: 222-30
23. Vuist WMJ, Buitenen FV, Hekman A, et al. Two distinct mechanisms of antitumour activity mediated by the combination of interleukin 2 and monoclonal antibodies. *Cancer Res* 1990; 50: 5767-72
24. Nakamura K, Kubo A. Biodistribution of iodine-125 labeled monoclonal antibody/interleukin 2 immunoconjugate in athymic mice bearing human tumour xenografts. *Cancer* 1997; 80 (12): 2650-5
25. Borriore P, Montacchini L, Beggiato, E, et al. Clinical and immunological studies in advanced cancer patients sequentially treated with anti CD3 monoclonal antibody (OKT3) and interleukin 2. *Leuk Lymphoma* 1996; 21: 325-30
26. Bock SN, Cameron RB, Kragel P, et al. Biological and antitumour effects of recombinant human macrophage colony stimulating factor in mice. *Cancer Res* 1991; 51: 2649-54
27. Stevenson GT, Glennie MJ. Surface immunoglobulin of B-lymphocytic tumours as a therapeutic target. *Cancer Surv* 1980; 4: 213-44
28. Eisenthal A, Rosenberg SA. Systemic induction of cells mediating antibody dependent cellular cytotoxicity following administration of interleukin-2. *Cancer Res* 1989; 49: 6953-9
29. Shiloni E, Eisenthal A, Sachs D, et al. Antibody dependent cellular cytotoxicity mediated by murine lymphocytes activated in recombinant interleukin-2. *J Immunol* 1987; 138: 1992-8
30. Gill I, Agah R, Hu E, et al. Synergistic antitumour effects of interleukin-2 and the monoclonal Lym-1 against human Burkitt lymphoma cells in vitro and in vivo. *Cancer Res* 1989; 49: 5377-9
31. Bajorin DF, Chapman PB, Wong G, et al. Phase I evaluation of a combination of a monoclonal antibody R24 and interleukin 2 in patients with metastatic melanoma. *Cancer Res* 1990; 50 (23) 7490-5
32. Sondel PM, Hank JA. Combination therapy with interleukin-2 and antitumor monoclonal antibodies. *Cancer J Sci Am* 1997; 3 (1 Suppl.): S121
33. Holz E, Raab R, Riethmuller G. Antibody-based immunotherapeutic strategies in colorectal cancer. *Recent Results Cancer Res* 1996; 142: 381-400
34. Surfus JE, Hank JA, Oosterwijk E, et al. Anti-renal cell carcinoma chimeric antibody G250 facilitates antibody-dependent cellular cytotoxicity with in vitro and in vivo interleukin-2-activated effectors. *J Immunother Emphasis Tumor Immunol* 1996; 19: 184-91
35. Data on file, IDEC Pharmaceuticals Corporation and Genentech Inc., 1996
36. Kelley MJ, Linnoila RI, Avis, IL, et al. Antitumour activity of a monoclonal antibody directed against gastrin-releasing peptide in patients with small cell lung cancer. *Chest* 1997; 112: 256-61
37. Modjtahedi H, Hickish T, Nicolson M, et al. Phase I trial and tumour localisation of the anti-EGFR monoclonal antibody ICR62 in head and neck or lung cancer. *Br J Cancer* 1996; 73: 228-35
38. Prewett M, Rockwell P, Rockwell RF, et al. The biologic effects of C225, a chimeric monoclonal antibody to the EGFR, on human prostate carcinoma. *J Immunother Emphasis Tumor Immunol* 1996; 19: 419-27
39. Buchsbaum DJ, Lawrence TS. New trends in the use of radio-immunoconjugates for the therapy of cancer. *Targeted Diagn Ther* 1990; 3: 215-55
40. Press OW, Eary JF, Appelbaum FR, et al. Phase II trial of 131I-B1 (anti-CD20) antibody therapy with autologous stem cell transplantation for relapsed B cell lymphomas. *Lancet* 1995; 346: 336-40
41. Press OW, Eary JF, Appelbaum FR, et al. Radiolabeled-antibody therapy of B-cell lymphoma with autologous bone marrow support. *N Engl J Med* 1993; 329: 1219-24
42. Denardo GL, Lamborn KR, Goldstein DS, et al. Increased survival associated with radiolabeled Lym-1 therapy for non-Hodgkin's lymphoma and chronic lymphocytic leukemia. *Cancer* 1997; 80 (12 Suppl.): 2706-11
43. Denardo SJ, Kramer EL, O'Donnell RT, et al. Radioimmunotherapy for breast cancer using indium-111/yttrium-90Bre-3: results of a phase I clinical trial. *J Nucl Med* 1997; 38: 1180-5
44. Kanellos J, Pietersz, GA, Cunningham Z, et al. Antitumour activity of aminopterin-monoconal antibody conjugates: in vitro and in vivo comparison with methotrexate monoclonal antibody conjugates. *Immunol Cell Biol* 1987; 65: 483-93
45. Goerlach A, Krauer KG, McKenzie IFC, et al. In vitro antitumour activity of 2'-deoxy-5-fluorouridine monoclonal antibody conjugates. *Bioconjug Chem* 1991; 2: 96-101
46. Pietersz GA. The linkage of cytotoxic drugs to monoclonal antibodies for the treatment of cancer. *Bioconjug Chem* 1990; 1: 89-95
47. Smyth MJ, Pietersz GA, Classon MJ, et al. The specific targeting of chlorambucil to tumours. *J Natl Cancer Inst* 1986; 76: 503-10
48. Peterson BH, DeHerdt SV, Scheck DW, et al. The human immune response to KS1/4-desacetylvinblastine (Ly256787) and KS1/4-deacetylvinblastine hydrazide (LY203728) in single and multiple dose clinical studies. *Cancer Res* 1991; 51: 2286-90
49. Blakey DC, Thorpe PE. An overview of therapy with immunotoxins containing ricin or its A-chain. *Antibody Immunoconjugates Radiopharm* 1988; 1: 1-16
50. Lambert JM, Goldmacher VS, Collinson AR, et al. An immunotoxin prepared with blocked ricin: a natural plant toxin adapted for therapeutic use. *Cancer Res* 1991; 51: 6236-42
51. Pietersz GA, Kanellos J, McKenzie IFC. Novel synthesis and in vitro characterization of a disulphide linked ricin-monoconal antibody devoid of galactose binding activity. *Cancer Res* 1988; 48: 4469-76
52. Camera L, Del-Vecchio S, Petrillo A, et al. Evaluation of therapeutic response using iodine-131-B72.3 monoclonal antibody in patients with ovarian carcinoma. *Eur J Nucl Med* 1991; 18: 269-73
53. Steward JSW, Hird V, Snook D, et al. Intraperitoneal Yttrium-90-labelled monoclonal antibody in ovarian cancer. *J Clin Oncol* 1990; 8: 1941-51
54. Alvarez RD, Partridge EE, Khazaeli MB, et al. Intraperitoneal radioimmunotherapy of ovarian cancer with 177-Lu-CC49: a phase I/II study. *Gynecol Oncol* 1997; 65: 94-101
55. Goldenberg DM, Horowitz JA, Sharkey RM, et al. Targeting dosimetry, and radioimmunotherapy of B-cell lymphomas with iodine-131-labelled LL2 monoclonal antibody. *J Clin Oncol* 1991; 9: 548-64
56. Vriesendorp HM, Herpst JM, Germack MA, et al. Phase I-II studies of yttrium labelled antiferritin for end-stage Hodg-

- kin's disease, including radiation therapy oncology group. *J Clin Oncol* 1991; 9: 918-28
57. Ychou M, Pelegrin A, Faurous P, et al. Phase-I/II radio-immunotherapy study with iodine-131 labeled anti-CEA monoclonal antibody F6 F(ab')₂ in patients with non-resectable liver metastases from colorectal cancer. *Int J Cancer* 1998; 75: 615-9
 58. Juweid ME, Sharkey RM, Behr T, et al. Radioimmunotherapy of patients with small-volume tumours using iodine-131-labeled anti-CEA monoclonal antibody NP-4 F(ab')₂. *J Nucl Med* 1996; 37: 1504-10
 59. Cox JP, Jankowski KJ, Katakly R, et al. Synthesis of a kinetically stable yttrium-90 labelled macrocycle-antibody conjugate. *J Chem Soc Chem Commun* 1989: 797-8
 60. Moi MK, Denardo SJ, Meares CF. Stable bifunctional chelates of metals used in radioimmunotherapy. *Cancer Res* 1990; 50 Suppl.: 789S-793
 61. Harrison A, Walker CA, Parker D, et al. The in vivo release of yttrium-90 from cyclic and acyclic ligand-antibody conjugates. *Nucl Med Biol* 1991; 18: 469-76
 62. Denardo GL, Kroger LA, Denardo SJ, et al. Comparative toxicity studies of yttrium-90 MX-DTPA and 2-IT-BAD conjugated monoclonal antibody (BrE-3). *Cancer* 1994; 73 Suppl.: 1012-22
 63. King DJ, Antoniow P, Owens RJ, et al. Preparation and preclinical evaluation of humanised A33 immunoconjugates for radioimmunotherapy. *Br J Cancer* 1995; 72: 1364-72
 64. Juweid M, Swayne LC, Sharkey RM, et al. Prospects of radioimmunotherapy in epithelial ovarian cancer: results with iodine-131-labeled murine and humanized MN-14 anti-carcinoembryonic antigen monoclonal antibodies. *Gynecol Oncol* 1997; 67: 259-71
 65. Ford CHJ, Newman CE, Johnson JR, et al. Localisation and toxicity study of a vindesine-antiCEA conjugate in patients with advanced cancer. *Br J Cancer* 1983; 47: 35-42
 66. Melino G, Hobbs JR, Radford M, et al. Drug targeting for 7 neuroblastoma patients using human polyclonal antibodies. *Protides Biol Fluids* 1984; 32: 413-6
 67. Takahashi T, Yamaguchi T, Kitamura K, et al. Clinical application of monoclonal antibody-drug conjugates for immunotargeting chemotherapy of colorectal carcinoma. *Cancer* 1988; 61: 881-8
 68. Oldham RK, Lewis M, Orr DW, et al. Adriamycin custom-tailored immunoconjugates in the treatment of human malignancies. *Mol Biother* 1988; 1: 103-13
 69. Tjandra JJ, Pietersz GA, Smyth MJ, et al. Phase I clinical trial of drug-monoclonal antibody conjugates in patients with advanced colorectal carcinoma-a preliminary report. *Surgery* 1989; 106: 533-45
 70. Orr D, Oldham R, Lewis M, et al. Phase I trial of mitomycin C immunoconjugate cocktails in human malignancies. *Mol Biother* 1989; 1: 229-40
 71. Schneck D, Butler F, Dugan W, et al. Phase I studies with a murine monoclonal antibody vinca conjugate (KS1.4-DAVLB) in patients with adenocarcinomas. *Antib Immunoconjugates Radiopharm* 1989; 2: 93-100
 72. Oldham RK, Lewis M, Orr DW, et al. Individually specified drug immunoconjugates in cancer treatment. *Int J Biol Markers* 1989; 4: 65-77
 73. Elias DJ, Hirshowitz L, Kline LE, et al. Phase I clinical comparative study of monoclonal antibody KS1/4 and KS1/4-methotrexate immunoconjugate in patients with non-small cell lung carcinomas. *Cancer Res* 1990; 50: 4154-9
 74. Data on file, Celltech plc (UK) and Wyeth-Ayerst research of Randor (USA), 1997
 75. Zein N, Sinha AM, McGahren, et al. Calicheamicin γ 1: an antitumour antibiotic that cleaves double-stranded DNA site specifically. *Science* 1998; 240: 1198-201
 76. Pimm MV. Drug monoclonal antibody conjugates for cancer therapy: potentials and limitations. *CRC Crit Rev Therap Drug Carrier Syst* 1988; 5: 189-227
 77. Byers VS, Rodvien R, Grant K, et al. Phase I study of monoclonal antibody-ricin A chain immunotoxin Xoma Zyme-791 in patients with metastatic colon cancer. *Cancer Res* 1989; 49: 6153-60
 78. Weiner LM, O'Dwyer J, Kitson J, et al. Phase I evaluation of an anti-breast carcinoma monoclonal antibody 260-F9-recombinant A chain immunoconjugate. *Cancer Res* 1989; 49: 4062-7
 79. Gould BJ, Borowitz MJ, Groves ES, et al. Phase I study of an anti-breast cancer immunotoxin by continuous infusion: report of a targeted toxic effect not predicted by animal studies. *J Natl Cancer Inst* 1989; 81: 775-81
 80. Oratz R, Speyer JL, Wernz JC, et al. Anti melanoma monoclonal antibody-ricin A chain immunoconjugate (XMME-001-RTA) plus cyclophosphamide in the treatment of malignant melanoma: results of a phase II trial. *J Biol Response Mod* 1990; 9: 345-54
 81. LeMaistre CF, Rosen S, Frankel A, et al. Phase I trial of H65-RTA immunoconjugate in patients with T-cell lymphoma. *Blood* 1991; 78: 1173-82
 82. Vitetta ES, Stone M, Amlot P, et al. Phase I immunotoxin trial in patients with B-cell lymphoma. *Cancer Res* 1991; 51: 4052-8
 83. Gonzalez R, Salem P, Bunn Jr PA, et al. Single dose murine monoclonal antibody ricin A chain immunotoxin in the treatment of metastatic melanoma: a phase I trial. *Mol Biother* 1991; 3: 192-6
 84. Engert A, Diehl V, Schnell R, et al. A phase-I study of an anti-CD25 ricin A-chain immunotoxin (RFT5-SMPT-dgA) in patients with refractory Hodgkin's lymphoma. *Blood* 1997; 89: 403-10
 85. Pai LH, Wittes R, Setser A, et al. Treatment of advanced solid tumours with immunotoxin LMB-1: an antibody linked to *Pseudomonas* exotoxin. *Nat Med* 1996; 2: 350-3
 86. Laske DW, Muraszko KM, Oldfield, EH, et al. Intraventricular immunotoxin therapy for leptomeningeal neoplasia. *Neurosurgery* 1997; 41: 1039-49
 87. Stone MJ, Sausville EA, Fay JW, et al. A phase I study of bolus versus continuous infusion of the anti-CD19 immunotoxin, IgG-HD37-dgA, in patients with B-cell lymphoma. *Blood* 1996; 88: 1188-97
 88. LoRusso PM, Lomen PL, Redman BG, et al. Phase I study of monoclonal antibody-ricin A chain immunoconjugate Xomazyme-791 in patients with metastatic colon cancer. *Am J Clin Oncol* 1995; 18: 307-12

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