

Immunotherapy with Radio-immune Conjugates

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Monoclonal antibodies have been used in a variety of ways in the management of cancer including diagnosis, monitoring, and treatment of disease. The U.S. Food and Drug Administration (FDA) has approved numerous monoclonals for the treatment of cancer (Table 13.1). Among the unmodified monoclonal antibodies, Panitumumab (Vectibix), cetuximab (Erbix) and bevacizumab (Avastin) are now marketed for metastatic colorectal cancer, trastuzumab (Herceptin) for breast cancers that overexpress HER-2 receptors, and alemtuzumab (Campath) for B-cell lymphocytic leukemia (B-CLL). Several other monoclonal antibodies are in late-stage clinical trials. With the general availability of these agents, it appears that antibody-based therapeutics have an established role in clinical oncology.

Radio-immunotherapy (RIT) utilizes an antibody labeled with a radionuclide to deliver cytotoxic radiation to a target cell. In cancer therapy, a monoclonal antibody¹ (maB) with specificity for a tumor-associated antigen is used to deliver a lethal dose of radiation to the tumor cells. The ability of the antibody to specifically bind to a tumor-associated antigen increases the dose delivered to the tumor cells while decreasing the dose to normal tissues. Whilst antibodies armed with drug conjugates and immunotoxins kill only the targeted cell, radionuclide conjugates can exert a bystander effect, destroying adjacent cells that lack antigen expression.² With external beam therapy, only a limited area of the body is irradiated. However, RIT, like cytotoxic chemotherapy, is a systemic treatment that, in principle, can eliminate metastatic disease throughout the body.

A number of issues must be addressed in designing an optimal systemic radio-immunotherapeutic agent, including (1) selection of the antigenic target to which the radio-immunoconjugate will bind to, (2) choice of a carrier molecule that will face the least barriers, and (3) choice of the radionuclide. We will address these issues separately.

TUMOR-ASSOCIATED ANTIGENS

The molecular abnormalities which are involved in neoplastic growth result in differences between malignant and non-malignant cells. These differences are exemplified in the DNA, RNA, proteins and other molecules, which in turn can be found intracellularly or displayed on the surface of tumor cells. Investigators have

TABLE 13.1. Monoclonal antibodies currently used in oncology

Antibody	Antigenic Target	Cancer Type	FDA Approval
Vectibix (Panitumumab)	EGFR	Colorectal carcinoma	2006
Erbitux (Cetuximab)	EGFR	Squamous cell carcinoma of the head and neck	2006
Avastin (Bevacizumab)	VEGF	Colorectal carcinoma	2004
		Non-small cell lung cancer Colorectal carcinoma	2006
CamPATH (Alemtuzumab)	CD52	B-cell chronic lymphocytic leukemia	2001
Zevalin (⁹⁰ Y-Ibritumomab)	CD20	Non-Hodgkin's lymphoma	2001
Bexxar (I ¹³¹ -Tositumomab)	CD20	Non-Hodgkin's lymphoma	2001
Mylotarg (Gemtuzumab Ozogamicin)	CD33	Acute myelogenous Leukemia	2000
Ontak (Denileukin difitox)	CD25	Cutaneous T-cell leukemia	1999
Herceptin (Trastuzumab)	HER-2	HER-2 positive breast cancer	1998
Rituxan (Rituximab)	CD20	Non-Hodgkin's lymphoma	1997

exploited the characteristics of these molecules and have devised means to make them more visible to the immune system, or make them serve as targets for directed therapy.³

Antigens which could be targeted for cancer therapy would ideally have high and homogeneous expression in tumors, minimal expression in normal tissues, little or no soluble form, and accessibility from the circulation.⁴ Such characteristics are, however, rarely found. Expression is heterogeneous, and there is the potential for loss of the antigenic target due to shedding, internalisation, modulation of its form, or down-regulation of its expression. In addition, the presence of the antigen on normal cells raises issues of cross-reactivity and toxicity, and compromises therapeutic effectiveness. All the factors mentioned are important issues to be considered in defining antigens on tumor cells and in designing optimum targeting strategies.

To date, a number of tumor-associated antigens have been identified.³ Cell surface antigens or receptors on normal cells may be overexpressed in tumors. Examples include interleukin-2 (IL-2) receptors,⁵ the epidermal growth factor (EGF) receptor (*c-erb-B1*)⁶ and the HER-2/neu (*c-erb-B2*) antigen.⁷ In certain instances, tumor cells, due to defects in their glycosylation pathways, express unusual carbohydrate moieties on their surface glycoproteins, – for example polymorphic epithelial mucin (PEM).⁸

Malignant cells have the tendency to become more primitive and as a result express 'oncofoetal' antigens on their surface,⁹ such as carcinoembryonic antigen

(CEA)¹⁰ and placental alkaline phosphatase (PLAP).¹¹ Intracellular protein targets for therapy include viral antigens and mutated proteins. In addition, targets include proteins which are not usually accessible in normal, viable cells, such as histones and cytokeratins, but are easy to target in necrotic, permeable tumor masses because of leaky vascularisation.¹²

Angiogenesis, although normal during fetal growth and wound healing, is an abnormal process occurring during tumor growth and metastasis.¹³ Novel approaches for destroying tumors by attacking their vasculature are being developed, exploiting the presence of antigens in neovascular endothelium. For example, endoglin and endosialin are two endothelial cell surface antigens that preferentially express on proliferating vascular cells, and might be exploited as targets for cytotoxic therapy.¹⁴ Vascular endothelial growth factors (VEGFs) and their receptors have been demonstrated to produce angiogenesis reduction when targeted with blocking molecules.¹⁵ Other angiogenic markers identified so far include three domains of fibronectin which are overexpressed in tumor-derived cells: IIICS, ED-A and ED-B.¹⁶

BARRIERS TO SUCCESSFUL ANTIBODY THERAPY

The most important limitation is antigen specificity. Few, if any, monoclonal antibodies react only with tumor cells and fail to react with normal tissues. Moreover, antigens that modulate and are shed into the circulation, such as CD10 in ALL, have generally proven to be poor targets for targeted therapy. An exception to this generalization has been observed with HER-2/*neu*, which has demonstrated substantial activity against breast cancer, alone and in combination with chemotherapy. The extracellular domain of HER-2/*neu* is cleaved and has been used as a marker for receptor overexpression.

Due to their size, monoclonal antibodies have slower kinetics of distribution and less tissue penetration than do conventional drugs.¹⁷ The success of an antibody to localize to tumors depends on several factors. Biodistribution studies indicate distance from blood vessels to be a factor of importance with respect to antigen recognition and binding. In addition, central areas of bulky disease have poor blood supply and increased intratumoral fluid pressure, making them less accessible to immunoconjugates.¹⁸ Furthermore, large masses can act as antigenic sinks, decreasing drug delivery to other tumor sites.¹⁹ Modeling studies led Juweid and colleagues to formulate the hypothesis of the binding-site barrier, which postulated that antibody molecules could be prevented from penetrating tumors by the very fact of their successful binding to peritumoral antigen. Intracavitary therapy has been used in an attempt to improve access of antibody to tumor cells, but the antibody generally penetrates only a few millimeters beneath the serosal surface.

Heterogeneity has been observed in antigen expression within and between cancers from different individuals. Cells that lack antigen expression cannot be effectively targeted. With unconjugated antibodies that lack “bystander” activity, a

combination of several reagents may be required to target all cells. This is where the use of radioimmunotargeting is advantageous.

The host's response to the foreign immunoglobulin is a major limitation. Because a large number of antibodies used clinically are derived from mice, they can induce the development of human anti-mouse antibodies (HAMAs). The presence of HAMAs can prevent effective delivery of murine monoclonal antibodies to tumor cells, particularly when multiple doses must be administered to obtain optimal antitumor activity. Genetic manipulation of murine monoclonal antibodies has been used to generate less immunogenic reagents. Chimeric (60% human) and humanized (95% human) antibodies have been engineered to retain the murine antigen-binding complementarity regions in association with human framework regions.²⁰ Although the immunogenicity of such antibodies can be substantially reduced and HAMA responses can be limited, their injection can still evoke an anti-idiotypic response. Unlike murine antibodies, human or humanized antibodies that contain the human Fc antibody portion trigger ADCC and complement-dependent cytotoxicity. The availability of antibodies derived entirely from humans, such as those isolated from combinatorial libraries using the process of phage display, has revolutionized therapeutic strategies.²¹ Genetic engineering has also been used to produce single-chain antigen binding proteins that have more favorable pharmacokinetic properties than intact immunoglobulin or Fab fragments.

CHOICE OF THE RADIONUCLIDE

The third component of an optimal radio-immunotherapeutic regimen to consider is the nature of the radionuclide used. Radionuclides used in monoclonal-based therapy, their physical half-lives, emissions, and path lengths are listed in Table 13.2.

To take advantage of tumor targeting, relatively short path lengths are desirable. Consequently, radio-isotopes have been chosen that emit alpha particles or beta particles rather than gamma rays. The path of beta emissions can range from 1 to 10 millimeters and exert a bystander effect on antigen-negative neighboring cells. Alpha particles have a very short path length but a very high rate of linear energy transfer (LET). The biologic effectiveness of such high LET radiation does not require the presence of oxygen, nor does it depend on dose rate.²² Overall, tumor response depends on multiple factors such as dose rate, cumulative radiation dose, and the actual radiosensitivity of the tumor.

Most published clinical studies used the β -emitting radionuclides ^{90}Y or ^{131}I . Such β -emitting radionuclides depend on cross-fire for their action on large tumor masses. However, as the tumor mass decreases, the benefit of the crossfire effect also decreases. With various small tumors including leukemias, the therapeutic effect of high-energy β -emitting radionuclides is limited because they yield a high dose of irradiation outside of the tumor volume as a result of the long path of the β -irradiation. For such forms of malignancy, the development of pre-targeting approaches²³ focuses on α -emitting radionuclides that are the most effective agents

TABLE 13.2. Radionuclides used in monoclonal-antibody-based cancer radiotherapy regimens

Isotope	Half-life (h)	Emission	Maximum Energy (keV)	Maximum Particle Range (mm)
^a Iodine-131 (¹³¹ I)	193	Beta	610	2.0
Yttrium-90 (⁹⁰ Y)	64	Beta	2280	12.0
Lutetium-177 (¹⁷⁷ Lu)	161	Beta	496	1.5
Copper-67 (⁶⁷ Cu)	62	Beta	577	1.8
^a Rhenium-186 (¹⁸⁶ Re)	91	Beta	1080	5.0
Rhenium-188 (¹⁸⁸ Re)	17	Beta	2120	11.0
Bismuth-212 (²¹² Bi)	1	Alpha	8780	0.09
Bismuth-213 (²¹³ Bi)	0.77	Alpha	>6000	<0.1
Astatine-211 (²¹¹ At)	7.2	Alpha	7450	0.08

^a They also have gamma emissions.

Source: Adapted from Chester KA, Hawkins RE. Clinical issues in antibody design. *Trends Biotechnol* 1995; **13**: 294–300.

for killing tumor cells without damaging adjacent normal tissues. This separates the antibody targeting from the delivery of the radionuclide. The antibody is first targeted to the tumor followed by clearance of the residual circulating antibody that is facilitated by a clearing agent. A radioactive agent is then administered for selective capture at the tumor site. The problem of pre-targeting strategies is their inherent complexity and the immunogenicity of the components, which are generally not of human origin.

Schemes for pretargeted RIT have occasionally used bispecific antibodies with specificities for both tumor and radionuclide chelator,²⁴ but more commonly, the very high-affinity interaction between biotin and avidin or streptavidin has been exploited. An infused antibody – streptavidin conjugate or fusion protein – is first allowed to localize to a tumor target. A clearing agent is then used to remove the remaining circulating conjugate. Delivery of a radionuclide is achieved with the use of a biotinylated chelator. The chelator, radionuclide complex, is either captured by the antibody – streptavidin bound to tumor cells – or cleared rapidly through the kidney due to its low molecular weight. Significant advantages of pretargeted therapy over conventional RIT include the much greater tumor-to-normal-tissue ratio, thereby lowering the whole-body exposure. The immunogenicity of streptavidin might, however, prevent the repeated treatment cycles that may be required for effective therapy. In addition, a rather large quantity of radionuclide must be administered to capture a small fraction of the radionuclide at the tumor target site.

β EMITTERS

In beta decay, a neutron inside the nucleus of an atom breaks down and changes to a proton, emits an electron, and then the atomic number goes up by one and the mass number remains unchanged.²⁵ β rays are more suited for tumors larger than 0.5cm

and are advantageous over α -particles in the sense that, because of their longer path length, not every cell needs to be targeted to be killed. The traversals of tumor cells by multiple β -particles result in enhanced killing by cross-fire, partially compensating for nonhomogeneity of antigen expression, whereas the short path length of α -particles increases the requirement for much greater homogeneity of targeting cells within a tumor. This limitation may be more significant for solid tumors, which are often poorly vascularised and have high interstitial pressure, as previously mentioned due to poor lymphatic drainage.

Although beta emissions can kill tumor cells, normal cells will also be affected by the circulating radio-isotopes to varying extents. For example, the bone marrow cells of patients who have a significant amount of lymphoma in the bone marrow are particularly sensitive to radiation damage.

^{131}I was the first isotope used in radiotherapy, but it was associated with low energy β particles, emitted unwanted γ radiation, the biological half-life of the conjugate in the tumor area was short due to the action of tissue dehalogenases.²⁶ In addition, myelosuppression may follow ^{131}I -antibody treatment from the radiation dose that the bone marrow receives from the circulating conjugate. Alternatively, ^{90}Y emits only β -particles of appropriate energy for therapy, but still presents problems associated with myelosuppression. The extent of heterogeneity of dose deposition in tumors is highly dependent on the antibody characteristics and radionuclide properties, and can enhance therapeutic efficacy through the selective dose delivery to the radiosensitive areas of the tumor. In a study by Flynn and colleagues where the aim was to assess the influence of radionuclide characteristics on the heterogeneity of dose deposition,¹³¹ I generally delivered a higher dose throughout the tumor even though the instantaneous dose-rate distribution for ^{90}Y was more uniform.²⁷

α EMITTERS

Alpha emissions have energies in the several MeV range with a high probability to produce cytotoxic DNA double-strand breaks. The range is, however, short enough to avoid damage to nontargeted regions, but a homogeneous antibody distribution is essential if a bystander effect is to be observed on antigen-negative cells. The interest in Bismuth-212 and Bismuth-213 has been steadily increasing due to their availability and to the fact that with Bismuth-212, the ^{212}Pb precursor (longer half-life) can be used as an *in vivo* ^{212}Bi -generator.²⁸ Astatine-211 has been conjugated to antibodies (rituximab) and demonstrated a very short half-life, short path length and a very high tumor to normal cell toxicity ratio *in vitro*.

Other studies have shown that radio-immunotherapy of micrometastatic disease, monocellular bloodborne malignancies (such as leukemias, lymphomas), and malignancies spread on the surface of body compartments (like neoplastic meningitis) using high linear energy transfer α -particles and monoclonal antibody fragments have therapeutic advantages over β radio-immunotherapy.²⁹ Such

micrometastases from the residual disease are life-threatening and lead to relapse and mortality from the postradical metastatic residual disease.

RADIO-IMMUNOTHERAPY IN HAEMATOLOGIC MALIGNANCIES

In haematopoietic neoplasms, which are more radiosensitive, lower radiation doses can induce greater tumor responses. Lymphomas are particularly attractive targets considering their inherent radiosensitivity as well as the presence of differentiation antigens at the lymphoma cell surface. Arming anti-CD20 antibodies with radionuclides has resulted in significant antitumour responses in patients with non-Hodgkin's lymphoma (NHL). Two anti-CD20 monoclonals – Zevalin (90Y-ibrutinomab) and Bexxar (131I-tositumomab) – were approved by the FDA in 2002 and 2003, respectively, for radio-immunotherapy of NHL patients either relapsed or refractory to chemotherapy and rituximab. Phase III clinical trials showed that in comparison to rituximab or chemotherapy, the enhanced targeted cytotoxicity provided by these radio-immunoconjugates translated into significantly higher overall (OR) and complete remissions (CR).³⁰ A phase II study with Bexxar as first-line therapy for stage III and IV follicular lymphoma resulted in CR of 75% and OR of 95%.³¹

It is important to note that prior to the introduction of rituximab and its yttrium-90 conjugate, Zevalin, there were no targeted therapies for lymphoma and the outcomes were poor for many patients. This is therefore a significant addition to the treatment options and it has been extremely effective in treating patients resistant to more conventional therapies.

Lym-1, a mAb that selectively targets malignant lymphocytes, also has induced therapeutic responses and prolonged survival in patients with NHL when labeled with iodine-131 (¹³¹I).^{32,33} The antibody Lym-1 is specific for a human leukocyte antigen (HLA-DR) expressed in >95% of B cell tumors. This murine MAb has not been humanized. Lym-1 has shown efficacy in patients who have failed chemotherapy, either with low-grade or aggressive forms of NHL.

The cell surface antigen CD33 is expressed on most myeloid leukemic blasts and leukemic progenitor cells. Its normal tissue expression is limited to committed normal myelomonocytic and erythroid progenitor cells and (at low levels) early hematopoietic stem cells. M195, a murine anti-CD33 MAb, has been used to deliver therapeutic doses of ¹³¹I in combination with busulfan or cyclophosphamide to eliminate disease before bone marrow transplantation.³⁴ HuM195, a humanized version of M195, has been employed as a vehicle for the RAIT of acute and chronic myelogenous leukemia. HuM195 RAIT resulted in minor responses in 8 of 12 patients treated with ⁹⁰Y-conjugated Mab and 13 of 18 patients treated with ²¹³Bi-conjugated Mab.^{35,36}

RADIO-IMMUNOTHERAPY IN SOLID TUMORS

Activity of radio-immunoconjugates has also been shown for solid tumors with varying success. Several molecules have been used against antigenic targets for

the detection and therapy of colorectal, breast, lung, ovarian and medullary thyroid cancers.³⁷

High-dose radio-immunotherapy followed by stem-cell haematologic rescue has resulted in delivery of higher radiation doses to tumors. Trials involving patients with solid tumors, including breast, gastrointestinal, and prostate cancer, produced variable antitumor responses, but these were not as impressive as the responses observed in haematologic malignancies.

BREAST CANCER

Breast cancer is the second most common cause of cancer death in women in the United States. Although more than 60% of patients can now be cured by initial treatment, the rest will die of their disease. Early detection of micrometastases and improved treatment using monoclonal antibodies may provide an effective means of increasing the prospects for survival. Radiolabelled monoclonal antibodies are currently being applied for the treatment of primary or metastatic breast cancer, in experimental, pre-clinical, or clinical trials, in combination with traditional external beam radiotherapy and/or chemotherapy. Antigen targets have included primarily Carcinoembryonic antigen (CEA), MUC1, and L6. Radio-immunotherapy comprises systemically administered monoclonal antibodies, linked to high-energy, beta-emitters. Radioactive antibodies, in the form of [⁹⁰Y]BrE-3, ⁹⁰Y-m170 and ⁹⁰Y-labelled L6 antibody, are applied with adjuvant autologous peripheral blood stem cells transfusion to prevent myelotoxicity. Partial or rarely complete responses to “hot” antibody treatment of breast cancer have been reported. Innovative strategies using this combined-modality treatment hold promise for better disease-free and survival rates.

BrE-3 antibody, a murine IgG1 monoclonal, reacts with an epitope on the tandem repeat of the peptide core of MUC-1.³⁸ A Phase I trial was performed to explore the use of [⁹⁰Y]BrE-3 murine Ab.³⁹ Although responses were observed, an immune response prevented further use of this Ab. A humanized version has been evaluated in a clinical trial, and 8 of 17 patients (47%) showed responses despite failing previous conventional therapies.⁴⁰

The anti-MUC1 Ab m170, radiolabeled with ⁹⁰Y and combined with paclitaxel, has progressed to dosimetric studies with measurable tumor regression and partial responses.⁴¹

L6 cell surface antigen is highly expressed in breast cancer and is related to a number of cell surface proteins with similar predicted membrane topology implicated in cell growth. ⁹⁰Y-DOTA-peptide-ChL6 resulted in excellent tumor targeting and an effective therapeutic index in preclinical studies.^{42,43}

CEA is expressed in normal tissues and in cancers, including breast carcinomas.⁴⁴ NP-4, a murine anti-CEA Ab labeled with ¹³¹I, resulted in therapeutic responses in a Phase I/II study. When 57 patients were treated with [¹³¹I]NP-4, modest antitumor activity was seen in 12 of 35 assessable patients, with one partial remission, four minor/mixed responses and seven instances of stabilization of progressing disease.⁴⁵

COLORECTAL CANCER

Some of the most advanced radio-immunoconjugates relate to gastrointestinal disease. The antigens targeted in colorectal cancer include Ep-CAM, TAG-72, A33, and CEA.

The Ep-CAM receptor has been used as a target for the NR-LU-10 antibody. The results of a Phase II clinical trial of [⁹⁰Y]DOTA-biotin pretargeted by NR-LU-10 Ab/streptavidin in patients with metastatic colon cancer were reported,⁴⁶ but the agent suffered from side effects such as bowel toxicity due to antigen cross-reactivity.

TAG-72 antigen has been targeted by the I¹³¹-CC49 antibody but failed to produce significant clinical responses.⁴⁷ The ⁹⁰Y-labelled antibody was evaluated in a Phase I clinical trial to avoid dehalogenation issues, but its potential use has been hampered by high hepatic doses.⁴⁸

The A33 antigen is a promising radioimmunotherapy target as it is highly and homogeneously expressed in 95% of all colorectal carcinomas. In a Phase I trial, colorectal patients were treated with a combination of [¹³¹I]huA33 and [¹²⁵I]huA33 one week before surgery.⁴⁹ No dose-limiting toxicity was observed and excellent tumor uptake was demonstrated. Higher doses were administered in a corresponding Phase I dose-escalation trial of [¹³¹I]huA33 with excellent targeting resulting in 4 of 15 patients having stable disease.⁵⁰

A Phase I/II clinical trial of ⁹⁰Y-labeled hMN14, a humanized radiolabelled Ab targeting CEA, was performed in patients with colorectal between 2000 and 2004.⁵¹ A radio-halogenated version of the same Ab, [¹³¹I]labetuzumab, gave impressive results in a Phase II trial in 19 colorectal cancer patients after salvage resection of liver metastases.⁵² The same antibody, Yttrium-90 labelled, was used in a clinical trial in the United States but was terminated for unspecified reasons, possibly due to the unsuitability of ⁹⁰Y for treating limited residual disease after surgery.⁵³

Another ⁹⁰Y-labeled anti-CEA Ab, T84.66, was tested in a Phase I trial in combination with 5-fluorouracil.⁵⁴ No objective responses were observed, but more than half of the patients shifted from progressive to stable disease.

OVARIAN CANCER

Two tumor associated monoclonal antibodies (human milk fat globule membrane protein antibodies) HMFG1 and HMFG2 directed against MUC-1 and labelled with ¹²³I have been used to detect primary and metastatic ovarian, as well as breast, and gastrointestinal neoplasms.^{55,56} ⁹⁰Y-labeled HMFG1 murine mAb (pemtumomab) has been used to treat patients with advanced ovarian cancer following conventional therapy.⁵⁷ Encouraging results were obtained in patients with minimal residual disease, with 50% complete remission several years after treatment. Following surgery, chemotherapy and intraperitoneal radio-immunotherapy, 78% of the 21 patients in complete remission survived for >10 years. Unfortunately, [⁹⁰Y]HMFG1 then failed to demonstrate a therapeutic effect in a multi-institution international randomized concurrently controlled Phase III clinical trial.

Ovarian cancer patients were also treated with intravenously administered ^{131}I -Labeled chimeric monoclonal antibody MOv18.⁵⁸ Therapeutic doses could be achieved without normal organ toxicity. Immunospecific localization of antibody on antigen-expressing tumors has been demonstrated, suggesting that further studies should be carried out.⁵⁹

Currently, there are no radiolabeled Abs in late-stage clinical development for ovarian cancer, although a number are currently in Phase I/II clinical trials, including [^{90}Y] HU3S193 at Memorial Sloan-Kettering Cancer Center and [^{90}Y] CC49 at the University of Alabama.

PROSTATE CANCER

Prostate antigenic targets have been targeted with radio-immunoconjugates with variable success. No major responses were observed in therapeutic studies targeting TAG-72 in prostate cancer patients.⁶⁰

A particularly promising target would seem to be prostate-specific membrane antigen (PSMA). The most well-known radiolabeled Ab to PSMA is [^{111}In] capromab pendetide (ProstaScint), which was approved 10 years ago by the FDA for imaging soft tissues, but not bone sites, of metastatic prostate cancer for presurgical staging or evaluation of PSMA relapse after local therapy.^{61,62} For presurgical patients with high-risk disease but negative bone CT and MRI scans, capromab was able to identify some patients with positive nodes, thereby sparing them not-indicated surgery.

Promising results have been obtained using ^{90}Y -J591 antibody in treating hormone-refractory metastatic prostate cancer.⁶³ This antibody targets the extracellular domain of PSMA. Patient recruitment is ongoing for a Phase II trial to study the efficacy of [^{177}Lu]DOTA-J591 in the treatment of metastatic prostate cancer.

In the combined modality radio-immunotherapy of prostate cancer for treating disseminated disease, chemotherapeutic doses have been employed which would otherwise not be tolerated with external beam radiation. O'Donnell and colleagues⁶⁴ published the combined effects of a radioimmunoconjugate ^{90}Y trium-DOTA-Peptide-ChL6 with taxanes in mice. They observed a 67% cure rate, whereas no mice were cured with radioimmunotherapy alone or chemotherapy alone. The doses used are achievable in humans and are expected to provide therapeutic synergy without increased toxicity.

LUNG CANCER

Lung cancer is the most common cancer in the world. Over half a million new cases are diagnosed annually in the world's three major markets. The disease has a poor prognosis and it is the main cause of cancer death in the UK with around 37,000 deaths every year.

Verluma is a $^{99\text{m}}\text{Tc}$ -labeled Fab fragment for identifying advanced-stage disease in patients with small-cell lung cancer.^{65,66} It was approved in 1996 but was recently

abandoned because, even though Verluma could accurately determine whether the disease was limited or extensive, it sometimes failed to image tumors and additional standard diagnostic tests were required.

In 2005, a Phase I study of [⁹⁰Y]CC49 in advanced non-SCLC patients yielded very disappointing results, warranting the development of a humanized version of CC49.⁶⁷ There were no objective tumor responses, and both immunogenicity and hematologic toxicities were problematic.

RENAL CANCER

Metastatic renal carcinoma has been treated with a ¹³¹I-labelled mouse monoclonal antibody (G250).⁶⁸ Thirty-three patients with measurable metastatic renal cell carcinoma were treated in a study by Divgi and colleagues. There were no major responses. On the basis of external imaging, ¹³¹I-labeled mouse monoclonal antibody G250 showed excellent localization to all tumors that were > or = 2 cm. Seventeen of 33 patients had stable disease, with tumor shrinkage observed in two patients. Antibody immunogenicity restricted therapy to a single infusion. A follow-up Phase I dose-escalation trial showed that fractionation did not significantly improve dose-limiting hematopoietic toxicity.⁶⁹

BRAIN CANCER

Brain cancer is one of the fastest growing and deadliest forms of cancer. According to the Central Brain Tumor Registry of the United States, each year in the U.S. alone, an estimated 35,500 new cases of primary brain tumors are diagnosed. Approximately 23% of all brain tumors are glioblastomas, which are only rarely cured.

In 2001, the U.S. Food and Drug Administration (FDA) has granted fast track status to Cotara^(TM) ([¹³¹I]chTNT Peregrine Pharmaceuticals, Inc.) for the treatment of recurrent glioblastoma multiforme. It is a radiolabeled monoclonal antibody that binds to the DNA exposed in necrotic zones. The clinical experience with [¹³¹I]chTNT to date has been recently reviewed by Shapiro et al.⁷⁰

Tenascin-C (TN-C) is an extracellular matrix glycoprotein that is expressed ubiquitously in high-grade gliomas but not in normal brain. A study of locoregional radio-immunotherapy of high-grade malignant gliomas was performed using anti-tenascin antibodies labelled initially with ¹³¹I and then with ⁹⁰Y.⁷¹ Using this technique, tumor growth could be retarded over relatively long periods of time. The glioblastoma median survival was prolonged to 25 months (¹³¹I group) or 31 months (⁹⁰Y group). The response rate (which comprised of PR, CR, and NED) was 47.1% (glioblastoma ¹³¹I group) or 40% (glioblastoma ⁹⁰Y group). In many cases a significant tumor shrinkage effect was observed. The use of ⁹⁰Y proved more favourable in bulky lesions and reduced the radioprotection problems.

Another study was performed that assessed the efficacy and toxicity of the ¹³¹I-labeled murine antitenascin monoclonal antibody 81C6 and determined its true

response rate among patients with newly diagnosed malignant glioma. Intratumoral administration of [^{131}I]81C6 has shown promise in a Phase I trial.⁷² In a more recent Phase II study at Duke University, the efficacy and toxicity of [^{131}I]81C6 infused directly into the resection cavity (intracavitary injection) were assessed in 33 patients with previously untreated malignant glioma.⁷³ Median survival achieved exceeded that of historical controls treated with conventional radiotherapy and chemotherapy, confirming the efficacy of labeled 81C6 for patients with newly diagnosed malignant glioma and supporting the case for carrying out a randomized phase III study.

A three-step avidin-biotin approach was used to target ^{90}Y -biotin to the tumor in patients with recurrent high grade glioma.⁷⁴ Encouraging results obtained in this phase I-II study prompted workers to apply the same approach in an adjuvant setting, to evaluate (1) time to relapse and (2) overall survival. Results indicated that radio-immunotherapy impeded tumor progression, prolonged time to relapse and increased overall survival.

PRETARGETING STRATEGIES

Radio-immunoconjugates can be specifically targeted to cancer cells through pre-targeting.⁷⁵ This scheme typically requires two or three separate components. In one scheme, the antibody component is first targeted to the tumor followed by clearance of the residual circulating antibody, often facilitated by a clearing agent. A cytotoxic agent is then administered for selective capture or activation at the tumor site. Pre targeting of radionuclides to tumors is particularly attractive in that it has the potential to greatly reduce the systemic toxicity of conventional radio-immunotherapy and cytotoxic chemotherapy, respectively. The problem of pretargeting strategies is their inherent complexity and the immunogenicity of the components that are not of human origin. Pre targeting strategies have advanced markedly, but many obstacles remain to be overcome if they are to provide significant new treatment options for cancer patients.

Significant advantages of pretargeted over conventional radio-immunotherapy include the much greater ratios of radioactivity in tumor versus non-tumor tissues, thereby lowering the whole body exposure to radioactivity. However, the immunogenicity of molecules such as streptavidin might prevent the repeated treatment cycles that would probably be necessary for effective therapy. A further problem for pretargeted radioimmunotherapy is the large quantity of radionuclide that must be administered in comparison to the minute proportion captured at the tumor target site.

CONCLUSION

Targeted radiotherapy of cancer using monoclonal antibodies has been an attractive concept for the past 25 years. However, real interest from clinical oncologists has

only been shown in the last few years following the impressive results using radio-immunotherapy to treat haematological tumors.

Key issues in radio-immunotherapy still remain to be elucidated, such as the genomic mechanism behind the cytotoxic effect observed. It has still not been clearly addressed whether cell death is due to an apoptotic or a necrotic mechanism and the reasons behind the apparent independence of extent of cell death from dose of radiation delivered. Other issues which remain controversial are the choice of radioisotope and the ideal half life that the radio-immunoconjugate should have in order to have maximum beneficial effect at the tumor site but to cause minimum damage to normal tissues. Nevertheless, we predict, that in at least some indications, radio-immunotherapy will be increasingly employed as a useful therapeutic option either as monotherapy or as a combination with conventional chemotherapy or radiotherapy.

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