SYMPOSIUM IN WRITING

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Advancing role of radiolabeled antibodies in the therapy of cancer

Abstract This review focuses on the use of radiolabeled antibodies in the therapy of cancer, termed radioimmunotherapy (RAIT). Basic problems concerned with the choice of antibody, radionuclide, and physiology of the tumor and host are discussed, followed by a review of the pertinent clinical publications of various radio-antibody constructs in the treatment of hematopoietic and solid tumors of diverse histopathology, grade, and stage, and in different clinical settings. Factors such as dose rate delivered, tumor size, and radiosensitivity play a major role in determining therapeutic response, while target-to-nontarget ratios and, particularly, circulating radioactivity to the bone marrow determine the principal dose-limiting toxicities. RAIT appears to be gaining a place in the therapy of hematopoietic neoplasms, such as non-Hodgkin's lymphoma: several agents are advancing in clinical trials toward registration, and one has recently been approved by the FDA. Although RAIT of solid tumors has shown less progress, use of pretargeting strategies, such as an affinity-enhancement system consisting of bispecific antibodies separating targeting from delivery of the radiotherapeutic, appears to enhance tumor-to-nontumor ratios, and may increase radiation doses to tumors more selectively than directly labeled antibodies.

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Introduction

Although more than 20 years have elapsed since the introduction of radioimmunodetection (RAID) and radioimmunotherapy (RAIT), involving the use of isotopes conjugated to monoclonal antibodies (mAbs) for imaging and therapy, respectively [53, 54, 56], it has only been in the last few years that this technology has begun to gain a role in the treatment of cancer because of its success in lymphoma patients. This article is intended to review the current status of cancer therapy with radiolabeled antibodies, and to complement and extend other recent reviews [46, 47, 49, 50, 60, 66, 77, 92, 133]. It is now appreciated that (1) many radionuclides and antibodies have potential applicability for this therapy, (2) antibody accretion remains the major limitation in delivering effective tumor radiation doses, and (3) multiple administrations and combinations with other treatment modalities will prove necessary, especially in the therapy of solid tumors. Whereas RAIT of hematopoietic neoplasms is becoming established as a new future therapy modality [32, 40, 43, 48, 67, 103, 104, 127], solid tumors have been less responsive, although targeting minimal or micrometastatic disease appears at present to be the most optimal approach in solid tumor therapy. The challenge of treating solid tumors has stimulated a number of approaches to improve the radiation dose delivered and to achieve a more uniform distribution of ionizing radiation, the ultimate goal being the delivery of tumoricidal doses while sparing normal tissues.

Basic challenges

Numerous reviews have discussed the dependence of RAIT on three basic factors: the antibody, the

radionuclide, and the target tumor and host [12, 14, 29, 34, 52, 58, 115, 117, 118, 129]. The most important variables affecting tumor response include cumulative radiation dose delivered to the tumor(s), dose rate, penetration, and tumor radiosensitivity. Tumor uptake and penetration have been the most challenging limitations, since accretion has been at such low levels as 0.001–0.01% of the injected dose of radiolabeled antibody per gram of tumor, resulting in tumor doses usually of < 1,500 cGy. The dose-limiting organ has been the bone marrow, which appears to have a dose limitation of about 150–200 cGy. Hence, improvements in accretion need to be achieved before the radiation doses needed for efficacy are achieved, and here various approaches have been pursued, such as the use of agents that increase tumor vascularization (flow) and permeation, antibody combinations, repeated applications, and pretargeting methodologies, to mention a few [5, 16, 19, 20, 21, 29, 30, 31, 34, 42, 47, 52, 57, 91, 99, 114, 129].

The selection of appropriate antibodies seems to be sufficient for the principal cancer types, and the earlier limitation of using immunogenic murine antibodies has been overcome with the introduction of chimeric, humanized, and fully human antibodies. The relation of different antibody forms to tumor uptake and residence time is summarized in Table 1, showing that the smaller the molecule, the faster and lower the uptake, as well as the shorter the residence time in tumor. This suggests that larger, intact immunoglobulin molecules may still be the optimal targeting agent in RAIT. However, initial dose rates appear to be much higher with monovalent

fragments, such as Fab, as compared to whole IgG, despite lower absorbed tumor doses at equitoxic dosing; this has also resulted, in animal models, in effective control of tumor growth because of high intratumoral dose rates [8, 10].

Even the selection of radionuclides available for RAIT has expanded in recent years, such that several useful candidates are available and have shown clinical promise (Table 2). The suitability of a radionuclide resides in its physical and chemical properties, its stability after conjugation to antibody, and the nature of its radiation (high or low linear energy transfer). The choice is also influenced by the clinical setting, such as tumor location, size, morphology, physiology, and radiosensitivity, as well as the kinetics of the antibody. For example, a large tumor would be more amenable to therapy with a deep-penetrating beta-emitter, and radionuclides with shorter path-lengths of penetration, with higher LET, would be more suitable for smaller or minimal disease. The need for a radiolabeling procedure to be simple, efficient, reproducible, and affordable is self-evident, but these criteria are not necessarily fulfilled by some of the radionuclides reported in some clinical trials. Table 2 lists many of the radionuclides that have been studied clinically, but at present only ^{131}I and ^{90}Y appear to fulfill the requirements for commercial development. Nevertheless, necessity and success with any radionuclide will ultimately result in improved availability and cost, as market conditions come into play when new therapies are needed for life-threatening diseases. Recently, much interest was provoked by the

Table 1 Targeting properties of different forms of antibodies[a] (reproduced from [50], with permission)

		IgG	F(ab')$_2$	Fab'	Diabody	scFv
Physical						
	Molecular wt.	150 k	100 k	50 k	40k	20k
Biological						
	Immune effector function	Yes	No	No	No	No
	Biol. $T_{1/2}$ blood	2–3 days	1–2 days	4 h	< 4 h	1 h
	Target organ	Liver	Liver	Kidney	Kidney	Kidney
Tumor binding						
	Uptake (1 = highest to 4 = lowest)	1	2	3	3	4
	Duration (1 = longest to 4 = shortest)	1	2	3	3	4
	Optimal accretion time	Days	Day	Hours	Hours	Hour

[a]Kindly provided by R.M. Sharkey, Belleville, N.J.

Table 2 Radionuclides of current interest in RAIT (reproduced from [5], with permission)

Isotope	Half-life (h)	Emission (for therapy)	Maximum energy (keV)	Maximum particle range (mm)
Iodine-131 (^{131}I)	193	Beta	610	2.0
Yttrium-90 (^{90}Y)	64	Beta	2,280	12.0
Lutetium-177 (^{177}Lu)	161	Beta	496	1.5
Copper-67 (^{67}Cu)	62	Beta	577	1.8
Rhenium-186 (^{186}Re)	91	Beta	1,080	5.0
Rhenium-188 (^{188}Re)	17	Beta	2,120	11.0
Bismuth-212 (^{212}Bi)	1	Alpha	8,780	0.09
Bismuth-213 (^{213}Bi)	0.77	Alpha	> 6,000	< 0.1
Astatine-211 (^{211}At)	7.2	Alpha	7,450	0.08

report showing that actinium-225, with a 10-day half-life, coupled to internalizing antibodies, showed in vitro and in vivo (in mice) destruction of human tumor cells by alpha-irradiation [88].

The excellent targeting of tumors with diverse pancarcinoma antibodies and the relatively good radiosensitivity of certain neoplasms encourage the pursuit of cancer radioimmunotherapy. However, there are still too few such studies reported, and none has yet progressed beyond phase I or II trials. This may be due both to the nature of the patient population being studied and to development requirements involving the radiolabeled antibodies. As in most phase I studies, usually patients with advanced disease are entered, and these have been heavily pretreated, resulting in unfavorable patients for assessing dose response or potential efficacy. Whereas small doses of antibody fragments, which are virtually nonimmunogenic, can be used for immunoscintigraphy, higher protein doses of intact immunoglobulins used in a therapy setting can result in immune responses, especially with repeated doses, if murine antibodies are used. Therefore, there has been an effort to use chimeric, humanized (CDR-grafted), and now even fully human immunoglobulins, and these appear to be well tolerated. More potent beta-emitting radionuclides, such as ^{90}Y, have also been used increasingly as stable chelation methods have developed [60]. The few clinical trials of RAIT thus far published have focused mostly on CEA, MUC1, TAG-72, L6, and Thomsen-Friedenreich (Tn) antibodies, and have been early studies by single groups of investigators.

Optimization of RAIT

Strategies to improve RAIT reduce to five basic goals: (a) enhance antibody uptake and distribution in tumor by increasing tumor vascular permeability and flow, using smaller molecules, and possibly exploiting pretargeting strategies; (b) decrease nontargeted antibody in the blood by in vivo clearance or ex vivo adsorption mechanisms, as well as the pretargeting approaches, (c) protect normal organs from radiotoxicity, e.g., by using hematopoietic growth factors and peripheral blood stem cell reconstitution, and by blocking readsorption of antibody fragments by the kidneys with cationic amino acids, amino sugars, and their polymers; (d) decrease the immunoglobulin's immunogenicity by humanization or use of human antibodies, or immunosuppressing the host; and (e) improve the radiation dose and dose rate in tumor without concomitantly increasing cumulative radiation in normal organs, which is accomplished by many of the other strategies and perhaps also by adjusting the antibody dose and the dose schedule (e.g., dose fractionation) of the radiopharmaceutical. Since these topics have been covered in other publications [5, 16, 20, 21, 29, 30, 34, 42, 47, 52, 57, 91, 99, 114, 129], they will be addressed here only briefly and mostly in reference to clinical studies.

Dose fractionation

Regardless of which antibody or radionuclide is used in RAIT, consideration of how this is administered is now receiving considerable attention. Should the radioimmunoconjugate be given in a bolus as a single, maximally tolerated dose, or should it be administered in smaller, fractionated doses, as now practiced with external beam radiation therapy? The convention of administering external beam radiotherapy by fractionated doses has suggested that the same improved effects could be achieved with fractionated RAIT. One possible reason for such an improvement is that fractionation of the amounts of radionuclide delivered may contribute to a more uniform distribution of radiation dose throughout the tumor, with different tumor regions radiated with each dose, especially if tumor size and cell numbers decrease with the first injections, making the remaining viable cells more amenable to subsequent cytotoxic irradiation. The evidence and rationale supporting the advantage of fractionated RAIT suggest that fractionated RAIT provides more uniform distribution of antibody and radiation dose, reduced toxicity, increased maximal tolerated dose and tumor radiation, and prolongation of tumor response [36, 91, 114]. Disadvantages of fractionated RAIT have also been listed, such as lower radiation dose rate, a more complicated therapy schedule, increased cost, and a potential delay in tumor regression. In a clinical study comparing dose fractionation with single doses of radiolabeled antibody, it was found that the former produced a modest increase in the therapeutic window, because bone marrow suppression was statistically significantly less than with fractionated RAIT [114]. Such dose fractionation, however, requires the use of a nonimmunogenic antibody, such as human or humanized (CDR-grafted) forms, since fractionated small doses of foreign immunoglobulin, such as murine antibodies, would result in human anti-murine antibodies that affect the kinetics of the radiolabeled antibody with subsequent doses [114].

Combination RAIT and chemotherapy

Most efforts to improve RAIT have focused on increasing the uptake of the radioimmunoconjugate, improving its penetration and distribution within the tumor, and enhancing tumor to nontumor localization ratios, so as to afford a more selective tumor targeting. Since these have been reviewed elsewhere, as discussed above, they will not be discussed again here. Even more than external beam irradiation, RAIT is potentially used optimally in combined therapy modalities, since the carrier antibody can also deliver other therapeutic agents, radiosensitizers, and vascularization and biological response modifiers. Since the foremost dose-limiting toxicity of RAIT is bone marrow suppression, hematopoietic cytokines and autologous blood stem cell

grafting can be combined with RAIT in order to overcome myelosuppression.

The combination of radiolabeled antibodies with drugs has received increased interest and investigation in recent years. Studies have been concerned with choice of antibody and radionuclide, drug, tumor type, sequence of the application of the two modalities, and relative doses of each. It is still too early to make generalizations, but current evidence suggests the beginning with the radiolabeled antibody may be more effective than initiating the drug first, followed by RAIT [42]. But it is not established yet whether this is true for all tumor systems and all RAIT and drug choices. It seems that the more radiosensitizing the drug, the better the combination performs, but less radiosensitizing agents have not been studied as much. Since combined modality RAIT is, in my view, a rational improvement over either modality alone, some of the interesting studies published will be discussed further. Most preclinical experiments involving xenografted human tumors in nude mice have shown evidence of improved therapeutic efficacy for the combination, but there is a paucity of data supporting any particular schedule for the two modalities. Except for the study by DeNardo et al. [42], few investigators have reported on the effect of scheduling of the two modalities, and how dose scheduling may be influenced by the tumor and its clinical setting. It has even been found that external beam irradiation can improve therapeutic efficacy when combined with radiolabeled CEA antibodies [27, 28]. In experimental systems, RAIT has also been found to affect tumor vascularization and vascular permeability, as well as hypoxia [16, 20, 21], which may be one mechanism explaining how subsequent therapies, including drugs, could be affected.

The few clinical studies reported on the combination of chemotherapy with RAIT indicate an acceptable toxicity and some antitumor activity, but as yet no additive or synergistic effects have been shown in a randomized trial. Another problem in such trials is the generally poor condition of the study subjects, most of whom have large, progressing tumors that have relapsed after prior chemotherapy. This is why patient selection, emphasizing more limited disease, is essential for the true assessment of RAIT alone or RAIT in combination with other modalities.

Pre-targeting strategies

In order to increase the tumor-to-background ratios in antibody targeting, several promising pretargeting strategies separating antibody targeting from radionuclide delivery are being developed. These methods are intended to minimize the systemic radiation resulting from prolonged circulation of antibodies directly conjugated with isotopes, so that delivery of the radionuclide is accomplished only after most antibody has cleared from normal tissues. In general, a nonradioactive antibody containing a second recognition site, such

as to a radiolabeled small molecule (or a hapten), is injected. At the time of maximal tumor accretion and circulatory clearance of the antibody, the relevant hapten bearing the radionuclide is injected as a second step. The radiolabeled hapten binds to the second recognition site of the tumor-localizing antibody, whereas unbound hapten is rapidly cleared from the body. Compared to directly labeled antibodies, these methods achieve higher tumor-to-blood and tumor-to-normal tissue ratios, but timing and the doses given are critical [4, 5, 31, 57, 58, 98, 99, 119].

One approach has been the noncovalent interaction between avidin or streptavidin and biotin, which have a high binding affinity, in the order of $K_a = 10^{15}$ M^{-1} [65]. Avidin or streptavidin conjugated to antibody is targeted first, followed by the administration of radiolabeled biotin. Conversely, the targeting antibody can be biotinylated and, after being injected, avidin or streptavidin is administered in order to bind to the antibody at the tumor. The final step involves injection of radiolabeled biotin, which attaches to avidin at the tumor. Modifications of both approaches are being made, including the use of clearing agents that reduce the amounts of the targeting antibody at nontumor sites [4]. Thus, these can involve two or multistep procedures, all intended to increase tumor-to-nontumor ratios. However, both endogenous biotin and the immunogenicity of avidin and streptavidin can be problematic [31, 57, 58, 98, 99, 119].

Despite impressive results in animal studies of biotin/avidin methods [4], clinical trials have been less encouraging. A phase I clinical dose-escalation study found that nonmyeloablative doses exceeding 200 mCi of ^{90}Y could be tolerated, with radioactivity in the tumor equaling that achieved with conventional RAIT [25, 26, 75]. However, untoward side-effects, particularly intestinal toxicity, limited further escalation and resulted in these particular studies being abandoned. Using another system involving CD20 antibody for the treatment of non-Hodgkin's lymphoma, doses up to 50 mCi/m^2 of ^{90}Y-DOTA biotin resulted in evidence of tumor regression [131]. Since this hematopoietic neoplasm is very radiosensitive, and has responded well to virtually all forms of RAIT, it is not clear whether this result represents a demonstration of the advantage of this pretargeting method or of the optimal results obtained with radiosensitive lymphomas. Nevertheless, in a preclinical study comparing conventionally labeled CD20 mAb with pretargeted RAIT with ^{90}Y, higher tumor-to-nontumor tissue ratios and markedly better therapeutic results were obtained with the pretargeting approach [105].

Paganelli and co-workers have pioneered a three-step pretargeting method that involves biotinylated antibody given in the first step, the administration of streptavidin or avidin to clear circulating antibody and to couple to biotinylated antibody localized at the tumor as the second step, and finally the administration of radiolabeled biotin [94, 98, 99]. In a phase I/II clinical trial of high-grade glioma, 48 patients with residual disease or

recurrence were treated with ^{90}Y-DOTA biotin at 60–80 mCi/m^2. The primary biotinylated antibody was an anti-tenascin mAb. An objective response of 25% and stable disease in 52% of the patients were reported. The mean absorbed dose to tumor, at the maximal tolerated dose, was 1,200 cGy per cycle. In some cases, the duration of response was more than 1 year, which is an encouraging finding in this tumor type [98]. A different pretargeting approach to increase the radiation dose delivered to tumor, as compared to blood and other normal tissues, has involved re-engineering of the targeting antibody molecule as a bispecific antibody (bsAb). This is made chemically or recombinantly from monovalent antibody fragments that target two different antigens, one at the tumor and the other a hapten chelate. After the bispecific antibody localizes in the tumor and clears from normal tissues, the second agent that binds selectively to the second arm of the antibody and delivers the radioactivity to the tumor is administered [5, 31]. A diagram of this method, termed the "affinity enhancement system" (AES), is presented in Fig. 1. In the most extensive work with this approach, the targeting antibody is against carcinoembryonic antigen (CEA) and the second arm recognizes a DTPA-indium chelate [5]. After the localization step, a bivalent hapten, which is a peptide incorporating two DTPA-indium moieties as well as tyrosine carrying a diagnostic or therapeutic radionuclide, is given. The novelty appears to include the use of a bivalent hapten, which forms a stable complex with two molecules of pretargeted bsAb at the tumor. Timing of the second injection of the radiolabeled hapten is important, in order to achieve high tumor-to-background ratios when there is little or no bsAb circulating in the blood. Excellent tumor imaging has been obtained with this AES method in several clinical trials [5, 6, 31, 78, 113]. Figure 2 is an image of a metastatic CEA-expressing carcinoma targeted by the radiolabeled hapten given as a second step in this AES system, showing excellent targeting against virtually no
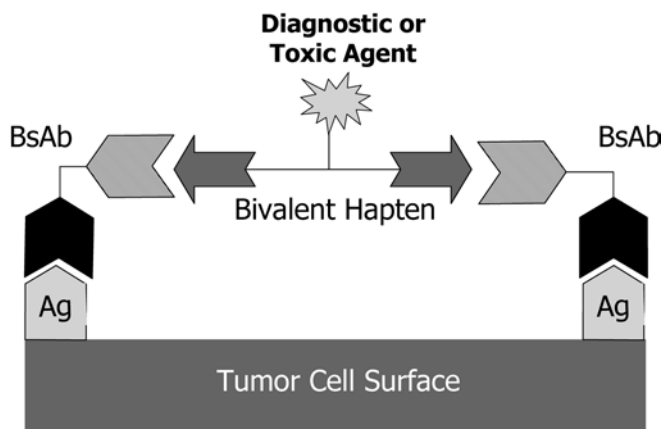
Given 50 mg/m^2 of BsAb

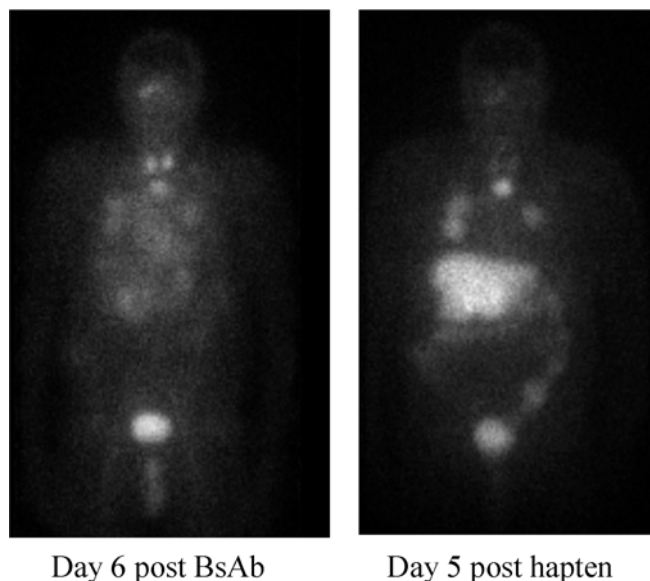Day 6 post BsAb · Day 5 post hapten

Fig. 2 Comparison of targeting with an ^{131}I-labeled anti-CEA bsAb (*left*) to that achieved with the ^{131}I-labeled hapten, 6 and 5 days after injection, respectively, in a patient with a CEA-producing cancer who had lesions in the chest and liver. Some normal thyroid and urinary bladder activity is also seen. (Reproduced from [31] with permission)

Fig. 1 Schematic diagram of bispecific antibody pretargeting system, using a bivalent hapten for binding to two arms of a tumor-localizing bispecific antibody. (Reproduced with permission from [31])

background radioactivity, as compared to the results when the bsAb is labeled. This involved a half-humanized (anti-CEA), half-murine (anti-hapten) bispecific antibody (Pentacea, IBC Pharmaceuticals, LLC, Morris Plains, N.J.) [31].

Using a different bsAb, excellent targeting results have been reported in a preclinical model of human renal carcinoma. With the G250 bispecific antibody and pretargeting, Boerman et al. [22] showed that tumor to blood ratios increased up to values as high as 3,500 at 72 h post radiochelate injection. At 20 h post injection, about 50% of the whole-body activity was localized in the tumor. Therapy experiments in animal models have also confirmed the efficacy of AES, including substantial cures of xenografted human colon carcinoma [45]. This AES approach appears to hold much promise for RAIT, but may also be applicable to a more selective and enhanced delivery of drugs to tumors.

The AES pretargeting technology, using an anti-CEA bispecific antibody and a ^{131}I-labeled hapten given 4 days later, has been studied in the treatment of patients with disseminated small cell lung cancer [128]. Doses of 1.48–6.66 GBq (40–180 mCi) of ^{131}I were administered, with hematological rescue with autologous stem cells being done at doses above 150 mCi. Tumor targeting was excellent, and the estimated tumor doses in six patients were 2.6–32.2 cGy/mCi. Among the 12 patients evaluated, two partial responses, one stabilization and nine progressions were observed, with efficacy and toxicity being dose-related.

Clinical results

Hematopoietic tumors

Various antibodies, labels, and treatment strategies have been studied, and most have shown evidence of efficacy in hematopoietic tumors, particularly non-Hodgkin's lymphoma (NHL). For example, antibodies against CD20, CD22, CD37, and HLA-DR antigens have been used with either ^{131}I, ^{90}Y, or, rarely, other radionuclides, such as ^{186}Re or ^{67}Cu. Most initial studies showed favorable results with indolent forms of NHL, but more recent trials have also shown efficacy in aggressive NHL. Four agents have been studied most often in NHL: ^{131}I-B1, ^{90}Y-2B8, ^{131}I/^{90}Y-LL2, and ^{131}I-Lym-1. A number of excellent reviews of progress in the RAIT of lymphomas have appeared in the past [32, 40, 43, 48, 67, 103, 104, 127].

The antitumor activity of RAIT is primarily due to the associated radioactivity of the radiolabel attached to the antibody, which emits continuous, exponentially decreasing, low-dose-rate irradiation with a heterogeneous dose deposition. In some cases, as is evidenced in lymphoma, the antibody itself may contribute to tumor destruction. There may also be an immune response of the host to tumor antigens released after antibody- or isotope-mediated cell destruction, as has been suggested in NHL treatment [41]. In summary, important considerations in the efficacy of RAIT include the nature of the antibody (specificity, affinity, avidity, dose, immunoreactivity, mechanism of action of naked antibody), the radiolabel (emission properties, half-life, stability of radioconjugate), the antigen targeted (location, modulation, stability, density, expression), and the nature of the target neoplasm (radiosensitivity, location, size, vascularization, immunogenicity, proliferative rate), as well as such factors as heterogeneity of dose deposition, dose rate effects and status of the host's bone marrow and normal organ functions following other forms of cytotoxic therapy. These considerations are illustrated well in the RAIT of NHL, but are also relevant to other neoplasms.

Non-Hodgkin's lymphoma

Bexxar (^{131}I-tositumomab; Corixa Corp., Seattle, Wash.) and Zevalin (^{90}Y-ibritumomab tiuxetan, IDEC-Y2B8; IDEC Pharmaceuticals, San Diego, Calif.) are the two most advanced RAIT products under regulatory review for NHL therapy. Both are murine antibodies directed against the CD20 antigen expressed on the surface of normal and malignant B lymphocytes. Bexxar is conjugated with ^{131}I, whereas Zevalin is labeled with ^{90}Y. Bexxar is used as an IgG2a murine mAb with cold murine antibody added, whereas Zevalin has the murine antibody labeled and cold human/mouse chimeric rituximab (Rituxan, IDEC/Genentech) added to the

product. Bexxar is given with a patient-specific dosimetric pretherapy study, whereas Zevalin has been developed so that this pretherapy dosimetry is not needed, and is administered on a body weight basis. Both products, however, require a pretherapy cold antibody dosing in order to improve tumor targeting, which involves a 1-h infusion of 450 mg of unlabeled antibody with Bexxar and a much longer infusion (4–6 h) of 450 mg of rituximab with Zevalin. Hence, Bexxar involves three injections and three imaging sessions and Zevalin requires only two injections, unless the early imaging with ^{111}In becomes required as a pretherapy step. Nevertheless, the time involved in treating a patient with these radiolabeled antibodies is even shorter than with nonradioactive rituximab, which is administered weekly over 4–8 weeks. Both products have shown higher and more durable responses than naked antibodies, but they also have dose-limiting toxicity, predominantly myelotoxicity. Infusional adverse reactions are minimal for Bexxar, as compared to Zevalin, and both show minimal nonhematological toxicities, with no hair loss or mucositis and generally minimal nausea. Because of the usually high release of ^{131}I from Bexxar, thyroid blockage is required, yet may pose a complication of hypothyroidism even with such blockage. Some patients have shown myelodysplasia after long-term follow-up following Bexxar, but they were also heavily pretreated with chemotherapy, which could have contributed to this complication.

Antibody responses to the injected antibody can have adverse consequences, including anaphylaxis. When murine antibodies are administered, a human anti-mouse antibody (HAMA) response is usual, but this is diminished in patients with NHL who have had prior chemotherapy. When they are chemotherapy-na, the HAMA response can be considerable, such as ∼60% for Bexxar [130]. Also of concern is that HAMA can alter murine-based immunoassays for analytes that may be important for patient management, as discussed elsewhere [63]. But most critical may be the altered biodistribution and targeting that would preclude readministration of the foreign protein. In fact, if HAMA is present, administration of a chimeric antibody or even humanized antibody may enhance the HAMA response (personal observations).

With the new Nuclear Regulatory Commission's regulations for ^{131}I providing that patients may be released if the total effective dose equivalent to another individual from exposure to a treated patient is < 500 mrem [121], Bexxar can be given in most (but not all) states of the U.S. on an outpatient basis, while Zevalin and other products using pure beta-emitters, such as ^{90}Y, can be used throughout the U.S. on an outpatient basis. Both products have been studied predominately as a single-cycle therapy.

A phase III clinical trial of Bexxar in follicular, low-grade and transformed low-grade NHL who were heavily pretreated and chemotherapy resistant has been completed [72], and gave a response rate of 65%, compared with 28% for the last chemotherapy. A complete

response (CR) rate of 30% was reported, with a median remission duration of almost 5 years [72].

Bexxar has also been studied in a phase II trial in previously untreated low-grade or transformed NHL patients [130]. Seventy-four of the 76 patients (97%) had an objective response, with 63% achieving a CR. None of the patients required hematological supportive therapy, but HAMA was observed in 64% of the patients. It was found that less heavily pretreated patients responded more favorably to RAIT, at least with Bexxar and probably also with other such agents.

Kaminski and associates recently summarized their experience with Bexxar in 53 chemotherapy-relapsed/refractory NHL patients [73]. They determined the maximal total-body dose for patients not requiring autologous stem cell transplantation (ASCT) as 75 cGy and post-ASCT as 45 cGy. Forty-two of 59 patients (71%) responded; 20 (34%) had CRs. Thirty-five (83%) of 42 with low-grade or transformed NHL responded versus 7 (41%) of 17 de novo intermediate-grade NHL patients ($P = 0.005$). The median progression-free survival was 12 months for all 42 responders, and 20.3 months for those with CR. Seven patients were still in CR 3–5.7 years later. Sixteen were retreated after progression; nine responded and five had a CR. Ten patients (17%) had HAMA elevations. Long-term, five patients developed elevated TSH levels, five were diagnosed with myelodysplasia and three with solid tumors.

Press and associates [106, 108], using myeloablative doses of the B1 antibody of Bexxar labeled with ^{131}I and combined with peripheral blood stem cell transplantation, showed that very high objective responses of 86%, with 79% CRs, could be achieved; 39% of the patients survived free of any recurrences for 5–10 years without any further therapy. This was extended in a study of 29 patients receiving therapeutic infusions of 280–785 mCi (10.4–29.0 GBq) of ^{131}I-murine B1 [80]. Fourteen of 29 patients remained in unmaintained remissions ranging from 27+ to 87+ months after RAIT. The estimated overall and progression-free survival rates were 68% and 42%, respectively, with a median follow-up time of 42 months [80]. Nonhematological dose-limiting toxicity was reversible cardiopulmonary insufficiency, which occurred in two patients at RAIT doses that delivered ≥27 Gy to the lungs. Late toxicity has been uncommon, except for elevated TSH levels found in about 60% of the subjects. Two patients developed second malignancies, but none have developed myelodysplasia [86].

When the Seattle group extended RAIT at myeloablative doses to include chemotherapy with etoposide and cyclophosphamide (followed by ASCT), an overall survival rate of 83% and a progression-free survival of 68% were observed after a median follow-up of 2 years [109]. These results compared favorably with those of a nonrandomized control group of patients treated at the same institution with the same doses of the drugs, but who received total-body irradiation instead of the radiolabeled antibody (overall survival 53% and progression-free survival of 36%). Of the 52 patients treated, four died of opportunistic infections. In another study by this group, using ^{131}I-CD20 murine mAb (tositumomab) at a median dose of 510 mCi (18.87 GBq), combined with high-dose etoposide (30–60 mg/kg), cyclophosphamide (60–100 mg/kg), and infusion of cryopreserved autologous stem cells, the respective complete and overall response rates were 91% and 100% among 11 patients with conventionally measurable mantle cell lymphoma that relapsed or was refractory to treatment [59]. Fifteen of the 16 treated are alive, with 12 having no progression of disease at 6–57 months from transplantation and 16–97 months from diagnosis. Overall survival at 3 years from transplantation is estimated at 93%, and progression-free survival is estimated at 61%. These appear to be the best results in this lymphoma type, which is a radiation-sensitive malignancy.

[90]Y-Zevalin (yttrium-90 ibritumomab tiuxetan, IDEC-Y2B8) was studied in a phase I/II dosimetry trial in relapsed/refractory NHL patients by Wiseman et al. [134]. Patients received ^{111}In-Zevalin on day 0 followed by a therapeutic dose of ^{90}Y-Zevalin on day 7, in a dose-escalation (7.4–15 MBq/kg, or 0.2–0.4 mCi/kg) mode. Both doses were preceded by an infusion of the chimeric, unlabeled rituximab antibody. Median estimated radiation absorbed dose was 3.4 Gy to liver, 2.6 Gy to lungs, and 0.38 Gy to kidneys, with the median estimated tumor radiation absorbed dose being 17 Gy. Thus, Zevalin administered at nonmyeloablative MTDs resulted in acceptable absorbed doses to normal organs.

Results of a prospective, randomized trial of Zevalin in 143 patients with relapsed/refractory low-grade, follicular or transformed NHL, compared to a standard course of rituximab, showed an overall objective response rate of 80% for the Zevalin group versus 56% in the group who received unlabeled rituximab ($P = 0.002$), with a 30% CR rate for Zevalin versus 16% CR for rituximab ($P = 0.04$) [136]. These investigators also determined that Zevalin given at nonmyeloablative doses of 0.4 mCi/kg (15 MBq/kg) delivers acceptable radiation absorbed doses to normal organs without the need for pretherapy-based dosimetry with ^{111}In-labeled Zevalin.

Another study was conducted to evaluate the response rate to Zevalin in follicular NHL patients who were refractory to rituximab (defined as those who failed to achieve an objective response or had progression of disease within 6 months of the most recent course of rituximab given weekly ×4 at 375 mg/m^2). In the analysis of the 54 patients, an overall objective response of 74% and a CR rate of 15% were achieved according to the International Workshop standards [32], or an objective response rate of 59% and a CR of 4% by the prior IDEC criteria [138]. Duration of response was significantly longer (7.7+ mos. vs 4 mos.) for Zevalin as compared to prior rituximab ($P < 0.01$). In an analysis of 211 patients receiving Zevalin, it was reported that 1.4% developed HAMA and one patient (0.5%) developed human anti-chimeric antibody (HACA) [137]. Zevalin

was the first radioimmunoconjugate to achieve regulatory approval, being licensed by the FDA in February of 2002 for the treatment of patients with follicular and transformed NHL.

A third antibody for NHL, targeting a different antigen, CD22, is also emerging as potentially a third radiotherapeutic [55], or as a second unlabeled product [81]. This CD22 antibody, first named EPB-2 and subsequently LL2, was first developed as a murine form [100]. It was then shown, by labeling the Fab' fragment with ^{99m}Tc (LymphoScan, Immunomedics, Inc., Morris Plains, N.J.), to target all forms, stages, and sites of NHL, with only normal spleen showing accretion of the antibody [7, 96]. Subsequently, the murine antibody was humanized, or CDR-grafted onto human framework regions of IgG (hLL2 or epratuzumab; Immunomedics, Inc.) to reduce the murine component to less than 10%, thus having more human components than the chimeric rituximab anti-CD20 antibody. The LL2 mAb has been determined to target the surface CD22 antigen and then internalize rapidly into the cell [120]. Later resumption of synthesis and expression of CD22 permits binding of the antibody and further internal processing. This internalization has enabled the attachment of radiometals for a higher residence time and, in turn, dose delivered, in the tumor [116]. One of the interesting observations of the first RAIT trial in NHL with the murine LL2 labeled with ^{131}I was the apparent efficacy of very low doses of radiation [55], confirmed also in further studies by us [61], as well as by Vose et al. [126] and Linden et al. [84]. Subsequent studies with a ^{90}Y form of hLL2 indicated antitumor activity at the first dose levels of a dose-escalation study, including patients who failed prior high-dose chemotherapy [62]. A clinical trial comparing the dosimetry and pharmacokinetics of hLL2 labeled with ^{131}I or ^{90}Y in patients with NHL showed the advantage of the ^{90}Y label with this antibody [70]. At present, a phase I/II study with myeloablative doses of ^{90}Y-hLL2 in patients with predominantly aggressive NHL, including those who had prior high-dose chemotherapy, is being conducted [62]. It is noteworthy that the ^{90}Y-labeled hLL2 is given as a single injection with a protein dose of about 100 mg in these studies, without the need for a prior predosing to improve its biodistribution. ^{111}In-hLL2 is given in advance for targeting and dosimetry purposes, but it is not anticipated that ^{90}Y-hLL2 will need individualized patient dosimetry [70], as it is not required for Zevalin [37, 134, 135, 136]. Another difference between hLL2 and the other radiolabeled antibodies used for NHL therapy is that this antibody has the humanized form labeled, whereas Zevalin and Bexxar have murine antibodies radiolabeled, thus involving the administration of a murine antibody with its potential immunogenicity and prospect of precluding repeated administrations.

The hLL2 antibody labeled with ^{90}Y is also being studied in a dose fractionation schedule, beginning with two doses given once weekly and expanded up to four weekly doses. Initial results show responses at the schedule of two to three weekly doses [83]. Another phase I trial is in progress with hLL2 labeled with ^{186}Re, which also allows simultaneous imaging and therapy (like ^{131}I), and is showing antitumor activity at the initial doses [102]. Finally, comparing myeloablative and conventional doses of ^{131}I-labeled CD20 (chimeric rituximab) and hLL2 antibodies in a small series of NHL patients, Behr et al. reported superior results with the high, myeloablative doses [15]. These various reports indicate that chimeric CD20 and humanized CD22 mAbs can be effective in NHL with diverse radiolabels, such as ^{131}I, ^{90}Y, and ^{186}Re, but it is premature to determine which label and dose schedule will prove best for the treatment of NHL, and how it will be incorporated in a management paradigm. A preclinical study of rituximab labeled with the alpha-emitter ^{211}At also supports its potential use with this radiolabel [3]. In addition to antibodies against CD20 and CD22, a recent experimental study suggests that radiolabeled CD19 antibodies may also be of value in the RAIT of NHL [87].

A fourth radiolabeled antibody product under development for the RAIT of NHL is Lym-1 (Oncolym, Peregrine Pharmaceuticals, Inc., Fullerton, Calif.), which targets the HLA-DR10β subunit expressed on most malignant B cells [82]. DeNardo and associates, whose work forms the basis of virtually all conclusions concerning the role of this antibody, have shown that it is useful for the treatment of NHL when labeled with ^{131}I or ^{67}Cu [35, 36, 39, 82]. In a low-dose trial of ^{131}I-Lym-1, 17 of 30 (57%) patients had durable responses, including three CRs. A maximum tolerated dose trial of this agent yielded responses in 11 of 21 (52%), including seven CRs [35]. Thrombocytopenia was the only dose-limiting toxicity. ^{67}Cu used as the radiolabel provides both imaging and a β-emitting therapeutic, and responses were shown in 7 of 12 (58%) NHL patients treated [97]. Since Lym-1 is a murine antibody, these investigators studied the HAMA response in their patients, and found a 28% response among 43 patients treated with multiple doses of the antibody, but with no evidence of anaphylactoid or related complications [38]. However, HAMA activity interrupted therapy in 6 of the 43 patients (14%). It is interesting that the median survival for the HAMA-positive patients was longer (18 months) than for those who did not develop HAMA (9 months). These authors have speculated that HAMA may contribute to the antitumor responses [41].

The various trials of RAIT in NHL lead to the following tentative general conclusions [48]:

1. Durable and major responses can be achieved, even following relapse to chemotherapy and with bulky tumors.
2. Low radiation doses can achieve objective tumor responses.
3. Administration of unlabeled antibody may improve biodistribution of the labeled antibodies, either as a predose or concomitantly.

4. High-dose therapy combined with autologous bone marrow or peripheral stem cell transplantation can result in higher overall response rates of longer duration than the application of nonmyeloablative doses.

5. Patients with low involvement of disease in the bone marrow, with a low tumor burden and without an enlarged spleen respond more favorably.

6. mAbs with radiometals, such as ^{90}Y, show better tumor dosimetry than ^{131}I-labeled antibodies, and the former do not appear to require the pretherapy dosimetry essential for ^{131}I-labeled antibodies.

7. When combined with certain chemotherapeutic agents and autologous stem cell transplants, RAIT may be more effective than any single modality.

8. RAIT appears to be more effective than use of the same antibody unlabeled.

9. Long-term side-effects may include hypothyroidism (with ^{131}I products), myelodysplasia, and, possibly, secondary neoplasms.

In general, fewer studies have been pursued with RAIT in malignancies other than NHL, but efficacy in Hodgkin's disease, T-cell leukemia, and acute myelocytic leukemia has been reported, as reviewed elsewhere [50, 67].

Solid tumors

The more radioresistant solid tumors have not been as responsive to RAIT as the hematopoietic neoplasms, and for this reason a number of strategies to improve results are being pursued. Clinically, the major interests have been colorectal, ovarian, breast, medullary thyroid, and brain cancers, with some early studies being reported also in urinary bladder cancer, prostate carcinoma, and other tumors, as summarized elsewhere [50]. Many different radionuclides, antibody forms, and methods to increase antibody accretion and penetration are under investigation, and in fact a number of approaches appear to be promising. On the other hand, methods to prevent or alleviate dose-limiting side-effects, such as myelosuppression, are also of interest because they potentially enable the administration of higher radiation doses. However, at this moment no radiolabeled antibody has yet shown sufficient antitumor activity in advanced metastatic disease of any solid tumor type to suggest that it represents a new therapy modality. Nevertheless, recent results in the therapy of small-volume or micrometastatic disease, such as in colorectal and ovarian cancers, suggest that these neoplasms, in the minimal disease setting, may be the best first opportunity for systemic RAIT under current limitations of the technology.

The principal antibodies being studied are against CEA, TAG-72, MUC1, and other glycoproteins [e.g., Le(y)], tenascin, and prostate-specific membrane antigen [50]. The majority are being used as directly labeled intact IgG immunoglobulins, labeled with either ^{131}I or ^{90}Y, and being either chimeric or humanized forms. Most have been studied as a single-dose therapy, but there is increasing evidence that a fractionated dose schedule is more efficacious [91, 114]. As mentioned earlier and as shown by a number of reviews of the progress of RAIT in solid tumors [46, 47, 49, 50, 60, 66, 77, 92, 133], advances have not been as impressive as in the hematopoietic malignancies, and at this time the role of RAIT in any single neoplasm is still not established. The recent excellent discussion of clinical RAIT by Knox and Meredith [77] has catalogued a number of studies in solid tumors, so this discussion will only select representative reports of interest. It should be noted that almost all of the trials reported are phase I–II dose-escalation studies, so that suboptimal doses were used in many cases. Indeed, the antibodies and their forms, doses of the antibodies and the radionuclides administered, stage of the disease studied, and radiation absorbed doses accreted in the tumors have varied considerably among the clinical trials. Most investigations have involved a single dose of ^{131}I- or ^{90}Y-labeled antibody, mainly at low but occasionally at myeloablative doses requiring hematopoietic support. The majority of trials have involved advanced cancer patients who had failed other forms of therapy, thus constituting a difficult patient populations in terms of therapeutic response. Although complete responses are rare, partial and minor responses, and durable disease stabilization, have been observed, suggesting that optimization of RAIT in future clinical trials could improve the prospects of RAIT in solid tumors. Examples of solid tumor RAIT will be given for central nervous system and colorectal cancers. The majority of human solid tumors express CEA, so that antibodies to this antigen have been studied in colorectal, pancreas, lung, breast, and medullary thyroid cancers [13]. In the study by Behr et al. [13], tumor doses were found to be inversely related to the tumor mass, and ranged between 2 and 218 cGy/mCi; doses between 44 and 268 mCi of ^{131}I-NP-4 murine anti-CEA antibody (Immunomedics, Inc.) were administered. Modest antitumor effects were seen in 12 of 35 assessable patients, comprising one partial response, four minor/mixed responses, and seven with stabilization of previously rapidly progressing disease. The authors proposed that small tumors are more suitable for RAIT, and that bulky tumors will probably require myeloablative doses. More modest progress is being made in the RAIT of breast cancer [51], although it seems that this would be an optimal tumor type for this technology, since radiation has a role in the management of this disease. A review of results in diverse solid tumor types has appeared elsewhere [50].

Brain and other CNS cancers

Results from numerous studies have shown that the best efficacy has been achieved by locoregional administration or by systemic administration for the treatment of small tumors or minimal disease. Brain and central

nervous system (CNS) tumors are particularly good candidates for locoregional therapy. Using anti-tenascin antibodies labeled initially with ^{131}I and more recently with ^{90}Y, Riva et al. [111] injected these into the tumor bed after surgery of malignant gliomas, and reported impressive growth control. The median survival time for patients with glioblastoma was prolonged to 25 months for the ^{131}I-labeled antibody and 31 months for the ^{90}Y group. In many cases, significant tumor shrinkage was observed. Compared with the ^{131}I-labeled antibody, the ^{90}Y-radioimmunoconjugate showed more favorable effects in bulky lesions, and had fewer radioprotection problems. Employing another anti-tenascin antibody labeled with ^{131}I and injected directly into surgically created resection cavities of patients with malignant gliomas, average absorbed doses in the tumor cavities were 41 Gy [1]. In yet another study with a different ^{131}I-anti-tenescin antibody (81C6) given at a dose of 120 mCi in the intraresection cavity of patients with newly diagnosed glioma, 11 of 33 patients were alive at a median follow-up of 93 weeks, showing an increase as compared to historical controls treated with conventional radiotherapy and chemotherapy [110]. Nine patients (27%) developed reversible hematologic toxicity, and treatment-related neurologic toxicity was reported in five patients (15%). These results encourage conducting a randomized trial with this agent.

Systemic or intra-arterial RAIT has also been explored for brain tumors with [131]I- and ^{125}I-labeled antibodies, and these have provided evidence of objective responses without significant toxicities [23, 71]. Both in studies involving established disease and in investigations of its use as an adjuvant therapy, ^{125}I-anti-epidermal growth factor receptor antibody 425 has been shown to be active in the treatment of patients with primary glioblastoma multiforme, the results having included a 20% objective response rate [23, 24, 93]. The intra-arterial route of administration did not appear to offer any advantage over i.v. infusions, as was also found by others [139].

In addition to using radiolabeled 3F8 antibody in neuroblastoma therapy, Cheung and co-workers have studied this RAIT for leptomeningeal cancer by an intraventricular administration route, with estimated radiation doses of 14.9–56 cGy/mCi to the cerebrospinal fluid and less than 2 cGy/mCi to blood and other organs outside the CNS [79]. Intrathecal RAIT has also been applied to patients with medulloblastoma and neuroblastoma, resulting in objective and durable responses in some of the patients, such as in 5 of 11 recurrent neuroblastoma patients, and a CR was noted in 3 of 15 recurrent primitive neuroectodermal tumor patients [74, 110].

Colorectal cancer

Experimental studies have shown that radiolabeled CEA antibodies can be curative of minimal metastatic disease [8, 10, 18, 19, 117]. Also, clinical findings have observed that the highest radiation doses delivered to tumors are inversely proportional to tumor size [14]. Similar calculations and predictions were made by Sgouros [115]. Clinical studies with humanized CEA antibodies labeled with ^{131}I confirmed these animal studies, since patients with colorectal cancer metastases of small volume, after unsuccessful chemotherapy, showed encouraging responses [9, 11]. In an ongoing trial of RAIT with humanized anti-CEA MN-14 IgG (Immunomedics, Inc) in an adjuvant setting following resection of metastatic colorectal cancer, seven of nine patients showed no relapse at up to 36 months, in contrast to 67% in a control group at the same institution [9].

Early studies with ^{131}I-labeled CEA and B72.3 murine antibodies in colorectal cancer showed modest antitumor effects at nonmyeloablative doses. Four of 15 patients showed an objective response with B72.3 and other antibodies [112], while CEA antibodies showed antitumor effects in 12/35 patients with colorectal and other CEA-expressing cancers [13]. Studies with diverse CEA antibodies have also shown modest therapeutic responses with nonmyeloablative doses of ^{131}I-labeled antibodies [12, 42, 68].

Buchegger and associates have suggested, in early clinical studies [27, 28], that RAIT in close association with external beam irradiation is more efficient in an adjuvant setting after surgery. Clinically, six patients with limited liver metastases from colorectal cancer were treated with RAIT using 20 mCi ^{131}I-labeled anti-CEA antibody F(ab')$_2$ fragments combined with fractionated external beam radiation of 20 Gy to the entire liver. Spontaneously reversible bone marrow toxicities of grades 3 and 4 and reversible liver toxicity of grades 1–3 were observed. Three of the patients showed stable disease and one had a partial response, while two had disease progression.

A phase II RAIT trial with ^{131}I-CC49, which is the second-generation murine B72.3 pancarcinoma antibody, reported no objective tumor responses at the MTD dose of 75 mCi/m^2 [94, 95]. Twelve of 13 patients developed HAMA at 6–8 weeks post infusion. High-dose RAIT with autologous stem cell replacement was then undertaken with ^{131}I-labeled murine mAb CC49 in 15 patients with gastrointestinal cancers in a dose-escalation study from 50 to 300 mCi/m^2 [124]. Tumor localization was excellent, the percent injected dose per kg of tumor ranged from 0.2 to 2.1, and the absorbed dose in metastatic tumor sites ranged from 630 to 3,300 cGy. These authors then tested the same antibody labeled with [90]Y [125], and found a heterogeneous liver and splenic uptake, photopenic lesions in the liver for metastases, and generally poor uptake of the antibody in metastases. The absorbed tumor doses ranged from 180 to 3,000 cGy, but tumor to normal liver dose ratios were less than 1. No objective responses were observed. Doses up to 0.5 mCi/kg could be administered with reversible grade IV myelotoxicity.

Another target for colorectal cancer RAIT is the A33 antigen, which is a transmembrane glycoprotein of the

immunoglobulin superfamily [64]. A study of 23 patients who failed prior chemotherapy were treated with escalating doses of ^{131}I-A33 murine mAb, and the MTD was found to be 75 mCi/m^2 in these heavily pretreated patients [132]. The antibody showed variable uptake in the normal bowel, and no objective responses.

The NR-LU-10 pancarcinoma antibody (NeoRx Corp) was also studied in colorectal cancer patients by the pretargeting scheme using a streptavidin conjugate of the antibody [75]. Twenty-five patients were treated with a single dose of 110 mCi/m^2 of ^{90}Y-DOTA-biotin, 24 h after a clearing agent was given to remove the NR-LU-10/streptavidin. Diarrhea was the most frequent grade 4 nonhematological toxicity. A modest overall response rate of 8% was reported, with four patients having stable disease with freedom from progression of 10–20 weeks. These results do not confirm the promising preclinical studies with the same reagents and technology [4].

Pretherapy dosimetry

Methods for estimating tumor and organ doses prior to RAIT have been derived from external beam radiation calculations but appear to be less accurate for RAIT, thus provoking some controversy on the role of this technology for treatment planning. In contrast to external beam therapy, there are fewer sample points and an inhomogeneous dose distribution; there can be a wide dose variability for different lesions in the same patient [77, 122].

Dose estimations for RAIT are made on the basis of calculating the volume of tumors and normal organs, the cumulative radioactivity estimated as accreted in the organs and tumors, and the pharmacokinetics of the radioactivity given with the antibody. Various methods have been used to gain these data, including serial gamma camera imaging and biopsy [44, 61, 70, 80, 89, 90, 94, 95, 122, 135], for most organs and tumors, but the bone marrow dose estimates have been based upon blood pharmacokinetics and/or imaging of bone in areas of active marrow, such as the spine or sacrum [122]. When a therapeutic isotope has a gamma imaging energy, then it can be used in tracer doses for pretherapy dose estimates. In the case of pure beta emitters, such as ^{90}Y, a surrogate gamma-imaging isotope, such as ^{111}In, is used to predict the therapeutic dose. Tracer studies often predict the doses obtained from subsequent RAIT well, but variations, even in the same patient, can be experienced [44, 90, 94].

A major problem with pretherapy dosimetry has been a failure to achieve a consistent dose-response relationship for RAIT. For example, tumor doses in patients with lymphoma show a tenfold range, from 0.5 to 5.4 mGy/MBq, and have had a variable correlation between the estimated dose delivered and the response [122]. But at the extremes there is evidence for a relationship between estimated tumor doses and response

rates [89, 135]. Nevertheless, RAIT appears to achieve responses at dose estimates which are far lower than those calculated for external beam therapy [63, 123]. Normal organ doses from RAIT have ranged from 0.2 to 2.2 mGy/MBq, with considerable variability between patients [122]. When very high doses of RAIT are given, such as in the myeloablative studies performed by the Seattle group, secondary organ toxicity involved cardiopulmonary complications in patients who received more than 27 Gy to the lungs [107]. Thus, it appears that the low-dose-rate irradiation given by RAIT is tolerated relatively well by normal organs [122]. An inverse relationship between tumor size and dose delivered has also been observed [14], indicating that small-volume tumors and micrometastases may be the best targets for current RAIT methods. Indeed, this is supported by both experimental [8, 10, 18, 117], and clinical studies [9, 11]. In a comprehensive evaluation of 119 tumors in 93 patients given the ^{131}I-NP-4 and -MN-14 anti-CEA murine mAbs, it was reported that an inverse logarithmic relationship exists between tumor size and antibody uptake [12]. The most important factor determining the radiation dose to the tumor was found to be the absolute tumor uptake of the radiolabel, and the second most important factor was the biological half-life of the antibody in the tumor. Different antibody affinities did not appear to affect tumor uptake. At comparable masses, colorectal and medullary thyroid cancers had significantly higher uptake of antibody, as well as tumor-to-red marrow dose ratios, than other cancer types. Thus, it appears that tumor uptake of the antibody is the most important dose-determining factor, so that both colorectal and medullary thyroid cancers seem to be good targets for CEA antibodies used in RAIT.

It is well known from external beam irradiation that higher dose rates result in higher therapeutic efficacy [2, 85], but this has not been investigated well with internal emitters [10]. Recent studies in experimental models [8, 10] have begun addressing this issue, and it seems that dose rate effects are very important, not only at the comparatively high levels experienced with external beam therapy but also in the lower ranges associated with internal emitters and RAIT.

Based on the observation that during the recovery period after anticancer myelosuppressive therapy, hematopoietic progenitor cells become mitotically active in order to replenish the bone marrow compartment, and remain hyperproliferative even after normalization of blood counts of leukocytes and platelets, Blumenthal et al. [17] conducted a retrospective study of the blood levels of several hematopoietic cytokines following a single dose of RAIT with CEA antibodies in CEA-expressing solid tumors. It was found that the plasma level of Flt3-ligand could predict excessive platelet toxicity caused by additional cytotoxic therapy. This encouraging report suggests that the measurement of this hematopoietic cytokine may be a reliable surrogate marker of the status of the bone marrow following cytotoxic therapy, thus perhaps predicting how aggressive

a therapy, whether RAIT or chemotherapy, may be undertaken in any individual patient. In a more recent study, the use of Flt3-L blood levels to adjust bone marrow dose estimates in RAIT-treated patients with solid tumors was demonstrated [123]. Studies of this kind may be more clinically relevant than current bone marrow dosimetry methods.

Conclusion

RAIT of cancer has had a more than 20-year history, and during this time profound advances have been achieved in the development of tumor-seeking, humanized antibodies, in radiochemistry with diverse radionuclides, in the mitigation of dose-limiting myelosuppression, and in the improved targeting and delivery of radiation doses to tumors of different types and in diverse locations. Hematopoietic neoplasms have shown the best responses to RAIT, despite the delivery of relatively low doses of radiation; this has resulted in several radiolabeled antibodies advancing toward commercialization for the treatment of NHL. These results are due, perhaps, to good vascularization, high antigen density on a more homogenous tumor cell population, and possibly the involvement of concomitant apoptotic and immune mechanisms. In contrast, solid tumors fail to receive the radiation doses required to achieve similar responses. Although RAIT of solid tumors represents the principal challenge of the future, it is already apparent that use of this modality in a minimal disease setting, in locoregional applications, in combination modalities, in fractionated dose schedules, and in pretargeting strategies shows sufficient promise to justify continued optimism for its future role in the management of cancer.

References

1. Akabani G, Cokgor I, Coleman RE, Gonzalez Trotter D, Wong TZ, Friedman HS, Friedman AH, Garcia-Turner A, Herndon JE, DeLong D, McLendon RE, Zhae XG, Pegram CN, Provenzale JM, Bigner DD, Zalutsky MR (2000) Dosimetry and dose-response relationships in newly diagnosed patients with malignant gliomas treated with iodine-131-labeled anti-tenascin monoclonal antibody 81C6 therapy. Int J Radiat Oncol Biol Phys 46:947–958
2. Amdur R, Bedfore J (1994) Dose-rate effects between 0.3 and 30 Gy/h in a normal and a malignant human cell line. Int J Radiat Oncol Biol Phys 30:83–90
3. Aurlien E, Larsen RH, Kvalheim G, Bruland OS (2000) Demonstration of highly specific toxicity of the alpha-emitting radioimmunoconjugate[211]At-rituximab against non-Hodgkin lymphoma cells. Br J Cancer 83:1375–1379
4. Axworthy DB, Reno JM, Hylarides MD, Mallett RW, Theodore LJ, Gustavson LM, Su F, Hobson LJ, Beaumier PL, Fritzberg AR (2000) Cure of human carcinoma xenografts by a single dose of pretargeted yttrium-90 with negligible toxicity. Proc Natl Acad Sci U S A 97:1802–1807
5. Barbet J, Kraeber-Bodere F, Vuillez JP, Gautherot E, Rouvier E, Chatal JF (1999) Pretargeting with the affinity enhancement system for radioimmunotherapy. Cancer Biother Radiopharm 14:153–166
6. Bardies M, Bardet S, Faivre-Chauvet A, Peltier P, Douillard JY, Mahe M, Fiche M, Lisbona A, Giacalone F, Meyer P, Gautherot E, Rouvier E, Barbet J, Chatal JF (1996) Bispecific antibody and iodine-131-labeled bivalent hapten dosimetry in patients with medullary thyroid or small-cell lung cancer. J Nucl Med 37:1853–1859
7. Baum RP, Niesen A, Hertel A, Adams S, Kojouharoff G, Goldenberg DM, Hor G (1994) Initial clinical results with technetium-99m-labeled LL2 monoclonal antibody fragment in the radioimmunodetection of B-cell lymphomas. Cancer 73:896–899
8. Behr TM, Blumenthal RD, Memtsoudis S, Sharkey RM, Gratz S, Becker W, Goldenberg DM (2000) Cure of metastatic human colonic cancer in mice with radiolabeled monoclonal antibody fragments. Clin Cancer Res 6:4900–4907
9. Behr TM, Liersch T, Greiner-Bechert L, Griesinger F, Behe M, Markus PM, Gratz S, Angerstein C, Brittinger G, Becker H, Goldenberg DM, Becker W (2002) Radioimmunotherapy of small volume disease of metastatic colorectal cancer. Cancer 94 (Suppl):1373–1381
10. Behr TM, Memtsoudis S, Sharkey RM, Blumenthal RD, Dunn RM, Gratz S, Wieland E, Nebendahl K, Schmidberger H, Goldenberg DM, Becker W (1998) Experimental studies on the role of antibody fragments in cancer radioimmunotherapy: influence of radiation dose and dose rate on toxicity and anti-tumor efficacy. Int J Cancer 77:787–795
11. Behr TM, Salib AL, Liersch T, Behe M, Angerstein C, Blumenthal RD, Fayyazi A, Sharkey RM, Ringe B, Becker H, Wormann B, Hiddemann W, Goldenberg DM, Becker W (1999) Radioimmunotherapy of small volume disease of colorectal cancer metastatic to the liver: preclinical evaluation in comparison to standard chemotherapy and initial results of a phase I clinical study. Clin Cancer Res 5:3232s–3242s
12. Behr TM, Sharkey RM, Juweid M, Dunn RM, Ying Z, Zhang CH, Siegel JA, Goldenberg DM (1997) Variables influencing tumor dosimetry in radioimmunotherapy of CEA-expressing cancers with anti-CEA and antimucin antibodies. J Nucl Med 38:409–418
13. Behr TM, Sharkey RM, Juweid ME, Dunn RM, Vagg RC, Ying Z, Zhang CH, Swayne LC, Vardi Y, Siegel JA, Goldenberg DM (1997) Phase I/II clinical radioimmunotherapy with an iodine-131-labeled anti-carcinoembryonic antigen murine monoclonal antibody IgG. J Nucl Med 38:858–870
14. Behr TM, Sharkey RM, Juweid MN, Dunn RM, Ying Z, Zhang CH, Siegel JA, Gold DV, Goldenberg DM (1996) Factors influencing the pharmacokinetics, dosimetry, and diagnostic accuracy of radioimmunodetection and radioimmunotherapy of carcinoembryonic antigen-expressing tumors. Cancer Res 56:1805–1816
15. Behr TM, Wormann B, Gramatzki M, Riggert J, Gratz S, Behe M, Griesinger F, Sharkey RM, Kolb HJ, Hiddemann W, Goldenberg DM, Becker W (1999) Low- versus high-dose radioimmunotherapy with humanized anti-CD22 or chimeric anti-CD20 antibodies in a broad spectrum of B cell-associated malignancies. Clin Cancer Res 5:3304s–3314s
16. Blumenthal RD, Kashi R, Sharkey RM, Goldenberg DM (1995) Quantitative and qualitative effects of experimental radioimmunotherapy on tumor vascular permeability. Int J Cancer 61:557–566.
17. Blumenthal RD, Lew W, Juweid M, Alisauskas R, Ying Z, Goldenberg DM (2000) Plasma FLT3-L levels predict bone marrow recovery from myelosuppressive therapy. Cancer 88:333–343

18. Blumenthal RD, Sharkey RM, Haywood L, Natale AM, Wong GY, Siegel JA, Kennel SJ, Goldenberg DM (1992) Targeted therapy of athymic mice bearing GW-39 human-colonic-cancer micrometastases with I-131 labeled monoclonal antibodies. Cancer Res 52:6036–6044

19. Blumenthal RD, Sharkey RM, Kashi R, Goldenberg DM (1989) Comparison of therapeutic efficacy and host toxicity of two different[131]I-labelled antibodies and their fragments in the GW-39 colonic cancer xenograft model. Int J Cancer 44:292–300

20. Blumenthal RD, Sharkey RM, Kashi R, Sides K, Stein R, Goldenberg DM (1997) Changes in tumor vascular permeability in response to experimental radioimmunotherapy: a comparative study of 11 xenografts. Tumour Biol 18:367–377.

21. Blumenthal RD, Taylor A, Osorio L, Ochakovskaya R, Raleigh JA, Papadoupoulou M, Bloomer WD, Goldenberg DM (2001) Optimizing the use of combined radioimmunotherapy and hypoxic cytotoxin therapy as a function of tumor hypoxia. Int J Cancer 94:564–571

22. Boerman OC, Kranenborg MH, Oosterwijk E, Griffiths GL, McBride WJ, Oyen WJ, de Weijert M, Oosterwijk-Wakka J, Hansen HJ, Corstens FH (1999) Pretargeting of renal cell carcinoma: improved tumor targeting with a bivalent chelate. Cancer Res 59:4400–4405

23. Brady LW, Markoe AM, Woo DV, Rackover MA, Koprowski H, Steplewski Z, Peyster RG (1990) Iodine 125-labeled anti-epidermal growth factor receptor 425 in the treatment of malignant astrocytomas: a pilot study. J Neurosurg Sci 34:243–249

24. Brady LW, Woo DV, Markoe A, Dadparvar S, Carlsson U, Rackover M, Peyster R, Emrich J, Miyamoto C, Steplewski Z, Koprowski H (1990) Treatment of malignant gliomas with[125]I-labeled monoclonal antibody against epidermal growth factor receptor. Antib Immunoconjug Radiopharm 3:169–179

25. Breitz HB, Fisher DR, Goris ML, Knox S, Ratliff B, Murtha AD, Weiden PL (1999) Radiation absorbed dose estimation for[90]Y-DOTA-biotin with pretargeted NR-LU-10/streptavidin. Cancer Biother Radiopharm 14:381–395

26. Breitz HB, Weiden PL, Beaumier PL, Axworthy DB, Seiler C, Su FM, Graves S, Bryan K, Reno JM (2000) Clinical optimization of pretargeted radioimmunotherapy with antibody-streptavidin conjugate and[90]Y-DOTA- biotin. J Nucl Med 41:131–140

27. Buchegger F, Allal AS, Roth A, Papazyan JP, Dupertuis Y, Mirimanoff RO, Gillet M, Pelegrin A, Mach JP, Slosman DO (2000) Combined radioimmunotherapy and radiotherapy of liver metastases from colorectal cancer: a feasibility study. Anticancer Res 20:1889–1896

28. Buchegger F, Roth A, Allal A, Dupertuis YM, Slosman DO, Delaloye AB, Mach JP (2000) Radioimmunotherapy of colorectal cancer liver metastases: combination with radiotherapy. Ann N Y Acad Sci 910:263–269

29. Buchsbaum DJ (2000) Experimental radioimmunotherapy. Semin Radiat Oncol 10:156–167

30. Burke PA, DeNardo SJ, Miers LA, Kukis DL, DeNardo GL (2002) Combined modality radioimmunotherapy. Promise and peril. Cancer 94 (Suppl):1320–1331

31. Chang CH, Sharkey RM, Rossi EA, Karacay H, McBride W, Hansen HJ, Chatal JF, Barget J, Goldenberg DM (2002) Molecular advances in pretargeting radioimmunotherapy with bispecific antibodies. Mol Cancer Ther 1:553–563

32. Cheson BD (2003) Radioimmunotherapy of non-Hodgkin lymphomas. Blood 101:391–398

33. Cheson BD, Horning SJ, Coiffier B, Shipp MA, Fisher RI, Connors JM, Lister TA, Vose J, Grillo-Lopez A, Hagenbeek A, Cabanillas F, Klippensten D, Hiddemann W, Castellino R, Harris NL, Armitage JO, Carter W, Hoppe R, Canellos GP (1999) Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. J Clin Oncol 17:1244–1253

34. DeNardo GL, DeNardo SJ (1995) Overview of obstacles and opportunities for radioimmunotherapy of cancer. In: Goldenberg DM (ed) Cancer therapy with radiolabeled antibodies. CRC Press, Boca Raton, Fla.

35. DeNardo GL, DeNardo SJ, Goldstein DS, Kroger LA, Lamborn KR, Levy NB, McGahan JP, Salako Q, Shen S, Lewis JP (1998) Maximum-tolerated dose, toxicity and efficacy of[131]I-Lym-1 antibody for fractionated radioimmunotherapy of non-Hodgkin's lymphoma. J Clin Oncol 16:3246–3256

36. DeNardo GL, DeNardo SJ, Lamborn KR, Goldstein DS, Levy NB, Lewis JP, O'Grady LF, Raventos A, Kroger LA, Macey DJ, McGahan JP, Mills SL, Shen S (1998) Low-dose, fractionated radioimmunotherapy for B-cell malignancies using[131]I-Lym-1 antibody. Cancer Biother Radiopharm 13:239–254

37. DeNardo GL, Juweid ME, White CA, Wiseman GA, DeNardo SJ (2001) Role of radiation dosimetry in radioimmunotherapy planning and treatment dosing. Crit Rev Oncol Hematol 39:203–218

38. DeNardo GL, Kroger LA, Mirick GR, Lamborn KR, DeNardo SJ (1995) Analysis of antiglobulin (HAMA) response in a group of patient with B-lymphocytic malignancies treated with[131]I-Lym-1. Int J Biol Markers 10:67–74

39. DeNardo GL, Kukis DL, Shen S, DeNardo DA, Meares CF, DeNardo SJ (1999)[67]Cu versus ^{131}I-labeled Lym-1 antibody: comparative pharmacokinetics and dosimetry in patients with non-Hodgkin's lymphoma. Clin Cancer Res 5:533–541

40. DeNardo SJ, Kroger LA, Denardo GL (1999) A new era for radiolabeled antibodies in cancer? Curr Opin Immunol 11:563–569

41. DeNardo SJ, Kroger LA, MacKenzie MR, Mirick GR, Shen S, DeNardo GL (1998) Prolonged survival associated with immune response in a patient treated with Lym-1 mouse monoclonal antibody. Cancer Biother Radiopharm 13:1–12

42. DeNardo SJ, Kukis DL, Kroger LA, O'Donnell RT, Lamborn KR, Miers LA, DeNardo DG, Meares CF, DeNardo GL (1997) Synergy of Taxol and radioimmunotherapy with yttrium-90-labeled chimeric L6 antibody: efficacy and toxicity in breast cancer xenografts. Proc Natl Acad Sci U S A 94:4000–4004

43. Dillman RO (2002) Radiolabeled anti-CD20 monoclonal antibodies for the treatment of B-cell lymphoma. J Clin Oncol 20:3545–35557

44. Eary JF, Press OW, Badger CC, Durack LD, Richter KY, Addison SJ, Krohn KA, Fisher DR, Porter BA, Williams DL (1990) Imaging and treatment of B-cell lymphoma. J Nucl Med 31:1257–1268

45. Gautherot E, Rouvier E, Daniel L, Loucif E, Bouhou J, Manetti C, Martin M, Le Doussal JM, Barbet J (2000) Pretargeted radioimmunotherapy of human colorectal xenografts with bispecific antibody and[131]I-labeled bivalent hapten. J Nucl Med 41:480–487

46. Goldenberg DM (1993) Monoclonal antibodies in cancer detection and therapy. Am J Med 94:297–312

47. Goldenberg DM (ed) (1995) Cancer therapy with radiolabeled antibodies. CRC Press, Boca Raton, Fla.

48. Goldenberg DM (2001) The role of radiolabeled antibodies in the treatment of non-Hodgkin's lymphoma: the coming of age of radioimmunotherapy. Crit Rev Oncol Hematol 39:195–201

49. Goldenberg DM (2001) Radioimmunotherapy. In: Freeman LM (ed) Nuclear medicine annual 2001. Lippincott, Williams & Wilkins, Philadelphia

50. Goldenberg DM (2002) Targeted therapy of cancer with radiolabeled antibodies. J Nucl Med 43:693–713

51. Goldenberg DM (2002) Studies of immunoscintigraphy and radioimmunotherapy in breast cancer. Semin Breast Dis 5:142–155

52. Goldenberg DM, Blumenthal RD, Sharkey RM (1990) Biological and clinical perspectives of cancer imaging and therapy with radiolabeled antibodies. Semin Cancer Biol 1:217–225

53. Goldenberg DM, DeLand F, Kim E, Bennett S, Primus FJ, van Nagell JR Jr, Estes N, DeSimone P, Rayburn P (1978) Use of radiolabeled antibodies to carcinoembryonic antigen for the detection and localization of diverse cancers by external photoscanning. N Engl J Med 298:1384–1388

54. Goldenberg DM, Gaffar SA, Bennett SJ, Beach JL (1981) Experimental radioimmunotherapy of a xenografted human colonic tumor (GW-39) producing carcinoembryonic antigen. Cancer Res 41: 4354–4360

55. Goldenberg DM, Horowitz JA, Sharkey RM, Hall TC, Murthy S, Goldenberg H, Lee RE, Stein R, Siegel JA, Izon DO (1991) Targeting, dosimetry, and radioimmunotherapy of B-cell lymphomas with iodine-131-labeled LL2 monoclonal antibody. J Clin Oncol 9:548-564

56. Goldenberg DM, Kim EE, DeLand, FH, Bennett S, Primus FJ (1980) Radioimmunodetection of cancer with radioactive antibodies to carcinoembryonic antigen. Cancer Res 40:2984–2992

57. Goodwin DA. Strategies for antibody targeting (1991) Antib Immunoconj Radiopharm 4:427–434

58. Goodwin DA, Meares CF, McCall MJ, McTigue M, Chaovapong W (1998) Pre-targeted immunoscintigraphy of murine tumors with indium-111-labeled bifunctional haptens. J Nucl Med 29:226–234

59. Gopal AK, Rajendran JG, Petersdorf SH, Maloney DG, Eary JF, Wood BL, Gooley TA, Bush SA, Durack LD, Martin PJ, Matthews DC, Appelbaum FR, Bernstein ID, Press OW (2002) High-dose chemo-radioimmunotherapy with autologous stem cell support for relapsed mantle cell lymphoma. Blood 99:3158–3162

60. Govindan SV, Goldenberg DM, Hansen HJ, Griffiths GL (2000) Advances in the use of monoclonal antibodies in cancer radiotherapy. Pharm Sci Techn Today 3:90–98

61. Griffith MH, Yorke ED, Wessels BW, DeNardo GL, Neacy WP (1992) Direct dose confirmation of quantitative autoradiography with micro-TLD measurements for radioimmunotherapy. J Nucl Med 33:2020–2023

62. Hajjar G, Burton J, Sharkey RM, Schuster S, Czuczman M, Lamonica D, Chatal J-F, Alavi A, Stadtmauer E, Leonard J, Coleman M, Plutchok J, Goldenberg DM (2001) Initial results of an ongoing phase I/II radioimmunotherapy trial with ^{90}Y-labeled epratuzumab (LymphoCide^{TM}; anti-CD22 monoclonal antibody) in relapsed refractory non-Hodgkin's lymphoma (NHL) (abstract). J Nucl Med 42 (Suppl):157P

63. Hansen HJ, Sullivan CL, Sharkey RM, Goldenberg DM (1993) HAMA interference with murine monoclonal antibody-based immunoassays. J Clin Immunoassay 16:294–299

64. Heath JK, White SJ, Johnstone CN, Catimel B, Simpson RJ, Moritz RL, Tu GF, Ji H, Whitehead RH, Groenen LC, Scott AM, Ritter G, Cohen L, Welt S, Old LJ, Nice EC, Burgess AW (1997) The human A33 antigen is a transmembrane glycoprotein and a novel member of the immunoglobulin superfamily. Proc Natl Acad Sci U S A 94:469–474

65. Hnatowich DJ (1994) The in vivo uses of streptavidin and biotin: a short progress report. Nucl Med Commun 15:575–577

66. Illidge TM, Brock S (2000) Radioimmunotherapy of cancer: using monoclonal antibodies to target radiotherapy. Curr Pharm Des 6:1399–1418

67. Jurcic JG, Scheinberg DA (1995) Radioimmunotherapy of hematological cancer: problems and progress. Clin Cancer Res 1:1439–1446

68. Juweid M, Sharkey RM, Behr TM, Swayne LC, Dunn R, Siegel J, Goldenberg DM (1996) Radioimmunotherapy of patients with small-volume tumors using ^{131}I-labeled anti-CEA monoclonal antibody NP-4 F(ab')$_2$. J Nucl Med 37:1504–1510

69. Juweid M, Sharkey RM, Markowitz A, Behr T, Swayne LC, Dunn R, Hansen HJ, Shevitz J, Leung SO, Rubin AD, Herskovic T, Hanley D, Goldenberg DM (1995) Treatment of non-Hodgkin's lymphoma with radiolabeled murine, chimeric, or humanized LL2, an anti-CD22 monoclonal antibody. Cancer Res 55:5899s–5907s

70. Juweid ME, Stadtmauer E, Hajjar G, Sharkey RM, Suleiman S, Luger S, Swayne LC, Alavi A, Goldenberg DM (1999) Pharmacokinetics, dosimetry and initial therapeutic results with ^{131}I and ^{111}In/^{90}Y-labeled humanized LL2 anti-CD22 monoclonal antibody in patients with relapsed, refractory non-Hodgkin's lymphoma. Clin Cancer Res 5:3292s–3303s

71. Kalofonos HP, Pawlikowska TR, Hemingway A, Courtenay-Luck N, Dhokia B, Snook D, Sivolapenko GB, Hooker GR, McKenzie CG, Lavender PJ (1989) Antibody guided diagnosis and therapy of brain gliomas using radiolabeled monoclonal antibodies against epidermal growth factor receptor and placental alkaline phosphatase. J Nucl Med 30:1636–1645

72. Kaminski M, Zelenetz AD, Press O, Saleh M, Leonard J, Fehrenbacher L, Stagg R, Kroll S (1998) Multicenter, phase III study of iodine-131 tositumomab (anti-B1 antibody) for chemotherapy-refractory low-grade or transformed low-grade non-Hodgkin's lymphoma (abstract 1296). Blood 92:1296

73. Kaminski MS, Estes J, Zasadny KR, Francis IR, Ross CW, Tuck M, Regan D, Fisher S, Gutierrez J, Kroll S, Stagg R, Tidmarsh G, Wahl RL (2000) Radioimmunotherapy with iodine^{131}I tositumomab for relapsed or refractory B-cell non-Hodgkin lymphoma: updated results and long-term follow-up of the University of Michigan experience. Cancer Res 96:1259–1266

74. Kemshead JT, Papanastassiou V, Coakham HB, Pizer BL (1992) Monoclonal antibodies in the treatment of central nervous system malignancies. Eur J Cancer 28:511–519

75. Knox SJ, Goris ML, Tempero M, Weiden PL, Gentner L, Breitz H, Adams GP, Axworthy D, Gaffigan S, Bryan K, Fisher DR, Colcher D, Horak ID, Weiner LM (2000) Phase II trial of yttrium-90-DOTA-biotin pretargeted by NR-LU-10 antibody/streptavidin in patients with metastatic colon cancer. Clin Cancer Res 6:406–414

76. Knox SJ, Goris ML, Wessels BW (1992) Overview of animal studies comparing radioimmunotherapy with dose equivalent external beam irradiation. Radiother Oncol 23:111–117

77. Knox SJ, Meredith RF (2000) Clinical radioimmunotherapy. Semin Radiat Oncol 10:73–93

78. Kraeber-Bodere F, Faivre-Chauvet A, Cerato E, Devillers A, Sharkey RM, Chang K, Goldenberg DM, Barbet J, Chatal JF (2001) Clinical optimization of two-step radioimmunotherapy (RAIT) using an ^{131}I-labeled anti-CEA/anti-DTPA bispecific antibody and an ^{131}I-labeled bivalent DTPA hapten in patients with CEA-expressing tumor (abstract 459). J Nucl Med 42:123P

79. Kramer K, Cheung NK, Humm JL, Dantis E, Finn R, Yeh SJ, Antunes NL, Dunkel IJ, Souwedaine M, Larson SM (2000) Targeted radioimmunotherapy for leptomeningeal cancer using ^{131}I-3F8. Med Pediatr Oncol 35:716–718

80. Larson SM, Pentlow KS, Volkow ND, Wolf AP, Finn RD, Lambrecht RM, Graham MC, Di Resta G, Bendriem B, Daghighian F (1992) PET scanning of iodine-124-3F9 as an approach to tumor dosimetry during treatment planning for radioimmunotherapy in a child with neuroblastoma. J Nucl Med 33: 2020–2023

81. Leonard JP, Coleman M, Schuster MW, Feldman EJ, Chadburn A, Ely S, Sharkey RM, Hansen HJ, Wegener WA, Goldenberg DM (2000) Immunotherapy of NHL with Epratuzumab (anti-CD22 monoclonal antibody): excellent tolerability with objective responses (abstract 60). Proc Am Soc Clin Oncol 19:17a

82. Lewis JP, DeNardo GL, DeNardo SJ (1995) Radioimmunotherapy of lymphoma: a UC Davis experience. Hybridoma 14:115–120

83. Linden O, Tennvall J, Cavallin-Stahl E, Linder KJ, Darte L, Ohlsson T, Hindorf C, Wingardh K, Strand SE (2000) A phase I/II trial with Y-90 hLL2 in recurrent B-cell lymphomas, preliminary results (abstract 67). Cancer Biother Radiopharm 15:413

84. Linden O, Tennvall J, Cavallin-Stahl E, Darte L, Garkavij M, Lindner KJ, Ljungberg M, Ohlsson T, Sjogreen K, Wingardh K, Strand SE (1999) Radioimmunotherapy using[131]I-labeled anti-CD22 monoclonal antibody (LL2) in patients with previously treated B-cell lymphomas. Clin Cancer Res 5:3287s–3291s

85. Ling C, Spiro I, Stickler R (1984) Dose-rate effect between 1 and 10 Gy/min in a mammalian cell culture. Br J Radiol 57:723–728

86. Liu SY, Eary JF, Petersdorf SH, Martin PJ, Maloney DG, Appelbaum FR, Matthews DC, Bush SA, Durack LD, Fisher DR, Gooley TA, Bernstein ID, Press OW (1998) Follow-up of relapsed B-cell lymphoma patients treated with iodine-131-labeled anti-CD20 antibody and autologous stem-cell rescue. J Clin Oncol 16:3270–3278

87. Ma D, McDevitt MR, Barendswaard E, Lai L, Curcio MJ, Pellegrini V, Brechbiel MW, Scheinberg DA (2002) Radioimmunotherapy for model B cell malignancies using[90]Y-labeled anti-CD19 and anti-CD20 monoclonal antibodies. Leukemia 16:60–66

88. McDevitt MR, Ma D, Lai LT, Simon J, Borchardt P, Frank RK, Wu K, Pellegrini V, Curcio MJ, Miederer M, Bander NH, Scheinberg DA (2000) Tumor therapy with targeted atomic nanogenerators. Science 294:1537–1540

89. Meredith RF, Buchsbaum DJ, Knox SJ (2000) Radionuclide dosimetry and radioimmunotherapy of cancer. In: Abrams AR, Fritzberg AR (eds) Radioimmunotherapy of cancer. Marcel Dekker, New York

90. Meredith RF, Khazaeli MB, Plott G, Wheeler R, Russell C, Shochat D, Norvitch M, Saletan S, LoBuglio A (1993) Comparison of diagnostic and therapeutic doses of[131]Lym-1 in patients with non-Hodgkin's lymphoma. Antib Immunoconj Radiopharm 6:1-11

91. Meredith RF, Khazaeli MB, Liu T, Plott G, Wheeler RH, Russell C, Colcher D, Schlom J, Shochat D, LoBuglio AF (1992) Dose fractionation of radiolabeled antibodies in patients with metastatic colon cancer. J Nucl Med 33:1648–1653

92. Meredith RF, LoBuglio AF, Spencer EB (1997) Recent progress in radioimmunotherapy for cancer. Oncology (Huntingt) 11:979–987

93. Miyamoto D, Brady LW, Rackover M, Emrich J, Class R, Bender H, Dadparvar F, Woo D, Young T, Eshleman J, Dilling T, Micaily B, Sceplewski Z, Koprowski H, Black P, Nair S, McCormack T (1995) Utilization of[125]I-monoclonal antibody in the management of primary glioblastoma multiforme. Radiat Oncol Invest 3:126–132

94. Murray JL, Macey DJ, Kasi LP, Rieger P, Cunningham J, Bhadkamkar V, Zhang HZ, Schlom J, Rosenblum MG, Podoloff DA (1994) Phase II radioimmunotherapy trial with[131]I-CC49 in colorectal cancer. Cancer (Suppl) 73:1057–1066

95. Murray JL (1995) Radioimmunotherapy of colorectal cancer. In: Goldenberg DM (ed) Cancer therapy with radiolabeled antibodies. CRC, Boca Raton, Fla.

96. Murthy S, Sharkey RM, Goldenberg DM, Lee RE, Pinsky CM, Hansen HJ, Burger K, Swayne LC (1992) Lymphoma imaging with a new technetium-99m labeled antibody, LL2. Eur J Nucl Med 19:394–401

97. O'Donnell RT, DeNardo GL, Kukis DL, Lamborn KR, Shen S, Yuan A, Goldstein DS, Mirick GR, DeNardo SJ (1999)[67]Copper-2-iminothiolane-6-[p-(bromocetamido)benzyl]-TETA-Lym-1 for radioimmunotherapy of non-Hodgkin's lymphoma. Clin Cancer Res 5:3330s–3336s

98. Paganelli G, Grana C., Chinol M, Stabin MG, Grana C, Prisco G, Robertson C, Tosi G, Paganelli G (1999) Antibody-guided three-step therapy for high grade glioma with yttrium-90 biotin. Eur J Nucl Med 26:348–357

99. Paganelli G, Magnani P, Fazio F (1993) Pretargeting of carcinomas with the avidin-biotin system. Int J Biol Markers 8:155–159

100. Pawlak-Byczkowska EJ, Hansen HJ, Dion AS, Goldenberg DM (1989) Two new monoclonal antibodies, EPB-1 and EPB-2, reactive with human lymphoma. Cancer Res 49:4568–4577

101. Pizer BL, Papanastassiou V, Moseley R (1991) Meningeal leukemia and medulloblastoma: preliminary experience with intrathecal radioimmunotherapy. Antib Immunoconj Radiopharm 4:753–761

102. Postema EJ, Mandigers CM, Oyen WJ, Raemaekers JM, Corstens FH (2000) Radioimmunotherapy of patients with non-Hodgkin's lymphoma with[186]Re-hLL2 (abstract 36). Cancer Biother Radiopharm 15:407

103. Postema EJ, Boerman OC, Oyen WJ, Raemaekers JM, Corstens FH (2001) Radioimmunotherapy of B-cell non-Hodgkin's lymphoma. Eur J Nucl Med 28:1725–1735

104. Press OW (1999) Radiolabeled antibody therapy of B-cell lymphomas. Semin Oncol 26:58–65

105. Press OW, Corcoran M, Subbiah K, Hamlin DK, Wilbur DS, Johnson T, Theodore L, Yau E, Mallett R, Meyer DL, Axworthy D (2001) A comparative evaluation of conventional and pretargeted radioimunotherapy of CD20-expressing lymphoma xenografts. Blood 98:2535–2543

106. Press OW, Eary JF, Appelbaum FR, Bernstein ID (1995) Treatment of relapsed B cell lymphomas with high-dose radioimmunotherapy and bone marrow transplantation. In: Goldenberg DM (ed) Cancer therapy with radiolabeled antibodies. CRC, Boca Raton, Fla.

107. Press OW, Eary JF, Applebaum FR, Martin PJ, Badger CC, Nelp WB, Glenn S, Butchko G, Fisher D, Porter B, Matthews DC, Fisher LD, Bernstein ID (1993) Radiolabeled-antibody therapy of B-cell lymphoma with autologous bone marrow support. N Engl J Med 329:1219–1224

108. Press OW, Eary JF, Appelbaum FR, Martin PJ, Nelp WB, Glenn S, Fisher DR, Porter B, Matthews DC, Gooley T (1995) Phase II trial of[131]I-B1 (anti-CD20) antibody therapy with autologous stem cell transplantation for relapsed B cell lymphomas. Lancet 346:336–340

109. Press OW, Eary JF, Gooley T, Gopal AK, Liu S, Rajendran JG, Maloney DG, Petersdorf S, Bush SA, Durack LD, Martin PJ, Fisher DR, Wood B, Borrow JW, Porter B, Smith JP, Matthews DC, Appelbaum FR, Bernstein ID (2000) A phase I/II trial of iodine-131-tositumomab (anti-CD-20), etoposide, cyclophosphamide, and autologous stem cell transplantation for relapsed B-cell lymphomas. Blood 96:2934–2942

110. Reardon DA, Akabani G, Coleman RE, Friedman AH, Friedman HS, Herndon JE 2nd, Cokgor I, McLendon RE, Pegram CN, Provenzale JM, Quinn JA, Rich JN, Regalado LV, Sampson JH, Shafman TD, Wikstrand CJ, Wong TZ, Zhao XG, Zalutsky MR, Bigner DD (2002) Phase II trial of murine (131)I-labeled antitenascin monoclonal antibody 81C6 administered into surgically created resection cavities of patients with newly diagnosed malignant gliomas. J Clin Oncol 20:1389–1397

111. Riva P, Franceschi G, Riva N, Casi M, Santimaria M, Adamo M (2000) Role of nuclear medicine in the treatment of malignant gliomas: the locoregional radioimmunotherapy approach. Eur J Nucl Med 27:601–609

112. Riva P, Marangolo M, Tison V, Armaroli L, Moscatelli G, Franceschi G, Spinelli A, Vecchietti G, Morigi P, Tassini R (1991) Treatment of metastatic colorectal cancer by means of specific monoclonal antibodies conjugated with iodine 131: a phase II study. Nucl Med Biol 18:109–119

113. Rouvier E, Gautherot E, Meyer P, Barbet J (1997) Targeting medullary thyroid carcinomas with bispecific antibodies and bivalent haptens. Results and clinical perspectives. Horm Res 47:163–167

114. Schlom J, Molinolo A, Simpson JF, Siler K, Roselli M, Hinkle G, Houchens DP, Colcher D (1990) Advantage of dose fractionation in monoclonal-antibody-targeted radioimmunotherapy. J Natl Cancer Inst 82:763–771

115. Sgouros G (1995) Radioimmunotherapy of micrometastases: sidestepping the solid tumor hurdle. J Nucl Med 36:1910–1912

116. Sharkey RM, Behr TM, Mattes MJ, Stein R, Griffiths GL, Shih LB, Hansen HJ, Blumenthal RD, Dunn RM, Juweid ME, Goldenberg DM (1997) Advantage of residualizing radiolabels for an internalizing antibody against the B-cell lymphoma antigen, CD22. Cancer Immunol Immunother 44:179–188

117. Sharkey RM, Blumenthal RD, Behr TM, Wong GY, Haywood L, Forman D, Griffiths GL, Goldenberg DM (1997) Selection of radioimmunoconjugates for the therapy of well-established or micrometastatic colon carcinoma. Int J Cancer 72:477–485

118. Sharkey RM, Blumenthal RD, Hansen HJ, Goldenberg DM (1990) Biological considerations for radioimmunotherapy. Cancer Res 50:964s–969s

119. Sharkey RM, Karacay H, Griffiths GL, Behr TM, Blumenthal RD, Mattes MJ, Hansen HJ, Goldenberg DM (1997) Development of a streptavidin-anti-carcinoembryonic antigen antibody, radiolabeled biotin pretargeting method for radioimmunotherapy of colorectal cancer. Studies in a human colon cancer xenograft model. Bioconjug Chem 8:595–604

120. Shih LB, Lu HH, Xuan H. Goldenberg DM (1994) Internalization and intracellular processing of an anti-B-cell lymphoma monoclonal antibody, LL2. Int J Cancer 56:538–545

121. Siegel JA (1998) Revised Nuclear Regulatory Commission's regulations for release of patients administered radioactive materials: outpatient iodine 131 anti-B1 therapy. J Nucl Med [Supp] 39:28–33

122. Siegel JA, Goldenberg DM, Badger CC (1999) Radioimmunotherapy dose estimation in patients with B-cell lymphoma. Med Phys 20:579–582

123. Siegel JA, Yeldell D, Goldenberg DM, Stabin MG, Sparks RB, Sharkey RM, Brenner A, Blumenthal RD (2003) Red marrow radiation dose adjustment using plasma FLT3-L cytokine levels: improved correlations between hematologic toxicity and bone marrow dose for radioimmunotherapy patients. J Nucl Med 44:67–76

124. Tempero M, Leichner P, Dalrymple G, Harrison K, Augustine S, Schlam J, Anderson J, Wisecarver J, Colcher D (1997) High-dose therapy with iodine-131-labeled monoclonal antibody CC49 in patients with gastrointestinal cancers: a phase I trial. J Clin Oncol 15:1518–1528

125. Tempero M, Leichner P, Baranowska-Kortylewicz J, Harrison K, Augustine S, Schlom J, Anderson J, Wisecarver J, Colcher D (2000) High-dose therapy with [90]yttrium-labeled monoclonal antibody CC49: a phase I trial. Clin Cancer Res 6:3095–3102

126. Vose JM, Colcher D, Gobar L, Bierman PJ, Augustine S, Tempero M, Leichner P, Lynch JC, Goldenberg D, Armitage JO (2000) Phase I/II trial of multiple dose [131]iodine-MAb LL2 (CD22) in patients with recurrent non-Hodgkin's lymphoma. Leuk Lymphoma 38:91–101

127. Vose JM (1999) Antibody-targeted therapy for low-grade lymphoma. Semin Hematol 36:15–20

128. Vuillez JP, Kraeber-Bodere F, Moro D, Bardies M, Douillard JY, Gautherot E, Rouvier E, Barbet J, Garban F, Moreau P, Chatal JF (1999) Radioimmunotherapy of small cell lung carcinoma with the two-step method using a bispecific anti-carcinoembryonic antigen/anti-diethylenetriaminepentaacetic acid (DTPA) antibody and iodine-131 di-DTPA hapten: results of a phase I/II trial. Clin Cancer Res 5:3259s–3267s

129. Wahl RL (1994) Experimental radioimmunotherapy. A brief overview. Cancer (Supp) 73:989–992

130. Wahl RL, Zasadny KR, Estes J, Kison PV, Regan DD, McCullough NT, Kroll S, Stagg B, Kaminski MS (2000) Single center experience with iodine I[131] tositumomab radioimmunotherapy for previously untreated follicular lymphoma (FL) (abstract 309). J Nucl Med 41:78–79

131. Weiden PL, Breitz HB, Press O, Appelbaum JW, Bryan JK, Gaffigan S, Stone D, Axworthy D, Fisher D, Reno J (2000) Pretargeted radioimmunotherapy (PRIT™) for treatment of non-Hodgkin's lymphoma (NHL): initial phase I/II study results. Cancer Biother Radiopharm 15:15–29

132. Welt S, Divgi CR, Kemeny N, Finn RD, Scott AM, Graham M, Germain JS, Richards EC, Larson SM, Oettgen HF (1994) Phase I/II study of iodine 131-labeled monoclonal antibody A33 in patients with advanced colon cancer. J Clin Oncol 12:1561–1571

133. Wilder RB, DeNardo GL, DeNardo SJ (1996) Radioimmunotherapy: recent results and future directions. J Clin Oncol 14:1383–1400

134. Wiseman GA, White CA, Stabin M, Dunn WL, Erwin W, Dahlbom M, Raubitschek A, Karvelis K, Schultheiss T, Witzig TE, Belanger R, Spies S, Silverman DH, Berlfein JR, Ding E, Grillo-Lopez AJ (2000) Phase I/II ^{90}Y-Zevalin (yttrium-ibritumomab tiuxetan, IDEC-Y2B8) radioimmunotherapy dosimetry results in relapsed or refractory non-Hodgkin's lymphoma. Eur J Nucl Med 27:766–777

135. Wiseman GA, White CA, Witzig TA, Stabin MG, Spies S, Silverman DH, Raubitschek A, Gordon L, Emmanouilides C, Janakiraman N (1999) Final dosimetry results of IDEC-Y2B8 Phase I/II [90]yttrium radioimmunotherapy trial in non-Hodgkin's lymphoma (NHL) (abstract 260). J Nucl Med 40:64

136. Witzig TE, Gordon LI, Cabanillas F, Czuczman MS, Emmanouilides C, Joyce R, Pohlman BL, Bartlett NL, Wiseman GA, Padre N, Grillo-Lopez AJ, Multani P, White CA (2002) Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. J Clin Oncol 20:2453–2463

137. Witzig TE, Gordon LI, Wiseman GA, Spies S, Emmanouilides C, Czuczman MS, Flinn IW, Barlett NL, Murray JL, Alkuzweny B, Multani PS, Grillo-Lopez AJ, White CA (2000) Zevalin radioimmunotherapy is safe and well tolerated in patients with relapsed or refractory, B cell non-Hodgkin's lymphoma (NHL) (abstract 3160). Blood (Part 1):731a

138. Witzig TE, White CA, Flinn IW, Gordon L, Emmanouilides C, Cripe LD, Saleh M, Czuczman MS, Wiseman GA, Spies S, silverman DH, Burt RW, Olejnik T, Grillo-Lopez AJ (2000) Zevalin™ radioimmunotherapy of rituximab-refractory follicular non-Hodgkin's lymphoma (abstract 2183). Blood 96 (Part 1):507a

139. Zalutsky MR, Moseley RP, Benjamin JC, Colapinto EV, Fuller GN, Coakham HP, Bigner DD (1990) Monoclonal antibody and F(ab')$_2$ fragment delivery to tumor in patients with glioma: comparison of intracarotid and intravenous administration. Cancer Res 50:4105–4110