Perspectives on Cancer Therapy with Radiolabeled Monoclonal Antibodies

Robert M. Sharkey, PhD; and David M. Goldenberg, ScD, MD

Garden State Cancer Center, Center for Molecular Medicine and Immunology, Belleville, New Jersey

With the approval of 2 radiolabeled antibody products for the treatment of non-Hodgkin's lymphoma (NHL), radioimmunotherapy (RIT) has finally come of age as a new therapeutic modality, exemplifying the collaboration of multiple disciplines, including immunology, radiochemistry, radiation medicine, medical oncology, and nuclear medicine. Despite the many challenges that this new therapy discipline has encountered, there is growing evidence that RIT can have a significant impact on the treatment of cancer. Although follicular NHL is currently the only indication in which RIT has been proven to be effective, clinical trials are showing usefulness in other forms of NHL as well as in other hematologic neoplasms. However, the treatment of solid tumors remains a formidable challenge, because the doses shown to be effective in hematologic tumors are insufficient in the more common epithelial cancers. Nevertheless, there has been progress in locoregional applications and in the treatment of minimal residual disease. There is also optimism that pretargeting procedures, including new molecular constructs and targets, will improve the delivery of radioactivity to tumors, do so with less hematologic toxicity, and become the next generation of RIT.

Key Words: Antibodies; colorectal cancer; non-Hodgkin's lymphoma; pretargeting; radioimmunotherapy

J Nucl Med 2005; 46:115S–127S

At the turn of the 19th century, Paul Ehrlich conceived the idea that "magic bullets" could effectively target compounds and eradicate sites of disease, but it was not until the early 1950s that this idea was first explored with an antibody conjugated to a radionuclide (*1*). Another quarter of a century would pass before antibody-based tumor localization was achieved clinically (*2*). This then led to the first radioimmunodetection products in the mid-1990s (*3*). In the first years of the 21st century, the first agent of this type was approved for the treatment of non-Hodgkin's lymphoma (NHL) (*4,5*). The first 30 y of this process were devoted mostly to the discovery and production of suitable antibodies, as well as developing radiolabeling technologies. Cellular biology led the way with the development of monoclonal antibodies and, more recently, with the engineering of antibodies in various configurations with reduced immunogenicity. It is worth noting that antitumor antibodies remain one of the best means for selective binding to suitable targets on cancer cells and have also stimulated the study of other delivery forms, such as oligonucleotides or aptamers (*6,7*). However, the use of antibodies in radioimmunotherapy (RIT) is still evolving, with the investigation of new molecular constructs, new radionuclides and radiochemistry, improved dosimetry, prediction of tumor response and host toxicities, and better targeting strategies to prevent or overcome host toxicities, particularly myelosuppression. The hope is that the advances made for RIT in hematologic malignancies will translate to progress in the therapy of more radioresistant solid tumors. The purpose of this article is not to review the many efforts and advances made in RIT, but to summarize our views of the current status and future prospects. Other reviews may be consulted for more comprehensive discussions of this subject (*4,5,8–14*).

HEMATOLOGIC MALIGNANCIES

[90]Y-ibritumomab tiuxetan (Zevalin; IDEC Pharmaceuticals Corporation) and ^{131}I-tositumomab (Bexxar; Corixa and GlaxoSmithKline Corporations) are currently the only radiolabeled antibodies approved for treatment of cancer. Each is registered for therapy of chemotherapy-refractive, follicular (low-grade) NHL, with or without transformation, and uses an antibody that is directed to CD20, an antigen that is abundantly present on a high percentage of both normal and malignant B-cells. Details of the treatment schemes for each of these approved agents are provided in Figure 1. Each is administered at radioactivity dose levels that result in severe myelosuppression that, for the most part, is reversible. A significant portion of patients in the registration trials for Zevalin and Bexxar, however, required supportive therapy, including platelet transfusions (22% and 15%, respectively), erythropoietin or epoetin alfa (8% and 7%, respectively), or filgrastim (13% and 12%, respectively) (*15,16*). The overall response rate for ^{131}I-tositumomab in rituximab-refractory patients was similar to that reported with ^{90}Y-ibritumomab tiuxetan, suggesting that the radionuclides are equally effective. However, the selection of the radionuclide has been shown to be more important for
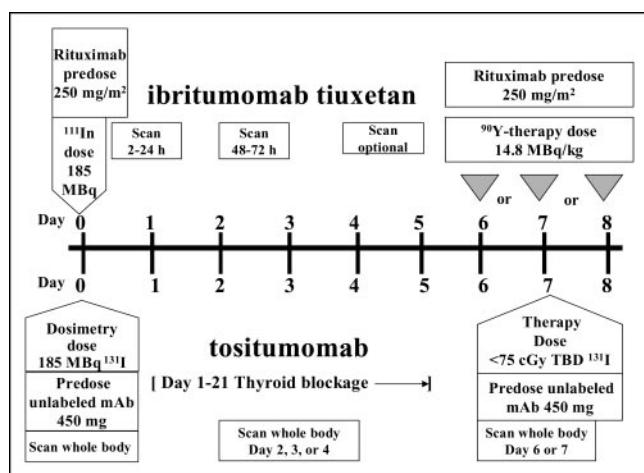
FIGURE 1. Comparison of administration conditions for ^{131}I-tositumomab) and ^{90}Y-ibritumomab tiuxetan. Reprinted with permission from Goldenberg, DM. Therapeutic use of radiolabeled antibodies: hematopoietic tumors. In: Ell PJ, Gambhir SS, eds. *Nuclear Medicine in Clinical Diagnosis and Treatment.* 3rd ed. London, UK: Churchill Livingstone; 2004:428–434.

other radiolabeled antibodies being investigated as potential therapeutics for NHL, because these antibodies are internalized and then rapidly catabolized, which favors the use of radionuclides that remain inside the cell (*17–20*). ^{90}Y-ibritumomab tiuxetan and ^{131}I-tositumomab each have unique requirements for use, which have been extensively reviewed in several papers (*21–26*).

Antitumor responses in NHL occur at very low absorbed doses (e.g., much less than 1,000 cGy). Koral et al. (*27,28*) reported a trend for higher absorbed doses to tumors that ultimately had a complete response, but others have disputed this relationship and even whether the lesions that responded were visualized by the radioimmunoconjugate (*29,30*). Although clear evidence for a dose–response relationship is lacking, it is likely present, but technical limitations in the way radiation dose and tumor size are measured create considerable inaccuracies in these estimates. Another variable is the fact that some antibodies used in these trials have therapeutic activity by themselves (*31,32*). Evidence suggests a role for anti-CD20 antibody in enhancing the therapeutic response from low-dose radiation (*33,34*). A randomized trial comparing the efficacy of a full course of rituximab (375 mg/m^2/week $\times$ 4) with that of ^{90}Y-ibritumomab tiuxetan (250 mg/m^2 rituximab/week $\times$ 2 with the radiolabeled murine anti-CD20) showed the radiolabeled antibody to be superior to the naked antibody (i.e., statistically improved overall and complete response rates), but the difference in median time to progression was not statistically significant. Nevertheless, durable responses have been reported for patients who achieved a complete response (*35,36*). A similarly randomized trial with the naked murine B1 anti-CD20 antibody used with ^{131}I-tositumomab was not performed. However, despite evidence that its mechanisms of action are somewhat different from those of rituximab,

B1 alone has been shown to be active in animal models (*37–39*). Furthermore, responses were described in patients after they received a pretherapy diagnostic imaging dose (*40,41*). Anti-CD22 and anti-human leukocyte antigen (anti-HLA) antibodies also have been shown to be active as naked antibodies (*42,43*). Thus, the antibody itself is likely contributing to the antitumor responses observed with some of the radiolabeled antibodies used in NHL. In contrast, evidence for this has not been documented for the antibodies used in RIT of solid tumors.

Although the acceptance of these approved treatment modalities appears to be slow, it is important to remember that this technology is still in the early stages of development. With several opportunities to improve the overall response and survival rates, this treatment modality should become more established. For example, RIT is currently used in patients in whom chemotherapy fails, but clinical studies using ^{131}I-tositumomab as a frontline treatment for NHL were highly encouraging, not only because of the excellent response rate, but also because the regimen had far fewer side-effects than chemotherapy (*44*). Press et al. (*45*) reported encouraging therapeutic results when ^{131}I-tositumomab was administered as a frontline therapy 4–6 wk after standard cyclophosphamide, adriamycin, vincristine, and prednisone treatment for follicular NHL. It is notable that in this trial hematologic toxicity was more severe with chemotherapy than with RIT. Most of the data for RIT are based on a single treatment, yet Kaminski et al. (*35*) reported that 9 of 16 patients re-treated with ^{131}I-tositumomab after progression responded a second time, with 5 attaining a complete response. Others have reported safety and efficacy of radioantibody treatment in patients who previously received another radioantibody therapy, and standard chemotherapy can also be given after nonmyeloablative radioantibody treatment without additional side effects (*46–48*). Thus, not only can multiple cycles of radioantibody treatment be given, but RIT can also be integrated safely with various treatment modalities as a means of further improving response.

Although each radioantibody treatment has established a dosing regimen that ultimately results in a majority of patients experiencing severe, dose-limiting thrombocytopenia and neutropenia, the tolerance of the therapy is related more to the patient's treatment history as it affects their bone marrow status than is the actual radiation dose delivered to the red marrow (*49*). Perhaps further refinement in optimizing the manner in which the radioactivity dose is assigned individually could ensure that each patient receives the maximum dose allowed and improves responses. A retrospective analysis of hematologic toxicity based on red marrow dose and the measurement of Flt-3 ligand, a biologic marker for bone marrow status, indicated that the inclusion of the biologic marker assessment could improve toxicity outcome prediction significantly compared with dosimetry alone, at least in patients with solid tumors (*50*). RIT is also being used in high-dose therapy regimens with

chemotherapy and external beam radiation (and possibly as a replacement for whole-body radiation) in cytoreductive marrow conditioning regimens (*51–56*).

Other radioantibody conjugates being tested clinically could improve responses or expand indications. A phase 2 trial with ^{131}I-rituximab (i.e., with the chimeric antibody labeled and not the murine antibody as in Zevalin) has been reported (*57*). In this study, 375 mg/m^2 of rituximab were given as a predose for both the imaging and subsequent therapy dose. An objective response rate of 71% was observed in 35 patients, including a complete response in 54% of patients, with a median duration of 20 mo. This response rate was similar to that reported with ^{131}I-tositumomab and ^{90}Y-ibritumomab tiuxetan, but the median duration was longer and appeared to have been better tolerated. Only 2 of 42 patients experienced a grade 4 hematologic toxicity. Early clinical trials examining the potential utility of radiolabeled epratuzumab (humanized anti-CD22 IgG; Immunomedics, Inc.) and Oncolym (anti-HLA-DR10; Peregrine Pharmaceuticals, Inc.) in NHL also have shown promising antitumor effects, including results in patients with aggressive forms of NHL (*30,58,59*).

Thus, there likely will be several opportunities to expand the application of radiolabeled antibodies in the treatment of NHL. However, because radiation, like chemotherapy, can have potential long-term effects, it could take several years before its full safety profile is known. In 5 clinical trials with ^{90}Y-ibritumomab tiuxetan in 349 patients, 3 cases of acute myeloid leukemia (AML) were reported in addition to 2 cases of myelodysplastic syndrome (MDS). MDS was also reported in 32 of 995 patients administered ^{131}I-tositumomab, with 27 mo as the median time to development. Therefore, the rate of MDS may continue to increase as the use of these agents expands. However, the probability for the development of MDS/AML in NHL patients given myeloablative doses of ^{131}I-anti-CD20 IgG ($n = 27$) was 0.076 at 8 y and 0.086 at 7 y in patients who received high-dose chemotherapy ($n = 98$), suggesting that the overall risk of developing secondary malignancies or complications with RIT may be no higher than that ascribed to chemotherapy (*60*). Patients administered ^{131}I-tositumomab also have a risk of developing mild-to-moderate hypothyroidism, must be given a thyroid blocking regimen at least 1 d in advance of the diagnostic injection, and must be maintained for at least 2 wk after the therapy regimen.

Patients with >25% bone marrow involvement are not currently referred for treatment with either ^{131}I-tositumomab or ^{90}Y-ibritumomab tiuxetan, because of increased risk of severe myelosuppression. This restriction exists primarily because these agents use β-emitting radionuclides with several-millimeter pathlengths that can cause collateral damage to the surrounding normal marrow. It is possible that smaller, fractionated doses of these radiolabeled antibodies could be given safely to reduce the infiltration of marrow. Indeed, studies have already shown that if the pathlength of the radionuclide is shortened RIT can be applied to blood-

borne (leukemias) or other hematologic malignancies primarily involving the bone marrow, potentially without the need for supportive measures (e.g., peripheral blood stem cell transplant).

In this regard, α-emitters are prime candidates for such applications and are being explored for the treatment of myeloid leukemia (*61–63*). An α-particle has a path length that will traverse several cell diameters, making it effective against small cell clusters, and its high linear energy transfer increases the probability for cell killing even when targeting low-density antigens. However, even Auger-emitting radionuclides have been shown to be highly effective in animal models with disseminated human NHL (*64*). Auger-emitters exert their activity mostly to the targeted cell and, therefore, could be ideally suited for treating micrometastatic disease, even in the bone marrow, because nontargeted cells would be unaffected. However, because of their low energy, a substantial number of Auger particles must be delivered to kill a cell. We have shown, for example, that an antibody to CD74 is capable of delivering large quantities of Auger-emitters inside the cell. This is not because of large numbers of antigen sites on the cell surface but because the antigen is internalized and constantly recycling, thereby transporting and emptying the antibody with its radioactive payload inside the cell and then returning to the cell surface, where it is available to bind additional antibodies (*65*). Although antibodies radiolabeled with α-emitters and Auger-emitters are typically considered for applications such as leukemia, they may have a role in the treatment of other hematologic malignancies (and potentially solid tumors). For example, a radiolabeled antibody conjugated to an α-emitter or Auger-emitter could be administered in patients with follicular lymphoma who have >25% bone marrow involvement, making these patients eligible for subsequent RIT with β-emitters or chemotherapy, particularly if these treatments can be shown to be effective at doses that would not affect the tolerance of subsequent therapy. It is also intriguing to speculate that α- or Auger-emitting antibodies could be given after these other treatments as a means of scavenging smaller pockets of disease that might not otherwise be as effectively treated with β-emitters or drugs.

SOLID CANCERS

Chemotherapy has been more successful in hematologic than in solid tumor malignancies, and this seems to be true for RIT as well. Clinical trials of NHL treatment have yielded reports of significant antitumor responses (in fact, complete responses) with the delivery of considerably less than 1,000 cGy to a tumor (*27,66*). Yet RIT in a variety of solid tumors has failed to elicit responses in patients with disease burdens similar to those treated in NHL trials, even with the delivery of as much as 3,000 cGy to the tumor using ^{131}I- or even ^{90}Y-labeled antibodies (*67,68*). With little evidence of clinically significant responses with RIT alone, other strategies to augment the targeting of the radiolabeled

antibody have been explored. For example, Meredith et al. (*69*) combined an anti-carcinoembryonic antigen (anti-CEA) antibody (COL-1) and the CC49 anti–tumor-associated glycoprotein (TAG)–72 IgG radiolabeled with ^{131}I, because by immunohistology the combination gave a more homogeneous distribution within the tumor than either antibody alone. In addition, patients received α-interferon, which had been reported to enhance CEA and TAG-72 expression in gastrointestinal tumors. Tumor imaging was judged to be excellent in most cases. The combination of anti-CEA and anti-TAG-72 antibodies together with interferon appeared to result in a modest increase in the absorbed dose to the tumor when compared with other trials that used only ^{131}I-CC49, but yielded no objective responses. Radiolabeled anti-TAG-72 antibodies have been used in other cancers, including breast, prostate, and ovarian cancer, but few if any objective responses have been observed in patients with bulky disease (*70–72*). Despite failing to observe objective antitumor responses with a ^{90}Y-anti-CEA antibody, Wong et al. (*73*) reported substantial reductions (e.g., 41%–68%) in a few colorectal cancer lesions (size, 2.0–6.5 cm in diameter) and some tumor stabilization with the delivery of <1,000 cGy. Thus, the challenge remains to understand the underlying mechanisms of the refractoriness of solid tumors to RIT.

In many respects, clinical testing has ignored what was observed in preclinical models over many years: RIT alone usually is not effective against bulky disease (*74*). Evidence in support of using RIT in minimal disease came from investigations of RIT in the treatment of a colon cancer in a lung metastasis model, where animals bearing multiple small foci of disease could be cured with RIT alone (*75*). RIT's efficacy in preventing the death of animals bearing these small microscopic colonies of colon cancer in the lung was significantly reduced if bulky disease (i.e., a large subcutaneous tumor) co-existed at the time of treatment (*76*). In addition, animal models have typically shown the uptake of radiolabeled antibodies to be highest on a per-gram basis in small tumors, which has been confirmed clinically (*77–80*). Despite these observations, RIT is still being tested in patients with multiple lesions that are mostly large (>5 cm in diameter). This is probably attributable to regulatory requirements that initial trials be conducted in patients with advanced disease for which other options have failed. In contrast, encouraging results are emerging with RIT in patients with minimal or occult disease. For example, after a phase 1 trial determining the maximum tolerated dose (MTD) and initial evidence of efficacy in either patients with small colorectal cancer metastases or patients given RIT after salvage resection of liver metastases (*81*), Liersch et al. (*82*) reported on an exploratory trial using an ^{131}I-humanized anti-CEA IgG in 22 patients who had undergone liver resection for metastatic colorectal cancer. The median survival from the time of liver resection of the 19 patients who could be assessed for response was 54 mo, which is encouraging compared with a median survival of

between 24 and 42 mo reported in 12 separate clinical trials involving more than 5,000 colorectal cancer patients after salvage surgery of liver metastases (*83*).

Although the majority of clinical trials to date have used whole IgG, several studies have suggested that antibody fragments would be better delivery vehicles. Although modeling/dosimetry studies predict that IgG is a better vehicle for therapy than an antibody fragment, empirical studies in animals show improved therapy with radiolabeled antibody fragments when compared with therapy with whole IgG (*84–90*). Enzymatically digested antibody fragments have been used clinically. In the future, engineered antibodies, such as single chains (single-chain variable fragment [scFv], ~25,000 Da) or diabodies (~50,000 Da), which are smaller monovalent and divalent binding proteins, respectively, or even one of a variety of other types of constructs, will be the focus of clinical testing as possible alternatives to intact IgG (*91–94*). Figure 2 depicts the commonly used antibody fragments as well as several of their molecularly engineered counterparts. Various candidate molecules and their properties are summarized in Table 1. Although $F(ab')_2$ and Fab' fragments have been used extensively in patients, less clinical data are available for the molecularly engineered products, so their precise targeting and distribution properties reflect findings in animal models.

The primary deterrent for using antibody fragments, particularly those with a molecular size <50,000 Da, is that they are cleared through the kidneys, which raises concern for renal toxicity, particularly when a radionuclide is used that is reabsorbed and retained by the kidney, such as a radiometal. Behr et al. (*95*) reported that a high predose of cationic amino acids could significantly reduce renal tubular reabsorption of radiometal-labeled (e.g., ^{99m}Tc- or ^{111}In-) Fab'. They showed subsequently in mice that with this procedure higher doses of a radiometal-labeled Fab' could be administered with less renal toxicity. However, bone marrow support was also required to escalate the radioactivity dose, indicating that, just as with an IgG, bone marrow toxicity is dose limiting even for an antibody fragment (*96*). Cationic amino acids have been used clinically in combination with ^{90}Y-1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid (DOTA), Tyr^3-octreate (^{90}Y-DOTATOC), a somatostatin peptide analog, to reduce renal accretion. Even with this method, care was taken not to exceed 2,500 cGy to the kidneys, because of late renal toxicity, and myelosuppression proved to be dose limiting (*97,98*). Having to contend with renal toxicity is also a significant deterrent for treatment with radiometal-labeled antibody fragments, because renal toxicity is not manifested until at least 3 mo after treatment and careful monitoring is required for more than 1 y (*99,100*). Because radioiodine is not retained in the kidneys, it is a more likely choice for labeling antibody fragments for studies. For example, clinical studies using a radioiodinated CH2-deletion construct, a type of engineered antibody fragment, are currently under way (*101*).
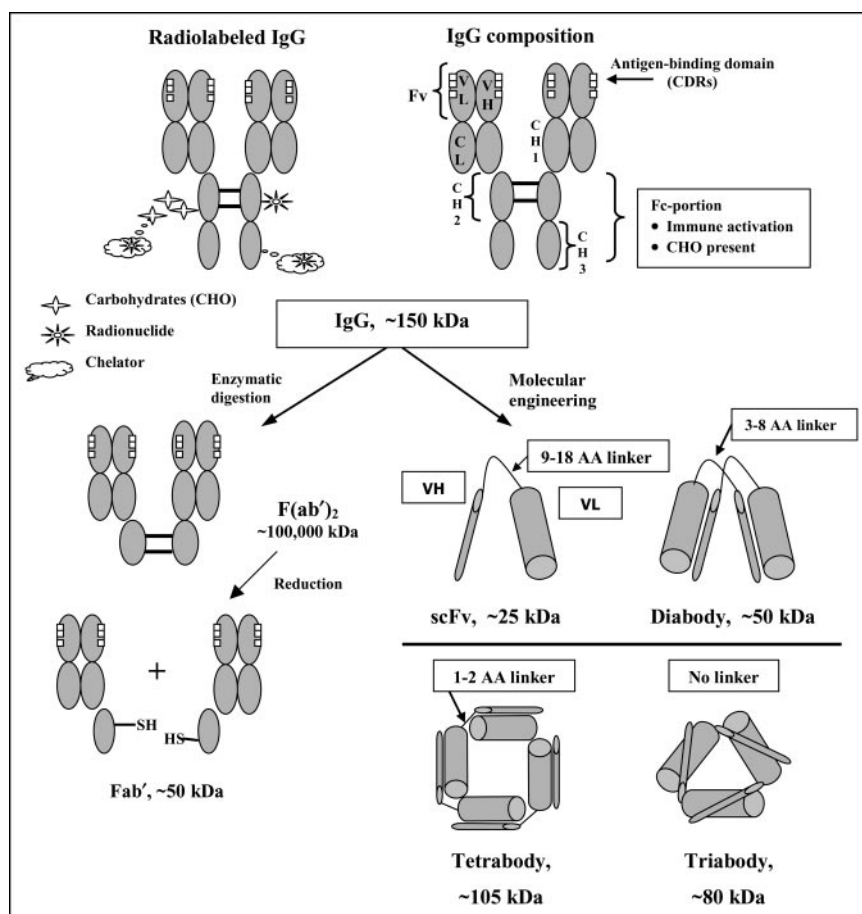
FIGURE 2. Schematic representation of various forms of antibody fragments prepared by enzymatic digestion or molecular engineering. The most commonly used form of antibody, IgG, has been radiolabeled by conjugating directly to the protein (e.g., radioiodination of tyrosine) or radiolabeling a coupled chelator. Chelators have been coupled directly to the protein or to the carbohydrates that reside on the Fc-portion of the molecule. Single chains are formed by linking the variable light (VL) and variable heavy (VH) chains with amino acid (AA) linker. Diabodies, triabodies, and even tetrabodies are formed spontaneously when smaller length AA chains are used to hold the VH and VL units together. Recombinant bispecific diabodies and other bispecific constructs can be prepared by pairing VH and VL of 2 antibodies with different specificities.

The use of RIT locally is another example of treating smaller tumors. The most prevalent locoregional treatment strategies include intraperitoneal delivery of antibodies for ovarian cancer and intracranial administration for brain cancers. The route of administration is based on preclinical data that showed an advantage for intraperitoneal over intravenous injection, particularly but not conditionally in the presence of malignant ascites (102–106). Colcher et al.

(107) showed in patients who were coadministered intraperitoneal and intravenous injections of ^{131}I-/^{125}I-B72.3 IgG that the majority of lesions (33 of 55) isolated in surgery had a 2-fold higher uptake for the intraperitoneally administered antibody, whereas 13 lesions were found to have a 2-fold higher uptake with the intravenously injected antibody. In 7 tumors uptake did not differ between the two administration routes. Pathologic assessment of these lesions led to the

TABLE 1
Comparison of Targeting Properties of Representative Forms of Antibody and Chemically Prepared or Engineered Fragments

Antibody/fragment	Estimated molecular weight (kDa)	Biologic properties		Tumor-binding properties		
		Half-time rank*	Target organ	Relative uptake rank†	Relative duration rank‡	Time to optimum accretion
IgG	150	1 (days)	Liver	1	1	Day(s)
F(ab')$_2$	100	2 (days)	Liver	2	2	Day
Fab'	50	3 (hours)	Kidneys	3	3	Hours
Diabody	40	3 (hours)	Kidneys	3	3	Hours
scFv	20	4 (hours)	Kidneys	4	4	Hour

*Relative biologic half-time. Numbers represent grading from slowest (1) to fastest (4). In parentheses is estimated time for 50% of antibody to clear from blood.
†Based on intravenous infusion. Numbers represent grading from highest (1) to lowest (4).
‡Numbers represent grading from longest (1) to shortest (4).

conclusion that peritoneal implants were more likely to benefit from intraperitoneal injection, whereas nonimplants (i.e., those metastases in the peritoneal cavity resulting from hematogeneous spread) were more likely to have higher uptake by intravenous injection. In this regard, it is possible that both routes of injection should be contemplated. We reported a complete response in a patient with ovarian cancer who presented with malignant ascites and peritoneal implants at the time of her intravenous treatment with 1,480 MBq/m^2 of ^{131}I-labeled anti-CEA IgG (108). Except for one other patient in this trial who experienced a brief minor response, all other enrolled patients with advanced disease progressed (109).

The most advanced agent in clinical testing for intraperitoneal RIT in ovarian cancer is pemtumomab (R1549; Antisoma plc), a ^{90}Y-labeled murine antihuman milk fat globulin 1 (MUC-1) murine antibody. This agent was tested in ovarian cancer patients with measurable and occult disease. The most promising data were from a phase 2 trial, in which 666 MBq/m^2 of this agent were administered intraperitoneally to 21 women with stage IC–IV ovarian cancer who had no detectable disease after surgery and completing a platinum-based chemotherapy regimen. Seventy-eight percent of these patients were alive 10 y after receiving this treatment, whereas the median survival for a historical control group (72 patients) was less than 4 y (110). However, according to a recent announcement by Antisoma, preliminary findings from a multicenter phase 3 trial showed no significant difference between ovarian cancer patients with no evidence of disease who were given a single intraperitoneal treatment of the ^{90}Y-labeled antibody and those in the control arm. It is important to keep in mind that this single experience does not necessarily detract from the prospects of RIT in this indication. An investigation of different antibodies, radionuclides, or specific treatment regimens with the radiolabeled antibody alone or in combination with other agents may still provide evidence that RIT could have a role in the management of occult disease. In this regard, another study using intraperitoneally administered ^{177}Lu-CC49 anti-TAG-72 IgG has noted a similar trend toward improved responses in ovarian cancer patients with minimal disease. For example, a response was observed in only 1 of 13 patients who had gross disease at the time of treatment, whereas 7 of 9 patients with nodules smaller than 1 cm did not progress until at least 21 mo. Of the 5 patients with occult disease, 4 were without evidence of disease for a range of 6 to 35 mo (111).

Using the intracranial route, clinical studies with an ^{131}I-labeled antitenascin antibody for the local treatment of glioblastoma multiforma have been very encouraging, with median survival rivaling that with brachytherapy or stereotactic radiosurgery but with a lower rate of radionecrosis (112,113). These investigators also reported a correlation between the radiation-absorbed dose delivered to the rim of the cavity, where the radioantibody was deposited, and various parameters that reflected a positive outcome for the patient (114). A similar intracranial approach has also been shown in animal models to be useful for treating brain metastases of other cancers (115). Quang et al. (116) also reported the successful use of ^{125}I-labeled antiepidermal growth factor receptor antibody in a phase 1 and 2 trial, where patients received the radiolabeled antibody after surgical resection of primary brain cancer and in concert with external beam therapy. Thus, intracavity treatment with radiolabeled antibodies is an approach that can be administered safely, alone or in combination with other treatment modalities, but appears to be most promising in minimal residual disease.

Nearly every form of cancer therapy at some time has been examined in combination with another form of treatment in the hope of improving efficacy, and RIT is no exception. Combination approaches fall into 2 categories: those designed to improve radioantibody distribution and uptake and those in which RIT is combined with other therapy modalities.

Some examples of the first category of combinations have already been cited, such as administering interferon to upregulate antigen expression and thereby improve targeting (70,71,117). Other reports have documented attempts to manipulate a tumor's vascular properties through hyperthermia, radiation, or biologically active compounds in an effort to enhance the amount of radiolabeled antibody targeted (118–123). Each of these methods has unique dosing and temporal requirements to optimize the targeting of the radiolabeled antibody. Whereas radiation and hyperthermia are designed to provide increased vascular permeability within the tumor, other systemically applied treatments with a variety of vasoactive agents have been used to enhance antibody uptake in tumors. Pedley et al. (124–126) observed improved targeting of antibodies using several agents known to affect the vasculature. The agents proved effective in killing or even curing established colorectal cancer xenografts when used in combination with radiolabeled antibodies. Kurizaki et al. (127) showed that the coadministration of an agonist peptide of human C5a with a radiolabeled antibody could improve antibody uptake and its therapeutic effects in nude mice. Other methods that are designed to inhibit neovascular formation also have been used effectively in combination with RIT (128–130). One innovative approach fuses gene therapy and RIT targeting by first introducing a gene product so that the tumor expresses a unique antigen. This antigen can then be targeted by a radiolabeled antibody (131).

Considerable attention has been given to combining RIT with chemotherapeutic agents. In such an approach, drugs that are known to be radiosensitizers or have other potential additive or synergistic properties are added to a full dose of RIT. The drug is given at a reduced dose or modified schedule primarily to enhance the therapeutic response of RIT over that with RIT alone. DeNardo et al. (132,133) were the first to report the enhancement of a subtherapeutic dose of paclitaxel when used in combination with a ^{90}Y-

labeled antibody in a nude mouse–human breast cancer model. Others have used this combination successfully in breast and prostate cancers (*134,135*). In addition to the taxanes, other chemotherapeutic agents have been reported to enhance the activity of RIT when used at submaximal doses (*136,137*). As with any combination modality, numerous dosing and scheduling issues are involved in optimizing the therapy. Several reports have reviewed this process, particularly as it relates to the p53 status of a given cell line (*138–140*). For example, starting with the SKOV-3 p53-null cell line, Blumenthal et al. (*140*) examined 3 p53-transfected cell lines that differed in their p53 mutation locus. They found considerable differences in each cell line's sensitivity to various drugs and to the drug–RIT combination. Several combinations were found to be antagonistic, highlighting the difficulty that may be encountered in the future with certain chemotherapy–RIT combinations as well as indicating ways in which prescreening of cancer biopsies could be used to optimize treatment planning (*140*).

The second approach, combining RIT with more conventional doses of drugs, rests on the assumption that RIT can enhance the chemotherapeutic regimen. However, this may prove difficult, because many conventional drugs are also myelosuppressive. Nevertheless, several reports in animal models have suggested promise for this approach (*141–146*).

Initial results of feasibility trials have been reported for combining intraperitoneal RIT with ^{177}Lu- or ^{90}Y-labeled anti-TAG-72 CC49 and chemotherapy in regional therapy of ovarian cancer as well as for ^{90}Y-labeled anti-CEA antibody combined with a continuous infusion treatment regimen of 5-FU in chemorefractive colorectal cancer (*147–149*). Although each of these regimens has been tolerated, with hematologic toxicity being dose limiting, it is too early to assess whether these combination modalities significantly enhance the effects of RIT for any specific chemotherapy regimen. However, based on studies in ovarian cancer, the trend continues to favor the treatment of less advanced disease with these combinations.

PRETARGETING: THE NEXT GENERATION OF RIT

Pretargeting evolved as a method to overcome the difficulties presented by high levels of radioactivity retained in the blood during RIT. Several types of pretargeting have been developed, but all strive to overcome the limitation of slow blood clearance of directly radiolabeled IgG by separating the targeting performed by the antibody from the subsequent delivery of the radionuclide (*149–156*).

Pretargeting was initiated with the notion that a bispecific monoclonal antibody (bsmAb) could be constructed with one binding arm directed to a target (e.g., a tumor antigen), and a second binding arm specific to a metal chelator (*157*). The metal chelator could then be radiolabeled and injected separately after the bsmAb had localized to the target antigen and cleared from the blood. This technique was first developed and tested clinically using a bispecific antibody composed of an anti-CEA Fab′ joined chemically with an antichelate Fab′ used in conjunction with the ^{111}In-labeled chelate (*158*). Le Doussal et al. (*159*) made a significant improvement to this initial pretargeting system by using a radiolabeled compound that included 2 haptens. This concept was called an affinity enhancement system, because a bsmAb bound monovalently to its target antigen would be cross-linked by the divalent hapten with a second bsmAb to form a divalent binding bridge to the tumor antigen (Fig. 3). Hnatowich et al. (*160*) were the first to describe an ingenious approach to pretargeting with avidin (mammalian produced) or streptavidin (bacterial product) in conjunction with biotin in a variety of configurations. This system appeared to be ideally suited for pretargeting, because the avidins could bind as many as 4 biotin molecules with an exceptionally high binding constant (10^{-15} mol/L). The reagents could be produced in plentiful amounts, were nontoxic, and were amenable to chemical modification for coupling to antibodies or radiolabeling.

Several configurations of an avidin/biotin pretargeting system were described, but 2 have prevailed (Fig. 3). One system, typically described as a 2-step approach, includes 3 agents: a streptavidin-conjugated antibody, a clearing agent, and the radiolabeled biotin. The clearing step is essential to the procedure, because the streptavidin–antibody conjugate, with its molecular weight of ∼200,000 Da, takes a considerable time to clear from the blood. This technique usually allows 1–2 d for the streptavidin antibody to localize to the tumor. The radiolabeled biotin is administered 1 d after the clearing step. The second approach, which has been referred to as a 3-step pretargeting procedure, also involves the use of 3 agents: a biotinylated antibody, a clearing/bridging agent, and radiolabeled biotin. In this method, avidin is used as a clearing agent, because it is glycosylated and has a natural tendency to clear from the blood by binding to sugar receptors in the liver. However, avidin will also bind to the tumor-localized, biotinylated antibody. Because avidin has up to 4 binding sites for biotin, it is still available to bind the radiolabeled biotin administered in the third injection (*161,162*).

Although it may be debated as to which technique is best, there is a growing consensus that pretargeting can deliver as much radioactivity to a tumor as a directly radiolabeled antibody but with much less exposure to the red marrow (*163,164*). In addition, as with antibody fragments, the pretargeting of the radiolabeled biotin or hapten/peptide occurs very quickly, and thus the dose rate to the tumor is also higher than that observed with a directly radiolabeled IgG. Moreover, all published investigations of pretargeting report far less renal accretion of radiolabeled biotin and hapten/peptides than with radiometal-labeled antibody fragments. Although the interaction of avidin/biotin is extremely strong, both the avidin/biotin and bsmAb pretargeting methods are bound to the tumor by the primary antitumor antibody, and the residence time in the tumor will likely reflect the antibody's binding affinity in both proce-
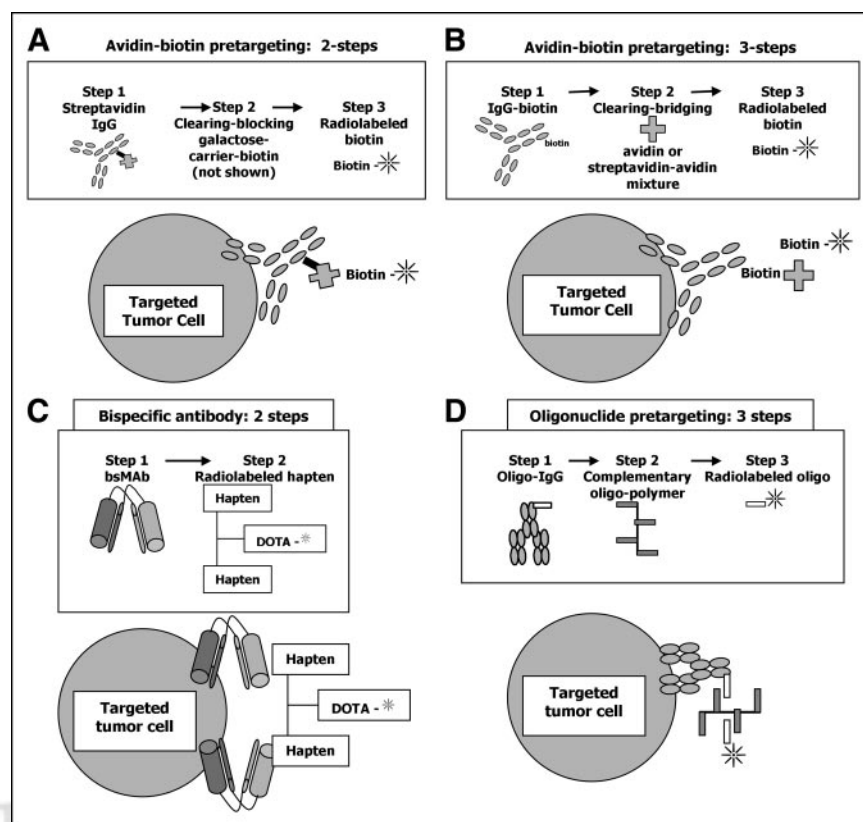
FIGURE 3. The next generation of radionuclide targeting; examples of pretargeting approaches: (A) Two-step streptavidin IgG/radiolabeled biotin procedure (*177,191*). (B) Three-step biotinylated IgG/radiolabeled biotin pretargeting procedure (*160,161*). (C) Bispecific antibody pretargeting procedure (*164*). (D) Oligonuclide pretargeting procedure (*165–167*).

dures. The avidins are immunogenic, whereas bsmAb can be humanized and, therefore, should be less immunogenic (*165*). Biotin occurs naturally in humans, but this does not seem to have hindered tumor targeting by these methods. Conjugates are prepared in a manner to protect against biotinylase activity.

A third type of pretargeting now explores the use of morpholinos, complementary synthetic DNA analogs, as bridging agents (*166–168*). These morpholino compounds should have low immunogenicity. When fully optimized, this approach could lead to further improvements in radionuclide targeting. Apart from the advantages and potential disadvantages for the bsmAb and avidin/biotin pretargeting systems that have been discussed in several reviews, all these approaches appear to hold promise as important advances in RIT (*155,156*).

Early imaging trials with the bsmAb and avidin/biotin pretargeting methods showed superior tumor-to-nontumor ratios in a shorter period of time than with directly radiolabeled antibody or fragments, which suggests that these methods could also be used for therapy (*169–173*). Axworthy et al. (*174*) were among the first to show that tumor uptake with a pretargeting procedure could be similar to that of a directly radiolabeled IgG while retaining superior tumor-to-nontumor ratios. Since this initial report, others have shown similar capabilities with either a bsmAb or an avidin/biotin pretargeting approach. Several investigators have reported improved therapy in a variety of models for solid tumors and hematologic malignancies when compared with results from directly radiolabeled antibodies or even antibody fragments (*175–186*). These promising preclinical studies resulted in several clinical therapy trials (most were phase 1 trials) designed to determine optimal targeting conditions, with subsequent accrual to determine the MTD. Despite the fact that preclinical studies show exceptionally rapid clearance of radioactivity from the blood, hematologic toxicity generally has been found to be dose limiting (*187–191*). In addition, reports from clinical trials using a ^{90}Y-DOTA-biotin in conjunction with the NR-LU-10-streptavidin conjugate indicated that patients experienced severe diarrhea. However, this was most likely caused by the antibody's specific binding to antigen in the lower GI tract and would not otherwise be expected with other pretargeting approaches (*192,193*). Renal toxicity was also observed. In all pretargeting approaches, the radiolabeled biotin or hapten-peptide is designed to clear quickly from the body by urinary excretion. Although renal retention of these agents is much lower than that seen with directly radiometal-labeled antibody fragments or even the radiolabeled somatostatin receptor peptides, renal toxicity is a concern.

Most clinical trials with pretargeting have involved patients with advanced cancer. Although modest but encouraging antitumor activity has been reported, these results continue to suggest that even with pretargeting, the treatment should be directed to use in patients with more radiosensitive tumors or minimal disease or in locoregional ap-

plications (*188–190*). For example, Paganelli et al. (*194*) reported a 25% objective response rate in patients with glioblastomas or astrocytomas after 2 treatments of a 3-step pretargeting procedure that used a biotinylated antitenascin antibody followed by an avidin chase/bridging step and, finally, by ^{90}Y-DOTA-biotin. All injections were given intracranially, and, although no hematologic toxicity was reported, neurotoxicity was dose limiting. Based on these promising data, a phase 1 and 2 trial was initiated in patients with high-grade gliomas. The study included 31 patients who underwent surgical debulking followed by radiotherapy, with 19 patients subsequently receiving the intracavity pretargeting procedure. All 12 of the patients who did not receive the additional pretargeting procedure died, with a median survival of 8 mo. For the patients who received the pretargeting RIT, median survival was 33.5 mo (*195*). Not surprisingly, antitumor responses have been observed more frequently in patients with NHL (*190,196,197*). At least in the experience of Weiden et al. (*190*), who used a pretargeting method involving a streptavidin–rituximab conjugate, a clearing agent, and ^{90}Y-DOTA-biotin, it appears that NHL patients might be able to tolerate only about 50% of the dose given to patients in the phase 2 trial reported by Knox et al. (*192*) in colorectal cancer patients, using the NR-LU-10-streptavidin conjugate followed by a clearing agent and ^{90}Y-DOTA-biotin. A clinical trial is currently underway to test a streptavidin-anti-CD20 scFv fusion protein and a ^{90}Y-DOTA-biotin in NHL and should thus reexamine this issue (*197*).

Although these early clinical trials with pretargeting have not yet produced substantial improvements in response rates in the indications in which these methods have been tested, the data continue to suggest that pretargeting can deliver at least similar, if not higher, radiation-absorbed doses to tumors per unit radioactivity administered. With evidence that higher levels of radioactivity can be administered using a pretargeting procedure than with a directly radiolabeled IgG, this should result in a higher total dose delivered to the tumors. In addition, because maximum radioactivity uptake occurs within the first few hours (whereas radiolabeled antibodies can take 1–2 d), the radiation absorbed dose rate is also increased for pretargeting. Whether these enhancements will be sufficient to improve responses remains to be determined. However, in circumstances in which a directly radiolabeled antibody can already produce significant antitumor response or improve survival, pretargeting should represent a means for reducing toxicity, particularly when the pretargeting method can deliver the same radiation dose to the tumor with less hematologic toxicity. Indeed, combinations of pretargeting approaches with chemotherapy may be better tolerated than when used with a directly radiolabeled antibody. Studies are examining combinations with chemotherapy as well as other enhancements used with directly radiolabeled antibodies that suggest that these methods will also improve radionuclide delivery with a pretargeting method (*198–200*). New approaches are being explored that could enhance the binding stability of antibodies to their ligands and further improve tumor retention (*201*). In many respects, pretargeting is still in an early stage of development, providing optimism that further advances will be forthcoming.

CONCLUSION

The history of RIT clearly indicates that the development of tumor-targeting radiolabeled antibodies is more complex than seeking a drug that affects a biochemical target. Yet in almost every preclinical model, as well as in some recent clinical studies comparing drugs with RIT in NHL, RIT has proven to be superior to chemotherapy in efficacy (*137,143,202*). It has also been shown that a combination of the 2 appears to be more effective than either modality alone (*132,134,138,144*). Therefore, scientific perspectives should be separated from commercial pressures in making an assessment of the current status and future prospects of RIT. Scientific evidence, in our view, supports the clinical prospects of this modality, but the slow adoption of the first RIT products by medical oncologists requires inducing them to become part of the management paradigm of RIT, both clinically and economically. In solid tumors, trials in minimal disease settings and involving combinations of RIT with chemotherapy should lead to better treatment outcomes. Support for such trials should come from government, regulatory, and industrial sources, with nuclear and radiation physicians championing these efforts.

REFERENCES

1. Pressman D, Korngold L. The in vivo localization of anti-wagner osteogenic-sarcoma antibodies. *Cancer.* 1953;6:619–623.
2. Goldenberg DM, DeLand F, Kim E, et al. Use of radiolabeled antibodies to carcinoembryonic antigen for the detection and localization of diverse cancers by external photoscanning. *N Engl J Med.* 1978;298:1384–1386.
3. Goldenberg DM, Juweid M, Dunn RM, Sharkey RM. Cancer imaging with radiolabeled antibodies: new advances with technetium-99m-labeled monoclonal antibody Fab' fragments, especially CEA-scan and prospects for therapy. *J Nucl Med Technol.* 1997;25:18–23.
4. Goldenberg DM. The role of radiolabeled antibodies in the treatment of non-Hodgkin's lymphoma: the coming of age of radioimmunotherapy. *Crit Rev Oncol Hematol.* 2001;39:195–201.
5. Goldenberg DM. Targeted therapy of cancer with radiolabeled antibodies. *J Nucl Med.* 2002;43:693–713.
6. Dean NM, Bennett CF. Antisense oligonucleotide-based therapeutics for cancer. *Oncogene.* 2003;22:9087–9096.
7. Britz-Cunningham SH, Adelstein SJ. Molecular targeting with radionuclides: state of the science. *J Nucl Med.* 2003;44:1945–1961.
8. Chanan-Khan A, Czuczman MS. Radioimmunotherapy in non-Hodgkin lymphoma. *Curr Opin Oncol.* 2002;14:484–489.
9. Press OW. Radioimmunotherapy for non-Hodgkin's lymphomas: a historical perspective. *Semin Oncol.* 2003;30:10–21.
10. Silverman DH, Delpassand ES, Torabi F, Goy A, McLaughlin P, Murray JL. Radiolabeled antibody therapy in non-Hodgkins lymphoma: radiation protection, isotope comparisons and quality of life issues. *Cancer Treat Rev.* 2004; 30:165–172.
11. Cheson BD. Radioimmunotherapy of non-Hodgkin lymphomas. *Blood.* 2003; 101:391–398.
12. Goldenberg DM. Advancing role of radiolabeled antibodies in the therapy of cancer. *Cancer Immunol Immunother.* 2003;52:281–296.
13. Russeva MG, Adams GP. Radioimmunotherapy with engineered antibodies. *Expert Opin Biol Ther.* 2004;4:217–231.
14. Bethge WA, Sandmaier BM. Targeted cancer therapy and immunosuppression using radiolabeled monoclonal antibodies. *Semin Oncol.* 2004;31:68–82.

15. Zevalin (ibritumomab tiuxetan) [package insert]. San Diego, CA: IDEC Pharmaceuticals Corporation; 2002.

16. Bexxar (tositumomab and ^{131}I-tositumomab) [package insert]. Seattle, WA: Corixa Corporation and Philadelphia, PA: GlaxoSmithKline; 2003.

17. Press OW, Shan D, Howell-Clark J, et al. Comparative metabolism and retention of iodine-125, yttrium-90, and indium-111 radioimmunoconjugates by cancer cells. *Cancer Res.* 1996;56:2123–2129.

18. Sharkey RM, Behr TM, Mattes MJ, et al. Advantage of residualizing radiolabels for an internalizing antibody against the B-cell lymphoma antigen, CD22. *Cancer Immunol Immunother.* 1997;44:179–188.

19. Juweid ME, Stadtmauer E, Hajjar G, et al. 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.* 1999;5:3292S–3303S.

20. DeNardo GL, DeNardo SJ, O'Donnell RT, et al. Are radiometal-labeled antibodies better than iodine-131-labeled antibodies: comparative pharmacokinetics and dosimetry of copper-67-, iodine-131-, and yttrium-90-labeled Lym-1 antibody in patients with non-Hodgkin's lymphoma. *Clin Lymphoma.* 2000;1:118–126.

21. Wagner HN Jr, Wiseman GA, Marcus CS, et al. Administration guidelines for radioimmunotherapy of non-Hodgkin's lymphoma with ^{90}Y-labeled anti-CD20 monoclonal antibody. *J Nucl Med.* 2002;43:267–272.

22. Siegel JA, Kroll S, Regan D, Kaminski MS, Wahl RL. A practical methodology for patient release after tositumomab and ^{131}I-tositumomab therapy. *J Nucl Med.* 2002;43:354–363.

23. Leonard JP, Siegel JA, Goldsmith SJ. Comparative physical and pharmacologic characteristics of iodine-131 and yttrium-90: implications for radioimmunotherapy for patients with non-Hodgkin's lymphoma. *Cancer Invest.* 2003;21:241–252.

24. Fink-Bennett DM, Thomas K. ^{90}Y-Ibritumomab tiuxetan in the treatment of relapsed or refractory B-cell non-Hodgkin's lymphoma. *J Nucl Med Technol.* 2003;31:61–68.

25. Siegel JA, Zimmerman BE, Kodimer K, Dell MA, Simon WE. Accurate dose calibrator activity measurement of ^{90}Y-ibritumomab tiuxetan. *J Nucl Med.* 2004;45:450–454.

26. Vose JM. Bexxar: novel radioimmunotherapy for the treatment of low-grade and transformed low-grade non-Hodgkin's lymphoma. *Oncologist.* 2004;9:160–172.

27. Koral KF, Dewaraja Y, Clarke LA, et al. Tumor-absorbed-dose estimates versus response in tositumomab therapy of previously untreated patients with follicular non-Hodgkin's lymphoma: preliminary report. *Cancer Biother Radiopharm.* 2000;15:347–355.

28. Koral KF, Kaminski MS, Wahl RL. Correlation of tumor radiation-absorbed dose with response is easier to find in previously untreated patients. *J Nucl Med.* 2003;44:1541–1543.

29. Sgouros G, Squeri S, Ballangrud AM, et al. Patient-specific, 3-dimensional dosimetry in non-Hodgkin's lymphoma patients treated with ^{131}I-anti-B1 antibody: assessment of tumor dose-response. *J Nucl Med.* 2003;44:260–268.

30. Sharkey RM, Brenner A, Burton J, et al. Radioimmunotherapy of non-Hodgkin's lymphoma with ^{90}Y-DOTA humanized anti-CD22 IgG (^{90}Y-epratuzumab): Do tumor targeting and dosimetry predict therapeutic response? *J Nucl Med.* 2003;44:2000–2018.

31. Rastetter W, Molina A, White CA. Rituximab: expanding role in therapy for lymphomas and autoimmune diseases. *Annu Rev Med.* 2004;55:477–503.

32. Coleman M, Goldenberg DM, Siegel AB, et al. Epratuzumab: targeting B-cell malignancies through CD22. *Clin Cancer Res.* 2003;9:3991S–3994S.

33. Hernandez MC, Knox SJ. Radiobiology of radioimmunotherapy with ^{90}Y ibritumomab tiuxetan (zevalin). *Semin Oncol.* 2003;30(suppl):6–10.

34. Du Y, Honeychurch J, Cragg MS, et al. Antibody-induced intracellular signaling works in combination with radiation to eradicate lymphoma in radioimmunotherapy. *Blood.* 2004;103:1485–1494.

35. Kaminski MS, Estes J, Zasadny KR, et al. 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. *Blood.* 2000;96:1259–1266.

36. Gordon LI, Molina A, Witzig T, et al. Durable responses after ibritumomab tiuxetan radioimmunotherapy for CD20^{+} B-cell lymphoma: long term follow-up of a phase I/II study. *Blood.* 2004;103:4429–4431.

37. Cragg MS, Glennie MJ. Antibody specificity controls in vivo effector mechanisms of anti-CD20 reagents. *Blood.* 2004;103:2738–2743.

38. Johnson P, Glennie M. The mechanisms of action of rituximab in the elimination of tumor cells. *Semin Oncol.* 2003;30(suppl):3–8.

39. Buchsbaum DJ, Wahl RL, Normolle DP, Kaminski MS. Therapy with unlabeled and ^{131}I-labeled pan-B-cell monoclonal antibodies in nude mice bearing Raji Burkitt's lymphoma xenografts. *Cancer Res.* 1992;52:6476–6481.

40. Kaminski MS, Zasadny KR, Francis IR, et al. Radioimmunotherapy of B-cell lymphoma with [^{131}I]anti-B1 (anti-CD20) antibody. *N Engl J Med.* 1993;329:459–465.

41. Kaminski MS, Zelenetz AD, Press OW, et al. Pivotal study of iodine I 131 tositumomab for chemotherapy-refractory low-grade or transformed low-grade B-cell non-Hodgkin's lymphomas. *J Clin Oncol.* 2001;19:3918–3928.

42. Leonard JP, Coleman M, Ketas JC, et al. Phase I/II trial of epratuzumab (humanized anti-CD22 antibody) in indolent non-Hodgkin's lymphoma. *J Clin Oncol.* 2003;21:3051–3059.

43. Dechant M, Bruenke J, Valerius T. HLA class II antibodies in the treatment of hematologic malignancies. *Semin Oncol.* 2003;30:465–475.

44. Kaminski M, Estes J, Tuck M, et al. Iodine I 131 tositumomab therapy for previously untreated follicular lymphoma [abstract]. *Proc ASCO.* 2000;19:5a.

45. Press OW, Unger JM, Braziel RM, et al. A phase 2 trial of CHOP chemotherapy followed by tositumomab/iodine I 131 tositumomab for previously untreated follicular non-Hodgkin lymphoma: Southwest Oncology Group Protocol S9911. *Blood.* 2003;102:1606–1612.

46. Rana TM. Post Bexxar relapse in NHL responds to Zevalin and can be safely accomplished [abstract]. *Proc Am Soc Clin Oncol.* 2003;22:613.

47. Tsai DE, Maillard I, Schuster SJ, et al. Use of ibritumomab tiuxetan anti-CD20 radioimmunotherapy in a non-Hodgkin's lymphoma patient previously treated with a yttrium-90-labeled anti-CD22 monoclonal antibody. *Clin Lymphoma.* 2003;4:56–59.

48. Ansell SM, Ristow KM, Habermann TM, Wiseman GA, Witzig TE. Subsequent chemotherapy regimens are well tolerated after radioimmunotherapy with yttrium-90 ibritumomab tiuxetan for non-Hodgkin's lymphoma. *J Clin Oncol.* 2002;20:3885–3890.

49. Wiseman GA, Kornmehl E, Leigh B, et al. Radiation dosimetry results and safety correlations from ^{90}Y-ibritumomab tiuxetan radioimmunotherapy for relapsed or refractory non-Hodgkin's lymphoma: combined data from 4 clinical trials. *J Nucl Med.* 2003;44:465–474.

50. Siegel JA, Yeldell D, Goldenberg DM, et al. 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.* 2003;44:67–76.

51. Pagel JM, Matthews DC, Appelbaum FR, Bernstein ID, Press OW. The use of radioimmunoconjugates in stem cell transplantation. *Bone Marrow Transplant.* 2002;29:807–816.

52. Gopal AK, Rajendran JG, Petersdorf SH, et al. High-dose chemo-radioimmunotherapy with autologous stem cell support for relapsed mantle cell lymphoma. *Blood.* 2002;99:3158–3162.

53. Matthews DC, Appelbaum FR, Eary JF, et al. Phase I study of ^{131}I-anti-CD45 antibody plus cyclophosphamide and total body irradiation for advanced acute leukemia and myelodysplastic syndrome. *Blood.* 1999;94:1237–1247.

54. Press OW, Eary JF, Gooley T, et al. A phase I/II trial of iodine-131-tositumomab (anti-CD20), etoposide, cyclophosphamide, and autologous stem cell transplantation for relapsed B-cell lymphomas. *Blood.* 2000;96:2934–2942.

55. Burke JM, Caron PC, Papadopoulos EB, et al. Cytoreduction with iodine-131-anti-CD33 antibodies before bone marrow transplantation for advanced myeloid leukemias. *Bone Marrow Transplant.* 2003;32:549–556.

56. Buchmann I, Bunjes D, Kotzerke J, et al. Myeloablative radioimmunotherapy with Re-188-anti-CD66-antibody for conditioning of high-risk leukemia patients prior to stem cell transplantation: biodistribution, biokinetics and immediate toxicities. *Cancer Biother Radiopharm.* 2002;17:151–163.

57. Turner JH, Martindale AA, Boucek J, Claringbold PG, Leahy MF. ^{131}I-Anti CD20 radioimmunotherapy of relapsed or refractory non-Hodgkin's lymphoma: a phase II clinical trial of a nonmyeloablative dose regimen of chimeric rituximab radiolabeled in a hospital. *Cancer Biother Radiopharm.* 2003;18:513–524.

58. Lindén O, Tennvall J, Cavallin-Ståhl E, et al. A phase I/II trial with Y-90 hLL2 in recurrent B-cell lymphomas. Preliminary results [abstract]. *Cancer Biother Radiopharm.* 2000;15:413.

59. O'Donnell RT, Shen S, Denardo SJ, et al. A phase I study of [90]Y-2IT-BAD-Lym-1 in patients with non-Hodgkin's lymphoma. *Anticancer Res.* 2000;20:3647–3655.

60. Gopal AK, Gooley TA, Maloney DG, et al. High-dose radioimmunotherapy versus conventional high-dose therapy and autologous hematopoietic stem cell transplantation for relapsed follicular non-Hodgkin lymphoma: a multivariable cohort analysis. *Blood.* 2003;102:2351–2357.

61. Jurcic JG, Larson SM, Sgouros G, et al. Targeted alpha particle immunotherapy for myeloid leukemia. *Blood.* 2002;100:1233–1239.

62. McDevitt MR, Ma D, Lai LT, et al. Tumor therapy with targeted atomic nanogenerators. *Science.* 2001;294:1537–1540.

63. Sgouros G, Ballangrud AM, Jurcic JG, et al. Pharmacokinetics and dosimetry of an alpha-particle emitter labeled antibody: ^{213}Bi-HuM195 (anti-CD33) in patients with leukemia. *J Nucl Med.* 1999;40:1935–1946.

64. Ochakovskaya R, Osorio L, Goldenberg DM, Mattes MJ. Therapy of disseminated B-cell lymphoma xenografts in severe combined immunodeficient mice with an anti-CD74 antibody conjugated with ^{111}indium, ^{67}gallium, or ^{90}yttrium. *Clin Cancer Res.* 2001;7:1505–1510.

65. Hansen HJ, Ong GL, Diril H, et al. Internalization and catabolism of radiolabelled antibodies to the MHC class-II invariant chain by B-cell lymphomas. *Biochem J.* 1996;320:293–300.

66. Wiseman GA, White CA, Sparks RB, et al. Biodistribution and dosimetry results from a phase III prospectively randomized controlled trial of Zevalin radioimmunotherapy for low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. *Crit Rev Oncol Hematol.* 2001;39:181–194.

67. Tempero M, Leichner P, Dalrymple G, et al. High-dose therapy with iodine-131-labeled monoclonal antibody CC49 in patients with gastrointestinal cancers: a phase I trial. *J Clin Oncol.* 1997;15:1518–1528.

68. Tempero M, Leichner P, Baranowska-Kortylewicz J, et al. High-dose therapy with ^{90}yttrium-labeled monoclonal antibody CC49: a phase I trial. *Clin Cancer Res.* 2000;6:3095–3102.

69. Meredith RF, Khazaeli MB, Plott WE, et al. Phase II study of dual ^{131}I-labeled monoclonal antibody therapy with interferon in patients with metastatic colorectal cancer. *Clin Cancer Res.* 1996;2:1811–1818.

70. Meredith R, Shen S, Macey D, et al. Comparison of biodistribution, dosimetry, and outcome from clinical trials of radionuclide-CC49 antibody therapy. *Cancer Biother Radiopharm.* 2003;18:393–404.

71. Meredith RF, Khazaeli MB, Macey DJ, et al. Phase II study of interferon-enhanced ^{131}I-labeled high affinity CC49 monoclonal antibody therapy in patients with metastatic prostate cancer. *Clin Cancer Res.* 1999;5:3254S–3258S.

72. Mulligan T, Carrasquillo JA, Chung Y, et al. Phase I study of intravenous Lu-labeled CC49 murine monoclonal antibody in patients with advanced adenocarcinoma. *Clin Cancer Res.* 1995;1:1447–1454.

73. Wong JYC, Chu DZ, Yamauchi DM, et al. A phase I radioimmunotherapy trial evaluating ^{90}yttrium-labeled anti-carcinoembryonic antigen (CEA) chimeric T84.66 in patients with metastatic CEA-producing malignancies. *Clin Cancer Res.* 2000;6:3855–3863.

74. Sharkey RM, Pykett MJ, Siegel JA, Alger EA, Primus FJ, Goldenberg DM. Radioimmunotherapy of the GW-39 human colonic tumor xenograft with ^{131}I-labeled murine monoclonal antibody to carcinoembryonic antigen. *Cancer Res.* 1987;47:5672–5677.

75. Sharkey RM, Weadock KS, Natale A, et al. Successful radioimmunotherapy for lung metastasis of human colonic cancer in nude mice. *J Natl Cancer Inst.* 1991;83:627–632.

76. Boerman OC, Sharkey RM, Blumenthal RD, Aninipot RL, Goldenberg DM. The presence of a concomitant bulky tumor can decrease the uptake and therapeutic efficacy of radiolabeled antibodies in small tumors. *Int J Cancer.* 1992;51:470–475.

77. Moshakis V, McIlhinney RA, Raghavan D, Neville AM. Localization of human tumour xenografts after i.v. administration of radiolabeled monoclonal antibodies. *Br J Cancer.* 1981;44:91–99.

78. Mayer A, Tsiompanou E, Flynn AA, et al. Higher dose and dose-rate in smaller tumors result in improved tumor control. *Cancer Invest.* 2003;21:382–388.

79. Siegel JA, Pawlyk DA, Lee RE, et al. Tumor, red marrow, and organ dosimetry for ^{131}I-labeled anti-carcinoembryonic antigen monoclonal antibody. *Cancer Res.* 1990;50:1039S–1042S.

80. Murray JL, Macey DJ, Kasi LP, et al. Phase II radioimmunotherapy trial with ^{131}I-CC49 in colorectal cancer. *Cancer.* 1994;73(suppl):1057–1066.

81. Behr TM, Liersch T, Greiner-Bechert L, et al. Radioimmunotherapy of small-volume disease of metastatic colorectal cancer. *Cancer.* 2002;94(suppl):1373–1381.

82. Liersch T, Behr TM, Becker W, et al. Effect of CEA radioimmunotherapy post salvage resection of colorectal cancer liver metastases: phase II safety and outcome results [abstract]. *Proc GI Cancer Symp.* 2003;160.

83. Fong Y. Surgical therapy of hepatic colorectal cancer metastasis. *CA Cancer J Clin.* 1999;49:231–255.

84. Williams LE, Wu AM, Yazaki PJ, et al. Numerical selection of optimal tumor imaging agents with application to engineered antibodies. *Cancer Biother Radiopharm.* 2001;16:25–35.

85. Casey JL, Pedley RB, King DJ, Green AJ, Yarranton GT, Begent RH. Dosimetric evaluation and radioimmunotherapy of anti-tumour multivalent Fab′ fragments. *Br J Cancer.* 1999;81:972–980.

86. Behr TM, Memtsoudis S, Sharkey RM, et al. Experimental studies on the role of antibody fragments in cancer radio-immunotherapy: influence of radiation dose and dose rate on toxicity and anti-tumor efficacy. *Int J Cancer.* 1998;77:787–795.

87. Behr TM, Blumenthal RD, Memtsoudis S, et al. Cure of metastatic human colonic cancer in mice with radiolabeled monoclonal antibody fragments. *Clin Cancer Res.* 2000;6:4900–4907.

88. Blumenthal RD, Sharkey RM, Kashi R, Goldenberg DM. 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.* 1989;44:292–300.

89. Vogel CA, Bischof-Delaloye A, Mach JP, et al. Direct comparison of a radioiodinated intact chimeric anti-CEA MAb with its F(ab′)$_2$ fragment in nude mice bearing different human colon cancer xenografts. *Br J Cancer.* 1993;68:684–690.

90. Goel A, Augustine S, Baranowska-Kortylewicz J, et al. Single-dose versus fractionated radioimmunotherapy of human colon carcinoma xenografts using ^{131}I-labeled multivalent CC49 single-chain Fvs. *Clin Cancer Res.* 2001;7:175–184.

91. Wu AM. Engineering multivalent antibody fragments for in vivo targeting. *Methods Mol Biol.* 2004;248:209–225.

92. Slavin-Chiorini DC, Kashmiri SV, Lee HS, et al. A CDR-grafted (humanized) domain-deleted antitumor antibody. *Cancer Biother Radiopharm.* 1997;12:305–316.

93. Goel A, Colcher D, Baranowska-Kortylewicz J, et al. Genetically engineered tetravalent single-chain Fv of the pancarcinoma monoclonal antibody CC49: improved biodistribution and potential for therapeutic application. *Cancer Res.* 2000;60:6964–6971.

94. Yazaki PJ, Wu AM, Tsai SW, et al. Tumor targeting of radiometal labeled anti-CEA recombinant T84.66 diabody and t84.66 minibody: comparison to radioiodinated fragments. *Bioconjug Chem.* 2001;12:220–228.

95. Behr TM, Sharkey RM, Juweid ME, et al. Reduction of the renal uptake of radiolabeled monoclonal antibody fragments by cationic amino acids and their derivatives. *Cancer Res.* 1995;55:3825–3834.

96. Behr TM, Sharkey RM, Sgouros G, et al. Overcoming the nephrotoxicity of radiometal-labeled immunoconjugates: improved cancer therapy administered to a nude mouse model in relation to the internal radiation dosimetry. *Cancer.* 1997;80(suppl):2591–2610.

97. Jamar F, Barone R, Mathieu I, et al. ^{86}Y-DOTA0-D-Phe1-Tyr3-octreotide (SMT487)—a phase 1 clinical study: pharmacokinetics, biodistribution and renal protective effect of different regimens of amino acid co-infusion. *Eur J Nucl Med Mol Imaging.* 2003;30:510–518.

98. Bodei L, Cremonesi M, Zoboli S, et al. Receptor-mediated radionuclide therapy with ^{90}Y-DOTATOC in association with amino acid infusion: a phase I study. *Eur J Nucl Med Mol Imaging.* 2003;30:207–216.

99. Moll S, Nickeleit V, Mueller-Brand J, Brunner FP, Maecke HR, Mihatsch MJ. A new cause of renal thrombotic microangiopathy: yttrium 90-DOTATOC internal radiotherapy. *Am J Kidney Dis.* 2001;37:847–851.

100. Cybulla M, Weiner SM, Otte A. End-stage renal disease after treatment with ^{90}Y-DOTATOC. *Eur J Nucl Med.* 2001;28:1552–1554.

101. Forero A, Meredith RF, Khazaeli MB, et al. A novel monoclonal antibody design for radioimmunotherapy. *Cancer Biother Radiopharm.* 2003;18:751–759.

102. Rowlinson G, Snook D, Busza A, Epenetos AA. Antibody-guided localization of intraperitoneal tumors following intraperitoneal or intravenous antibody administration. *Cancer Res.* 1987;47:6528–6531.

103. Ward BG, Wallace K. Localization of the monoclonal antibody HMFG2 after intravenous and intraperitoneal injection into nude mice bearing subcutaneous and intraperitoneal human ovarian cancer xenografts. *Cancer Res.* 1987;47:4714–4718.

104. Wahl RL, Barrett J, Geatti O, et al. The intraperitoneal delivery of radiolabeled monoclonal antibodies: studies on the regional delivery advantage. *Cancer Immunol Immunother.* 1988;26:187–201.

105. Griffin TW, Collins J, Bokhari F, et al. Intraperitoneal immunoconjugates. *Cancer Res.* 1990;50:1031S–1038S.

106. Kinuya S, Li XF, Yokoyama K, et al. Intraperitoneal radioimmunotherapy in treating peritoneal carcinomatosis of colon cancer in mice compared with systemic radioimmunotherapy. *Cancer Sci.* 2003;94:650–654.

107. Colcher D, Esteban J, Carrasquillo JA, et al. Complementation of intracavitary and intravenous administration of a monoclonal antibody (B72.3) in patients with carcinoma. *Cancer Res.* 1987;47:4218–4224.

108. Juweid M, Sharkey RM, Alavi A, et al. Regression of advanced refractory ovarian cancer treated with iodine-131-labeled anti-CEA monoclonal antibody. *J Nucl Med.* 1997;38:257–260.

109. Juweid M, Swayne LC, Sharkey RM, et al. Prospects of radioimmunotherapy in

epithelial ovarian cancer: results with iodine-131-labeled murine and humanized MN-14 anti-carcinoembryonic antigen monoclonal antibodies. *Gynecol Oncol.* 1997;67:259–271.

110. Epenetos AA, Hird V, Lambert H, Mason P, Coulter C. Long term survival of patients with advanced ovarian cancer treated with intraperitoneal radioimmunotherapy. *Int J Gynecol Cancer.* 2000;10:44–46.

111. Alvarez RD, Partridge EE, Khazaeli MB, et al. Intraperitoneal radioimmunotherapy of ovarian cancer with ^{177}Lu-CC49: a phase I/II study. *Gynecol Oncol.* 1997;65:94–101.

112. Bigner DD, Brown MT, Friedman AH, et al. Iodine-131-labeled antitenascin monoclonal antibody 81C6 treatment of patients with recurrent malignant gliomas: phase I trial results. *J Clin Oncol.* 1998;16:2202–2212.

113. Reardon DA, Akabani G, Coleman RE, et al. 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.* 2002;20:1389–1397.

114. Akabani G, Cokgor I, Coleman RE, et al. 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.* 2000;46:947–958.

115. Grossi PM, Ochiai H, Archer GE, et al. Efficacy of intracerebral microinfusion of trastuzumab in an athymic rat model of intracerebral metastatic breast cancer. *Clin Cancer Res.* 2003;9:5514–5520.

116. Quang TS, Brady LW. Radioimmunotherapy as a novel treatment regimen: ^{125}I-labeled monoclonal antibody 425 in the treatment of high-grade brain gliomas. *Int J Radiat Oncol Biol Phys.* 2004;58:972–975.

117. Greiner JW, Guadagni F, Noguchi P, et al. Recombinant interferon enhances monoclonal antibody-targeting of carcinoma lesions in vivo. *Science.* 1987;235: 895–898.

118. Stickney DR, Gridley DS, Kirk GA, Slater JM. Enhancement of monoclonal antibody binding to melanoma with single dose radiation or hyperthermia. *Nat Cancer Inst Monogr.* 1987;3:47–52.

119. Hauck ML, Dewhirst MW, Bigner DD, Zalutsky MR. Local hyperthermia improves uptake of a chimeric monoclonal antibody in a subcutaneous xenograft model. *Clin Cancer Res.* 1997;3:63–70.

120. Vogel CA, Galmiche MC, Buchegger F. Radioimmunotherapy and fractionated radiotherapy of human colon cancer liver metastases in nude mice. *Cancer Res.* 1997;57:447–453.

121. Kinuya S, Yokoyama K, Hiramatsu T, et al. Optimal timing of administration of hyperthermia in combined radioimmunotherapy. *Cancer Biother Radiopharm.* 2000;15:373–379.

122. Ruan S, O'Donoghue JA, Larson SM, et al. Optimizing the sequence of combination therapy with radiolabeled antibodies and fractionated external beam. *J Nucl Med.* 2000;41:1905–1912.

123. Saga T, Sakahara H, Nakamoto Y, et al. Enhancement of the therapeutic outcome of radio-immunotherapy by combination with whole-body mild hyperthermia. *Eur J Cancer.* 2001;37:1429–1434.

124. Pedley RB, Boden JA, Boden R, et al. Ablation of colorectal xenografts with combined radioimmunotherapy and tumor blood flow-modifying agents. *Cancer Res.* 1996;56:3293–3300.

125. Pedley RB, Hill SA, Boxer GM, et al. Eradication of colorectal xenografts by combined radioimmunotherapy and combretastatin a-4 3-O-phosphate. *Cancer Res.* 2001;61:4716–4722.

126. Pedley RB, El-Emir E, Flynn AA, et al. Synergy between vascular targeting agents and antibody-directed therapy. *Int J Radiat Oncol Biol Phys.* 2002;54: 1524–1531.

127. Kurizaki T, Okazaki S, Sanderson SD, et al. Potentiation of radioimmunotherapy with response-selective peptide agonist of human C5a. *J Nucl Med.* 2002;43:957–967.

128. Li XF, Kinuya S, Yokoyama K, et al. Benefits of combined radioimmunotherapy and anti-angiogenic therapy in a liver metastasis model of human colon cancer cells. *Eur J Nucl Med Mol Imaging.* 2002;29:1669–1674.

129. Burke PA, DeNardo SJ, Miers LA, Lamborn KR, Matzku S, DeNardo GL. Cilengitide targeting of alpha(v)beta(3) integrin receptor synergizes with radioimmunotherapy to increase efficacy and apoptosis in breast cancer xenografts. *Cancer Res.* 2002;62:4263–4272.

130. Kinuya S, Kawashima A, Yokoyama K, et al. Anti-angiogenic therapy and radioimmunotherapy in colon cancer xenografts. *Eur J Nucl Med.* 2001;28: 1306–1312.

131. Buchsbaum DJ. Imaging and therapy of tumors induced to express somatostatin receptor by gene transfer using radiolabeled peptides and single chain antibody constructs. *Semin Nucl Med.* 2004;34:32–46.

132. DeNardo SJ, Kukis DL, Kroger LA, et al. Synergy of taxol and radioimmuno-

therapy with yttrium-90-labeled chimeric L6 antibody: efficacy and toxicity in breast cancer xenografts. *Proc Natl Acad Sci USA.* 1997;94:4000–4004.

133. DeNardo SJ, Kroger LA, Lamborn KR, et al. Importance of temporal relationships in combined modality radioimmunotherapy of breast carcinoma. *Cancer.* 1997;80(suppl):2583–2590.

134. Clarke K, Lee FT, Brechbiel MW, Smyth FE, Old LJ, Scott AM. Therapeutic efficacy of anti-Lewis(y) humanized 3S193 radioimmunotherapy in a breast cancer model: enhanced activity when combined with taxol chemotherapy. *Clin Cancer Res.* 2000;6:3621–3628.

135. O'Donnell RT, DeNardo SJ, Miers LA, et al. Combined modality radioimmunotherapy for human prostate cancer xenografts with taxanes and [90]yttrium-DOTA-peptide-ChL6. *Prostate.* 2002;50:27–37.

136. Ng B, Kramer E, Liebes L, et al. Radiosensitization of tumor-targeted radioimmunotherapy with prolonged topotecan infusion in human breast cancer xenografts. *Cancer Res.* 2001;61:2996–3001.

137. Gold DV, Modrak DE, Schutsky K, Cardillo TM. Combined [90]yttrium-DOTA-labeled PAM4 antibody radioimmunotherapy and gemcitabine radiosensitization for the treatment of a human pancreatic cancer xenograft. *Int J Cancer.* 2004;109:618–626.

138. Burke PA, DeNardo SJ, Miers LA, Kukis DL, DeNardo GL. Combined modality radioimmunotherapy: promise and peril. *Cancer.* 2002;94(suppl):1320–1331.

139. Blumenthal RD, Leone E, Goldenberg DM. Tumor-specific dose scheduling of bimodal radioimmunotherapy and chemotherapy. *Anticancer Res.* 2003;23: 4613–4619.

140. Blumenthal RD, Leone E, Goldenberg DM, Rodriguez M, Modrak D. An in vitro model to optimize dose scheduling of multimodal radioimmunotherapy and chemotherapy: effects of p53 expression. *Int J Cancer.* 2004;108:293–300.

141. Chalandon Y, Mach JP, Pelegrin A, Folli S, Buchegger F. Combined radioimmunotherapy and chemotherapy of human colon carcinoma grafted in nude mice, advantages and limitations. *Anticancer Res.* 1992;12:1131–1139.

142. Tschmelitsch J, Barendswaard E, Williams C Jr, et al. Enhanced antitumor activity of combination radioimmunotherapy (^{131}I-labeled monoclonal antibody A33) with chemotherapy (fluorouracil). *Cancer Res.* 1997;57:2181–2186.

143. Cardillo TM, Blumenthal R, Ying Z, Gold DV. Combined gemcitabine and radioimmunotherapy for the treatment of pancreatic cancer. *Int J Cancer.* 2002;97:386–392.

144. Gold DV, Schutsky K, Modrak D, Cardillo TM. Low-dose radioimmunotherapy (^{90}Y-PAM4) combined with gemcitabine for the treatment of experimental pancreatic cancer. *Clin Cancer Res.* 2003;9:3929s–3937s.

145. Behr TM, Wulst E, Radetzky S, et al. Improved treatment of medullary thyroid cancer in a nude mouse model by combined radioimmunochemotherapy: doxorubicin potentiates the therapeutic efficacy of radiolabeled antibodies in a radioresistant tumor type. *Cancer Res.* 1997;57:5309–5319.

146. Stein R, Chen S, Reed L, Richel H, Goldenberg DM. Combining radioimmunotherapy and chemotherapy for treatment of medullary thyroid carcinoma: effectiveness of dacarbazine. *Cancer.* 2002;94:51–61.

147. Meredith RF, Alvarez RD, Partridge EE, et al. Intraperitoneal radioimmunochemotherapy of ovarian cancer: a phase I study. *Cancer Biother Radiopharm.* 2001;16:305–315.

148. Alvarez RD, Huh WK, Khazaeli MB, et al. A phase I study of combined modality [90]yttrium-CC49 intraperitoneal radioimmunotherapy for ovarian cancer. *Clin Cancer Res.* 2002;8:2806–2811.

149. Wong JY, Shibata S, Williams LE, et al. A phase I trial of ^{90}Y-anti-carcinoembryonic antigen chimeric T84.66 radioimmunotherapy with 5-fluorouracil in patients with metastatic colorectal cancer. *Clin Cancer Res.* 2003;9:5842–5852.

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

151. Barbet J, Kraeber-Bodere F, Vuillez JP, Gautherot E, Rouvier E, Chatal JF. Pretargeting with the affinity enhancement system for radioimmunotherapy. *Cancer Biother Radiopharm.* 1999;14:153–166.

152. Goodwin DA, Meares CF. Advances in pretargeting biotechnology. *Biotechnol Adv.* 2001;19:435–450.

153. Gruaz-Guyon A, Janevik-Ivanovska E, Raguin O, De Labriolle-Vaylet C, Barbet J. Radiolabeled bivalent haptens for tumor immunodetection and radioimmunotherapy. *Q J Nucl Med.* 2001;45:201–206.

154. McQuarrie SA, Xiao Z, Miller GG, Mercer JR, Suresh MR. Modern trends in radioimmunotherapy of cancer: pretargeting strategies for the treatment of ovarian cancer. *Q J Nucl Med.* 2001;45:160–166.

155. Chang CH, Sharkey RM, Rossi EA, et al. Molecular advances in pretargeting radioimmunotherapy with bispecific antibodies. *Mol Cancer Ther.* 2002;1:553–563.

156. Boerman OC, van Schaijk FG, Oyen WJ, Corstens FH. Pretargeted radioimmunotherapy of cancer: progress step by step. *J Nucl Med.* 2003;44:400–411.

157. Reardan DT, Meares CF, Goodwin DA, et al. Antibodies against metal chelates. *Nature.* 1985;316:265–268.

158. Stickney DR, Anderson LD, Slater JB, et al. Bifunctional antibody: a binary radiopharmaceutical delivery system for imaging colorectal carcinoma. *Cancer Res. 1991*;51:6650–6655.

159. Le Doussal JM, Martin M, Gautherot E, Delaage M, Barbet J. In vitro and in vivo targeting of radiolabeled monovalent and divalent haptens with dual specificity monoclonal antibody conjugates: enhanced divalent hapten affinity for cell-bound antibody conjugate. *J Nucl Med.* 1989;30:1358–1366.

160. Hnatowich DJ, Virzi F, Rusckowski M. Investigations of avidin and biotin for imaging applications. *J Nucl Med.* 1987;28:1294–1302.

161. Paganelli G, Pervez S, Siccardi AG, et al. Intraperitoneal radio-localization of tumors pre-targeted by biotinylated monoclonal antibodies. *Int J Cancer.* 1990; 45:1184–1189.

162. Paganelli G, Magnani P, Zito F, et al. Three-step monoclonal antibody tumor targeting in carcinoembryonic antigen-positive patients. *Cancer Res.* 1991;51: 5960–5906.

163. Paganelli G, Chinol M. Radioimmunotherapy: is avidin-biotin pretargeting the preferred choice among pretargeting methods? *Eur J Nucl Med Mol Imaging.* 2003;30:773–776.

164. Goldenberg DM, Chang CH, Sharkey RM, et al. Radioimmunotherapy: is avidin-biotin pretargeting the preferred choice among pretargeting methods? *Eur J Nucl Med Mol Imaging.* 2003;30:777–780.

165. Rossi EA, Sharkey RM, McBride W, et al. Development of new multivalent-bispecific agents for pretargeting tumor localization and therapy. *Clin Cancer Res.* 2003;9:3886S–3896S.

166. Liu G, Liu C, Zhang S, et al. Investigations of ^{99m}Tc morpholino pretargeting in mice. *Nucl Med Commun.* 2003;24:697–705.

167. Liu CB, Liu GZ, Liu N, et al. Radiolabeling morpholinos with ^{90}Y, ^{111}In, ^{188}Re and ^{99m}Tc. *Nucl Med Biol.* 2003;30:207–214.

168. Liu G, He J, Dou S, et al. Pretargeting in tumored mice with radiolabeled morpholino oligomer showing low kidney uptake. *Eur J Nucl Med Mol Imaging.* 2004;31:417–424.

169. Kalofonos HP, Rusckowski M, Siebecker DA, et al. Imaging of tumor in patients with indium-111-labeled biotin and streptavidin-conjugated antibodies: preliminary communication. *J Nucl Med.* 1990;31:1791–1796.

170. Peltier P, Curtet C, Chatal JF, et al. Radioimmunodetection of medullary thyroid cancer using a bispecific anti-CEA/anti-indium-DTPA antibody and an indium-111-labeled DTPA dimer. *J Nucl Med.* 1993;34:1267–1773.

171. Le Doussal JM, Chetanneau A, Gruaz-Guyon A, et al. Bispecific monoclonal antibody-mediated targeting of an indium-111-labeled DTPA dimer to primary colorectal tumors: pharmacokinetics, biodistribution, scintigraphy and immune response. *J Nucl Med.* 1993;34:1662–1671.

172. Dosio F, Magnani P, Paganelli G, Samuel A, Chiesa G, Fazio F. Three-step tumor pre-targeting in lung cancer immunoscintigraphy. *J Nucl Biol Med.* 1993;37:228–232.

173. Modorati G, Brancato R, Paganelli G, Magnani P, Pavoni R, Fazio F. Immunoscintigraphy with three-step monoclonal pretargeting technique in diagnosis of uveal melanoma: preliminary results. *Br J Ophthalmol.* 1994;78:19–23.

174. Axworthy DB, Fritzberg AR, Hylarides MD, et al. Preclinical evaluation of an anti-tumor monoclonal antibody/streptavidin conjugate for pretargeted ^{90}Y radioimmunotherapy in a mouse xenograft model [abstract]. *J Immunother.* 1994; 16:158.

175. Gautherot E, Bouhou J, Le Doussal JM, et al. Therapy for colon carcinoma xenografts with bispecific antibody-targeted, iodine-131-labeled bivalent hapten. *Cancer.* 1997;80(suppl):2618–2623.

176. Gautherot E, Le Doussal JM, Bouhou J, et al. Delivery of therapeutic doses of radioiodine using bispecific antibody-targeted bivalent haptens. *J Nucl Med.* 1998;39:1937–1943.

177. Kraeber-Bodere F, Faivre-Chauvet A, Sai-Maurel C, et al. Toxicity and efficacy of radioimmunotherapy in carcinoembryonic antigen-producing medullary thyroid cancer xenograft: comparison of iodine 131-labeled F(ab′)$_2$ and pretargeted bivalent hapten and evaluation of repeated injections. *Clin Cancer Res.* 1999; 5(suppl):3183S–3189S.

178. Axworthy DB, Reno JM, Hylarides MD, et al. Cure of human carcinoma xenografts by a single dose of pretargeted yttrium-90 with negligible toxicity. *Proc Natl Acad Sci USA*. 2000;97:1802–1807.

179. Gautherot E, Rouvier E, Daniel L, et al. Pretargeted radioimmunotherapy of human colorectal xenografts with bispecific antibody and ^{131}I-labeled bivalent hapten. *J Nucl Med.* 2000;41:480–487.

180. Press OW, Corcoran M, Subbiah K, et al. A comparative evaluation of conventional and pretargeted radioimmunotherapy of CD20-expressing lymphoma xenografts. *Blood.* 2001;98:2535–2543.

181. Yao Z, Zhang M, Axworthy DB, et al. Radioimmunotherapy of A431 xenografted mice with pretargeted B3 antibody-streptavidin and ^{90}Y-labeled 1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid (DOTA)-biotin. *Cancer Res.* 2002;62:5755–5760.

182. Zhang M, Yao Z, Garmestani K, et al. Pretargeting radioimmunotherapy of a murine model of adult T-cell leukemia with the alpha-emitting radionuclide, bismuth 213. *Blood.* 2002;100:208–216.

183. Subbiah K, Hamlin DK, Pagel JM, et al. Comparison of immunoscintigraphy, efficacy, and toxicity of conventional and pretargeted radioimmunotherapy in CD20-expressing human lymphoma xenografts. *J Nucl Med.* 2003;44:437–445.

184. Zhang M, Zhang Z, Garmestani K, et al. Pretarget radiotherapy with an anti-CD25 antibody-streptavidin fusion protein was effective in therapy of leukemia/lymphoma xenografts. *Proc Natl Acad Sci USA.* 2003;100:1891–1895.

185. Lewis MR, Wang M, Axworthy DB, et al. In vivo evaluation of pretargeted ^{64}Cu for tumor imaging and therapy. *J Nucl Med.* 2003;44:1284–1292.

186. Sharkey RM, Karacay H, Richel H, et al. Optimizing bispecific antibody pretargeting for use in radioimmunotherapy. *Clin Cancer Res.* 2003;9:3897S–3913S.

187. Cremonesi M, Ferrari M, Chinol M, et al. Three-step radioimmunotherapy with yttrium-90 biotin: dosimetry and pharmacokinetics in cancer patients. *Eur J Nucl Med.* 1999;26:110–120.

188. Kraeber-Bodere F, Bardet S, Hoefnagel CA, et al. Radioimmunotherapy in medullary thyroid cancer using bispecific antibody and iodine 131-labeled bivalent hapten: preliminary results of a phase I/II clinical trial. *Clin Cancer Res.* 1999;5:3190S–3198S.

189. Vuillez JP, Kraeber-Bodere F, Moro D, et al. 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.* 1999;5:3259S–3267S.

190. Weiden PL, Breitz HB, Press O, et al. Pretargeted radioimmunotherapy (PRIT) for treatment of non-Hodgkin's lymphoma (NHL): initial phase I/II study results. *Cancer Biother Radiopharm.* 2000;15:15–29.

191. Kraeber-Bodere F, Faivre-Chauvet A, Ferrer L, et al. Pharmacokinetics and dosimetry studies for optimization of anti-carcinoembryonic antigen x anti-hapten bispecific antibody-mediated pretargeting of iodine-131-labeled hapten in a phase I radioimmunotherapy trial. *Clin Cancer Res.* 2003;9:3973S–3981S.

192. Knox SJ, Goris ML, Tempero M, et al. Phase II trial of yttrium-90-DOTA-biotin pretargeted by NR-LU-10 antibody/streptavidin in patients with metastatic colon cancer. *Clin Cancer Res.* 2000;6:406–414.

193. Breitz HB, Fisher DR, Goris ML, et al. Radiation absorbed dose estimation for ^{90}Y-DOTA-biotin with pretargeted NR-LU-10/streptavidin. *Cancer Biother Radiopharm.* 1999;14:381–395.

194. Paganelli G, Bartolomei M, Ferrari M, et al. Pre-targeted locoregional radioimmunotherapy with ^{90}Y-biotin in glioma patients: phase I study and preliminary therapeutic results. *Cancer Biother Radiopharm.* 2001;16:227–235.

195. Grana C, Chinol M, Robertson C, et al. Pretargeted adjuvant radioimmunotherapy with yttrium-90-biotin in malignant glioma patients: a pilot study. *Br J Cancer.* 2002;86:207–212.

196. Weiden PL, Breitz HB. Pretargeted radioimmunotherapy (PRIT) for treatment of non-Hodgkin's lymphoma (NHL). *Crit Rev Oncol Hematol.* 2001;40:37–51.

197. Forero A, Weiden PL, Vose JM, et al. Phase I trial of a novel anti-CD20 fusion protein in pretargeted radioimmunotherapy for B-cell non-Hodgkin's lymphoma. *Blood.* 2004;104:227–236.

198. Paganelli G, Orecchia R, Jereczek-Fossa B, et al. Combined treatment of advanced oropharyngeal cancer with external radiotherapy and three-step radioimmunotherapy. *Eur J Nucl Med.* 1998;25:1336–1339.

199. Kraeber-Bodere F, Sai-Maurel C, Campion L, et al. Enhanced antitumor activity of combined pretargeted radioimmunotherapy and paclitaxel in medullary thyroid cancer xenograft. *Mol Cancer Ther.* 2002;1:267–274.

200. Graves SS, Dearstyne E, Lin Y, et al. Combination therapy with pretarget CC49 radioimmunotherapy and gemcitabine prolongs tumor doubling time in a murine xenograft model of colon cancer more effectively than either monotherapy. *Clin Cancer Res.* 2003;9:3712–3721.

201. Meares CF, Chmura AJ, Orton MS, Corneillie TM, Whetstone PA. Molecular tools for targeted imaging and therapy of cancer. *J Mol Recognit.* 2003;16:255–259.

202. Blumenthal RD, Sharkey RM, Natale AM, Kashi R, Wong G, Goldenberg DM. Comparison of equitoxic radioimmunotherapy and chemotherapy in the treatment of human colonic cancer xenografts. *Cancer Res.* 1994;54:142–151.

The Journal of NUCLEAR MEDICINE

Perspectives on Cancer Therapy with Radiolabeled Monoclonal Antibodies

Robert M. Sharkey and David M. Goldenberg

J Nucl Med. 2005;46:115S-127S.

This article and updated information are available at:
http://jnm.snmjournals.org/content/46/1_suppl/115S

Information about reproducing figures, tables, or other portions of this article can be found online at:
http://jnm.snmjournals.org/site/misc/permission.xhtml

Information about subscriptions to JNM can be found at:
http://jnm.snmjournals.org/site/subscriptions/online.xhtml

SNMMI | SOCIETY OF NUCLEAR MEDICINE AND MOLECULAR IMAGING