Treatment of Non-Hodgkin’s Lymphoma (NHL) With Radiolabeled Antibodies (mAbs)

Gerald L. DeNardo, MD

Most patients with non-Hodgkin’s lymphoma (NHL) achieve remission but, despite newer drugs, the natural history of this disease has not improved during the last 20 years. Less than one half of patients with aggressive NHL are cured, and few of those with low-grade NHL are curable. Furthermore, NHL becomes progressively more chemoresistant while remaining responsive to external beam radiation therapy. Radioimmunotherapy (RIT) is a logical strategy for the treatment of NHL because this disease is multifocal and radiosensitive. Because of their remarkable effectiveness for RIT, 2 anti-CD20 monoclonal antibodies (mAbs), one labeled with $^{111}$In for imaging or $^{90}$Y for therapy and a second labeled with $^{131}$I for imaging and therapy, have been approved for use in patients with NHL. These drugs have proven remarkably effective and safe. Evidence for the importance of the radionuclide is manifested by the data in the randomized pivotal phase III trial of $^{90}$Y-ibritumomab that revealed response rates were several times greater in the $^{90}$Y-ibritumomab arm than in the rituximab arm. A second drug for RIT, $^{131}$I-tositumomab, was compared in a pivotal trial with the efficacy of the last chemotherapy received by each patient. Once again, response rates were much higher for RIT. Both $^{90}$Y-ibritumomab and $^{131}$I-tositumomab require preinfusion of several hundred milligrams of unlabeled anti-CD20 mAb to obtain “favorable” biodistribution, that is, targeting of NHL. Response rates for other mAbs and radionuclides in NHL also have been high but these drugs have not reached the approval stage. These drugs can be used safely by physicians who have suitable training and judgment. Unlike chemotherapy, RIT is not associated with mucositis, hair loss, or persistent nausea or vomiting. Although hematologic toxicity is dose limiting, hospitalization for febrile neutropenia is uncommon. Randomized trials of RIT in different formulations have not been conducted, but there is evidence to suggest that the mAb, antigen, radionuclide, chelator, linker, and dosing strategy may make a difference in the outcome.

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monly multifocal on presentation, often not cured by standard treatment, radiosensitive, and has remarkably specific antigens (Ags). Additionally, these patients are immunocompromised, making repeated dosing possible. RIT uses a radionuclide attached to the mAb to localize radiation. Targeting of the mAb allows it to find and attach itself to the surface of NHL cells, carrying the radionuclide to these cells. Radionuclide emissions destroy not only the NHL cell to which the mAb is attached but also surrounding cells to which mAbs have not bound. A disadvantage is that surrounding healthy cells also can be damaged.

Tiuxetan is a second-generation metal chelator that is co-

Historical Background of NHL and RIT

Trials of RIT began in the 1950s when 131I-labeled rabbit polyclonal mAbs were given to patients with metastatic melanoma; CR occurred in 1 patient.11 In the 1970s, Ettinger and coworkers12 treated patients with cholangiocarcinomas and hepatomas with 131I-anti-CEA and 131I-antiferritin polyclonal Abs in combination with external beam radiation therapy and chemotherapy; a decrease in tumor size was observed in 6 of 9 patients. After the introduction of hybridoma technology in 1975,13 mAbs of defined specificity could be produced in gram quantities. Carrasquillo and coworkers14 then treated patients with radiolabeled mAbs to human melanoma associated Ag; 2 of 3 patients treated with higher doses showed an effect from treatment. Soon thereafter, DeNardo and coworkers15 published the original description of RIT in a patient with NHL (Fig. 1).

Nadler and coworkers16 were first to treat a patient with NHL with a mAb (Table 1). Miller and coworkers17 generated antidiotypic mAbs to treat individual patients with B-lymphocyte NHL; this trial was successful, although patients relapsed as the result of idiotypic variants. At about the same time, DeNardo and coworkers18,19,20 used RIT successfully for patients with NHL and chronic lymphocytic leukemia. Since then, others have confirmed the potential of several different radiolabeled mAbs for treatment of NHL. Press and coworkers21 took a straightforward approach to bone marrow toxicity. Patients with NHL were given 131I-anti-CD20 mAbs in a single dose expected to cause marrow aplasia. Previously harvested autologous bone marrow was subsequently reinfused, thus permitting escalation of the radionuclide dose.

McLaughlin and coworkers3 demonstrated the efficacy of chimeric anti-CD20 mAbs for immunotherapy for patients with low-grade NHL in a pivotal trial that led to approval of rituximab. Thereafter 90Y-ibritumomab and 131I-tositumomab were approved for RIT.3,6

RIT with ANTI-CD20 mAbs

General

Anti-CD20 mAbs react with greater than 95% of B lymphocytes and greater than 90% of B-lymphocyte NHL. Rituximab, a chimeric mAb, works by enlisting immune systems to destroy NHL cells to which it binds and also causes direct NHL cell death by apoptosis. After pretreatment with rituximab to obtain “favorable biodistribution,” 90Y-ibritumomab, the mouse parent of rituximab attached to the radionuclide 90Y, is used for RIT (Table 2). Prenfusion of unlabeled anti-CD20 mAb is essential because of the high density of Ag on normal lymphocytes; less unlabeled mAb is associated with poorer detection of known NHL.21

Another mouse mAb against the CD20 Ag has also been approved for the treatment of patients with NHL. 131I-tositumomab requires pretreatment with 475 mg of unlabeled anti-CD20 mAb to increase the radiation dose to NHL and reduce the radiation dose to normal tissue. More than one half of patients with low-grade or transformed low-grade NHL treated with a single nonmyeloablative dose of 131I-tositumomab for salvage achieve a response, and many of these patients have CR. A phase II trial in previously untreated patients with NHL showed an ORR of 100% and toxicity rarely in excess of grade 2.22 Press and coworkers23 have shown that a single dose of 131I-anti CD20 mAb was remarkably effective in patients with relapsed NHL when bone marrow transplantation (BMT) was used to permit administration of large doses of 131I. Tumor doses ranged from 27 to 92 Gy. With a median follow up of 2 years, there was a 62% progression-free survival rate and 93% overall survival rate. Toxicity included 3 serious infections (1 of which led to death), and 3 cases of cardiopulmonary toxicity.

90Y-Ibritumomab (Zevalin®)

The murine IgG1 mAb, ibritumomab, the parent of the chimeric mAb, rituximab, has been attached to the linker chelator tiuxetan (MX-DTPA) and 90Y to form 90Y-ibritumomab. Ibritumomab is a mAb to the CD20 Ag that is expressed on the surface of most normal and malignant B-lymphocytes. Tiuxetan is a second-generation metal chelator that is co-
valently bound to the mAb and chelates the radionuclides, $^{90}$Y for treatment and $^{111}$In for imaging. $^{90}$Y is a pure beta emitter with a mean path length in soft tissue of approximately 5 mm. $^{90}$Y-ibritumomab can be safely administered as an outpatient procedure, consisting of an infusion on day 1 for imaging and a second infusion for treatment within 1 to 2 weeks. Imaging is performed to confirm the expected biodistribution of the $^{111}$In-ibritumomab. Biodistribution is evaluated by using a series of whole-body images that are obtained after the administration of $^{111}$In-ibritumomab. Patients first receive an infusion of rituximab 250 mg/m$^2$ to decrease circulating B-lymphocytes and improve tumor targeting. If expected biodistribution is observed, patients proceed to the therapeutic dose of $^{90}$Y-ibritumomab. The therapeutic dose also is preceded by an infusion of rituximab 250 mg/m$^2$. It has been shown that predosing with unlabeled mAb improves the biodistribution of the radiolabeled mAb, from visualization of 18% of NHL sites without a predose to visualization of 92% of NHL sites after administration of 1 or 2.5 mg/kg.\textsuperscript{21}

In a dose escalation, phase I-II trial (7.4 to 14.8 MBq/kg; 0.2 to 0.4 mCi/kg), Knox and coworkers\textsuperscript{21} treated patients with recurrent low- and intermediate-grade NHL with $^{90}$Y-ibritumomab. The ORR after a single dose of $^{90}$Y-ibritumomab was 67% in low-grade and 82% in aggressive NHL. The MTD was

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**Table 1** Highlights of Historical Background of RIT for Patients with NHL\textsuperscript{*}

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
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</thead>
<tbody>
<tr>
<td>1980</td>
<td>Original description of immunotherapy using anti-CD20 mAbs, Nadler</td>
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<tr>
<td>1982</td>
<td>Original description of effective immunotherapy using anti-idiotypic mAbs, Miller</td>
</tr>
<tr>
<td>1987</td>
<td>Original description of RIT in NHL (and CLL) using $^{131}$I-Lym-1 mAbs, DeNardo</td>
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<tr>
<td>1989</td>
<td>Original description of myeloablative RIT using $^{131}$I-anti CD20 MAbs, Press</td>
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<tr>
<td>1990</td>
<td>Original description of use of RIT using $^{90}$Y anti idiotypic mAbs, Parker</td>
</tr>
<tr>
<td>1998</td>
<td>Approval of rituximab based on pivotal trial, McLaughlin</td>
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<tr>
<td>2002</td>
<td>Approval of ibritumomab tiuxetan based on pivotal trial, Witzig</td>
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<td>2001</td>
<td>Approval of tositumomab based on pivotal trial, Kaminski</td>
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<tr>
<td>2000</td>
<td>Original description of pretargeted RIT, Weiden</td>
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*Reference numbers in parentheses.
14.8 MBq/kg (0.4 mCi/kg); transient myelosuppression was the primary adverse event. Witzig and coworkers\textsuperscript{24} determined that a dose of rituximab of 250 mg/m\textsuperscript{2} was adequate for improving targeting of \textsuperscript{90}Y-ibritumomab and that the maximum tolerated single dose was 14.8 MBq/kg (0.4 mCi/kg) in patients with a platelet count of at least 150,000/mL and 11.1 MBq (0.3 mCi/kg) in those with a count between 100,000 to 149,000/mL. In a pivotal phase III trial, \textsuperscript{90}Y-ibritumomab was compared with rituximab in patients with relapsed or refractory follicular, low-grade, or transformed NHL.\textsuperscript{5} Patients were randomized to one treatment with \textsuperscript{90}Y-ibritumomab 14.8 MBq/kg (0.4 mCi/kg) after infusion of rituximab 250 mg/m\textsuperscript{2} or to rituximab 375 mg/m\textsuperscript{2}/w for each of 4 weeks. \textsuperscript{90}Y-ibritumomab produced significantly higher overall (80\% versus 56\%; \( P < 0.002 \)) and complete (30\% versus 16\%; \( P = 0.04 \)) response rates than did rituximab (Fig. 2).\textsuperscript{5}

The CR/CRu rate was 34\% in the \textsuperscript{90}Y-ibritumomab patients and 20\% in the rituximab patients (\( P = 0.04 \)). The efficacy of \textsuperscript{90}Y-ibritumomab also has been evaluated in patients with rituximab-refractory follicular NHL.\textsuperscript{25} The ORR and CR were 74\% and 15\%, respectively.

Response rates with \textsuperscript{90}Y-ibritumomab are high and the drug is well tolerated, with delayed, reversible myelosuppression being the dose-limiting toxicity.\textsuperscript{3,25,26} Responses have been achieved in patients with bulky tumors, older patients, and those that have failed rituximab. Only patients with adequate bone marrow reserves and less than 25\% lymphoma marrow involvement have been approved for treatment (Fig. 3). \textsuperscript{90}Y-ibritumomab dosing is weight-based. The administered dose of \textsuperscript{90}Y-ibritumomab usually is between 0.7 to 1.1 GBq (21-30 mCi) and is never more than 1.2 GBq (32 mCi). The risk of radiation exposure is minimal.\textsuperscript{27} Imaging can be predicted by substituting \textsuperscript{111}In as surrogate for imaging.

\textsuperscript{90}Y-ibritumomab is indicated for the treatment of patients with relapsed or refractory low-grade, follicular, or transformed B-lymphocyte NHL, including patients with rituximab-refractory follicular NHL. A total of 152 patients with NHL have received treatment after receiving \textsuperscript{90}Y-ibritumomab. Therapies given after \textsuperscript{90}Y-ibritumomab have proven safe and effective. ORRs were comparable to those in patients with NHL not previously treated with \textsuperscript{90}Y-ibritumomab.

\textbf{131I-Tositumomab (Bexxar\textsuperscript{®})}

Another drug approved by the Food and Drug Administration for NHL RIT is \textsuperscript{131}I-tositumomab, which targets the same CD20 Ag. Tositumomab, an IgG2a mouse mAb to which \textsuperscript{131}I Table 2 Overview of anti-CD20 RIT Using \textsuperscript{131}I-Tositumomab or \textsuperscript{90}Y-Ibritumomab

<table>
<thead>
<tr>
<th>Property</th>
<th>Tositumomab (Bexxar\textsuperscript{®})</th>
<th>Ibritumomab Tiuxetan (Zevalin\textsuperscript{©})</th>
</tr>
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<tbody>
<tr>
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<td>Mouse anti-CD20 mAb</td>
<td>Mouse anti-CD20 mAb</td>
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<tr>
<td>mAb predose</td>
<td>Mouse anti-CD20 mAb</td>
<td>Chimeric anti-CD20 mAb</td>
</tr>
<tr>
<td>Response and toxicity</td>
<td>Similar</td>
<td>Similar</td>
</tr>
<tr>
<td>Dosing</td>
<td>Dosimetry</td>
<td>Body weight and baseline platelet count \textsuperscript{90}Y</td>
</tr>
<tr>
<td>Radionuclide</td>
<td>\textsuperscript{131}I</td>
<td>Slower</td>
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<tr>
<td>Radionuclide clearance</td>
<td>Faster*</td>
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</tr>
<tr>
<td>Radionuclide half-life</td>
<td>8 days</td>
<td>Beta‡</td>
</tr>
<tr>
<td>Radionuclide emissions</td>
<td>Beta and gammat</td>
<td>Beta‡</td>
</tr>
</tbody>
</table>

*May reduce radiation exposure to normal and NHL cells.
†Allows monitoring radiation that goes to the targeted sites in a process called dosimetry
‡May require \textsuperscript{111}In as surrogate for imaging.

\textsuperscript{3}May reduce radiation exposure to normal and NHL cells.

\textsuperscript{1}May require \textsuperscript{111}In as surrogate for imaging.

Figure 2 Results for the pivotal phase III trial of \textsuperscript{90}Y-ibritumomab RIT and rituximab immunotherapy in relapsed/refractory low-grade, follicular, or transformed NHL. Patients randomized into the \textsuperscript{90}Y-ibritumomab arm were given a tracer dose of 185 MBq (5 mCi) \textsuperscript{111}In-ibritumomab on day 0 and then a therapeutic dose of 14.8 MBq/kg (0.4 mCi/kg) \textsuperscript{90}Y-ibritumomab on day 7. Both ibritumomab doses were preceded by an infusion of 250 mg/m\textsuperscript{2} of rituximab. Patients randomized into the rituximab arm received a standard course of rituximab (375 mg/m\textsuperscript{2} weekly \( \times 4 \)). The efficacy analysis performed on 143 patients enrolled in this trial showed an ORR of 80\% for \textsuperscript{90}Y-ibritumomab versus 56\% for rituximab (\( P = 0.002 \)). CRR was 30\% for \textsuperscript{90}Y-ibritumomab versus 16\% for rituximab (\( P = 0.04 \)). (Graphics generated from data in Witzig et al.\textsuperscript{7})
is attached, produces beta emissions for treatment and gamma emissions for imaging and dosimetry. In a pivotal phase III trial in patients with low-grade or transformed low-grade NHL, 65% of patients responded to $^{131}$I-tositumomab, whereas only 28% of the same patients responded to their previous regimen of salvage chemotherapy. Although not approved, patients who are ineligible because of extensive marrow involvement might meet this eligibility requirement by marrow cytoreduction using rituximab or chemotherapy before $^{90}$Y-ibritumomab or $^{131}$I-tositumomab. Alternatively, $^{131}$I-tositumomab might be used because the residence time and range of $^{131}$I in the marrow are shorter than those of $^{90}$Y. If the bone marrow eligibility requirement is met then the patient must have a favorable (expected) biodistribution by imaging to proceed to $^{90}$Y-ibritumomab therapy. Imaging is rarely the basis for ineligibility. The anterior image of the patient demonstrates favorable (expected) biodistribution and $^{111}$In-ibritumomab uptake in enlarged pelvic and inguinal lymph nodes. The patient with unfavorable (altered) biodistribution shows excessive renal uptake of radionuclide, interpreted to be caused by documented hydronephrosis secondary to tumor obstruction. Both images were obtained about 68 hours after $^{111}$In-ibritumomab. mAb, monoclonal antibody; NHL, non-Hodgkin’s lymphoma; RIT, radioimmunotherapy. (Reproduced with permission from DeNardo et al.52) (Color version of figure is available online.)

**RIT With Other mAbs**

Other mAbs and radionuclides have been investigated but have not reached the approval stage. Among these are Lym-1...
(Oncolym™), which targets HLA-DR10 β, and epratuzumab, which targets CD22.

Approximately 90% of B-lymphocyte NHL malignancies react with Lym-1. Lym-1, an IgG2a mAb with high affinity for a discontinuous epitope on the beta subunit of the human leukocyte Ag (HLA-DR), selectively binds a noncirculating Ag that is highly expressed on the surface of human malignant B-lymphocytes but less so on normal B-lymphocytes. The membrane Ag bound by Lym-1 is not significantly internalized after mAb exposure, nor is it shed into the blood of patients with NHL. 131I-Lym-1, as a single agent, has had significant response rates in all grades of NHL (Fig. 5A). To use the favorable characteristics of radiometals for RIT, trials have been conducted with 67Cu-2IT-BAT-Lym-1 and 111In/90Y-2IT-BAD-Lym-1. Patients given 67Cu-2IT-BAT-Lym-1 (0.9 to 2.2 GBq/m2; 25 to 60 mCi/m2 per dose), the lower dose being used when NHL was detected in the bone marrow, had an ORR of 58%. Myelotoxicity was dose-limiting with 90Y-2IT-BAD-Lym-1, just as it has been in studies of 131I-Lym-1 and 67Cu-2IT-BAT-Lym-1.

Both mLL2 and hLL2 (epratuzumab) are anti-CD22 mAbs that are rapidly internalized on binding to B-lymphocytes Humanized 90Y-epratuzumab (90Y-hLL2; Lymphocide™) has shown high tumor-to-normal organ radiation dose ratios and response rates in patients with relapsed/refractory NHL (Fig. 5B). A variety of trials have been conducted using (1) mLL2 or hLL2; (2) any of several radionuclides; (3) single or multiple doses; (4) nonmyeloablative or myeloablative approaches; and (5) phase I-II or phase II trials. Response rates and toxicities for RIT have been similar to those for other anti-lymphoma mAbs described here, except that response rates for 131I-anti-CD22 mAbs have been less.

Figure 4 (A) Lym-1, a mouse mAb that targets a discrete epitope of HLA-DR-10β expressed on malignant B-lymphocytes, was studied labeled with 131I, 67Cu, or 90Y. The first of a series of phase I-II, fractionated trials in patients with NHL, using multiple 1.1 or 2.2 GBq (30 or 60 mCi) doses of 131I-Lym-1, showed a 57% ORR. Standard dose escalation trial in cohorts of patients with NHL reached a maximum tolerated dose (MTD) of 131I-Lym-1 of 3.7 GBq/m2 (100 mCi/m2) for each of the first 2 doses of 131I-Lym-1 given 4 weeks apart and produced an ORR of 52% and CR rate of 33%; 100% of the cohort entered at the MTD dose level had CRs. When labeled with 67Cu or 90Y, the efficacy was similar to those for 131I-Lym-1. (Graphics generated from data in DeNardo et al.9,10) (B) Epratuzumab, humanized anti-CD22 mAb, has been studied in phase I-II trials labeled with 90Y, 131I, or 186Re and in a phase II trial labeled with 90Y. Response rates for 131I and 186Re-labeled anti-CD22 mAbs were lower than those for 90Y because this mAb is internalized. (Graphics generated from data in other sources.8,32,33,37)
Toxicity

RIT has proved safe. Treatment-related deaths and hospitalizations for toxicity have been rare. Patients prefer RIT to alternative therapies.

Allergic Reactions

Approximately one fifth of patients developed fevers, rigors, chills, and diaphoresis during and after infusions of mAbs. The reactions typically lasted no more than a few hours. Pruritus and urticaria were observed in as many as 18% of patients. Bronchospasm and anaphylaxis were observed in 2% and 1% of patients, respectively. Rapid infusions and the administration of a large quantity of mAbs, particularly those targeting white blood cells, increase the likelihood of an allergic reaction. To reduce the risk of an allergic response, patients are commonly medicated with acetaminophen and diphenhydramine before and during the infusion of mAbs.

Human Antimouse mAbs

After exposure to mAbs that are foreign proteins, patients may develop human Abs against the foreign protein. A human antimouse Ab (HAMA) response is commonly induced by mouse mAbs and can result in rapid clearance of subsequently administered mAbs from the circulation, reducing tumor uptake and therapeutic efficacy. There is considerable variability in the development of a HAMA response among patients. Chimeric and humanized Abs induce distinctly less Ab responses in patients.

Myelosuppression

Because of the radiosensitivity of bone marrow, myelosuppression, manifested most commonly by thrombocytopenia, has been dose-limiting. Despite occasionally severe thrombocytopenia and neutropenia, bleeding and infection have been rare. In high-dose RIT with BMT, Press and coworkers reported severe posthypotension, life-threatening congestive cardiomyopathy and hemorrhagic pneumonitis in individual patients after a single infusion of 131I-CD20 mAb that delivered 27 Gy or more to the lungs. When increasing doses of 90Y labeled mAb have been administered to dogs, hepatotoxicity has occurred because of retention of 90Y in the liver after mAb catabolism. Because 131I is rapidly cleared, serious hepatic toxicity has been reported less often than pulmonary toxicity in trials in which high levels of 131I have been delivered.

Concepts

General

The exquisite specificity and unlimited variability of mAbs has ushered in a revival of the concept of the “magic bullets” of Paul Ehrlich. mAbs bind with high specificity and affinity to Ags. Indeed, mAbs are specific for a discrete region, or epitope, of the Ag, thus leading to less cross-reactivity with nonmalignant tissues than polyclonal Abs.

In an effort to improve the results for unconjugated mAbs, mAbs have been conjugated with radionuclides. Radionuclides provide the following advantages: (1) beta emissions kill adjacent cells, regardless of whether they express the target Ag (“bystander” effect); and (2) radiation is not subject to drug resistance. RIT can be regarded as “smart” radiation therapy because systemic, tumor targeted RIT, can deliver as much as 50 times more radiation to malignant cells than that to the normal cells. The highest response rates have been achieved in patients with B-lymphocyte NHL. Beta emissions have ranges of 100 to 500 cell diameters providing a bystander effect on nearby cells that can overcome sources of heterogeneity. To reduce radiation to normal tissues, researchers have used pretargeted RIT. The method encompasses a delivery system in which the radionuclide is injected separately from the mAb, thereby minimizing exposure of normal tissues to circulating radionuclide. Weiden and coworkers, using a streptavidin/biotin 3-step pretargeting approach with an anti-CD20 mAb in patients with NHL, obtained tumor to whole body ratios that were 2 to 3 times higher than those achieved with conventional anti-CD20 RIT.

Does the Radionuclide Make a Difference?

The choice of radionuclide for RIT depends on the type of radiation emitted, its path length in tissue, its elimination, and its half-life. For diagnostic purposes, the most important types of decay are gamma emissions that can be detected by imaging. In contrast, beta particles deposit their energy in the vicinity of the decay. Alpha particles deposit energy over a shorter range than beta particles so that nearby cells are spared. Auger electrons have an even shorter range of energy deposition, so that intranuclear deposition is optimal for cell killing. Beta particles have a small probability of releasing enough energy along their track to produce DNA breaks. Approximately 200 DNA double-strand breaks per cell are required to sterilize 99% of a malignant cell population. High linear energy transfer alpha radiation is densely ionizing and more efficiently produces DNA breaks. Because of their short range in tissues, alpha (and Auger electrons) have little bystander effect.

131I is eliminated from tissues as the mAb is catabolized. If the Ag undergoes internalization within the NHL cell after mAb binding, “dehalogenation” is a problem. Radiometals like 90Y are retained (residualized) in tissues once the mAb has been catabolized. A residualizing radionuclide increases tumor radiation dose but also potentially increases the radiation dose to normal tissues, such as the liver, where proteins are catabolized. An additional disadvantage of 90Y is that, if freed from the chelated mAb, it accumulates in bone, thereby increasing radiation to the marrow. DeNardo and coworkers compared the radiation dosimetry of 67Cu, 131I and 90Y labeled-Lym-1 in patients with NHL. The therapeutic index (ratio of radiation doses to tumor and normal tissues) was most favorable for 67Cu.

Does the Ag Make a Difference?

Several of the many Ags that are present on NHL cells have been targeted in trials of radiolabeled mAbs. These include...
CD19, CD20, CD22, CD37, MHC class II allele HLA-DR10, and immunoglobulin idiotype Ags. CD20, CD22, and HLA-DR Ags are restricted to B-lymphocytes and are present on approximately 90% of B-lymphocytic NHL and normal B lymphocytes but not on stem cells, pre-B-lymphocytes, and plasma cells. HLA-DR and CD20 Ags are present at high densities on malignant lymphocytes, whereas CD22 is less abundant. In addition to high densities on malignant lymphocytes, CD20 Ag also is found in high densities on normal B lymphocytes; therefore, a larger anti-CD20 mAb dose is required to target NHL tissue. Because Lym-1 has preferential reactivity with malignant lymphocytes, only small amounts of Lym-1 are required to achieve optimal targeting of NHL.

**Does the mAb Make a Difference?**

For immunotherapy, chimeric or humanized mAbs, because of their slower clearance rates, are preferred but, for RIT, slow blood and body clearance can be a disadvantage. The choice of mAb for RIT depends to some extent on the radionuclide used; a mouse mAb is cleared from the patient faster than a chimeric or humanized mAb.

Anti-CD20 mAbs are cytotoxic for both malignant and mature lymphocytes. RIT, using rituximab, induces lymphopenia. Because B-lymphocyte precursors do not express CD20 Ag, B-lymphocytes return to normal at about 6 months after treatment. Anti-HLA-DR and CD22 mAbs also have been shown to be cytotoxic for malignant B lymphocytes.41,42 Additionally, mAbs against a common Ag may bind to different epitopes of the Ag, thereby having quite different characteristics. Another issue concerns human antiglobulin Ab responses. An Ab response in the patient may make it difficult to continue to treat the patient. If repeat doses are anticipated, a chimeric or humanized mAb may be favored.

**Does the Chelator Make a Difference?**

Radionuclides like $^{131}$I are covalently bound to mAbs, usually on a tyrosine residue, whereas metallic radionuclides are bound by a chelate that has been conjugated to lysyl residues of the mAb. Although mAb specific, the addition of less than 4 chelators does not seem to alter immunoreactivity.43 Recently developed macrocyclic chelators for radionuclides, such as $^{111}$In and $^{90}$Y, provide greater stability in vivo. DeNardo and coworkers investigated the effect of 2 different $^{90}$Y chelators, methylbenzylidiethylene-triaminepentaacetic acid (MX-DTPA) and bromoacetamidobenzyl-1,4,7,10-tetraazacyclododecane-$N,N,N,N^\prime,N^\prime,N^\prime,N^\prime$-tetraacetic acid (DOTA). The LD$_{50}$ in mice was higher when the latter was used as the $^{90}$Y chelator compared with MX-DTPA (Fig. 6). For both chelators, bone marrow toxicity was the cause of death. Whole-body autoradiography revealed substantially greater uptake of $^{90}$Y in the skeleton when MX-DTPA was used as the chelator. (Graphics generated from data in DeNardo et al.44)

**Figure 6** Survival of mice given $^{90}$Y-MX-DTPA (●) or 2 to 2IT-BAD (○) chelated mAb. All drug mortality occurred within 30 days after treatment. Predicted probability of mortality rate was calculated using logistic regression analysis. Data points represent 9 to 19 mice; bars, 95% confidence interval. The LD$_{50}$ for $^{90}$Y-MX-DTPA chelated mAb was 8.1 MBq (218 μCi) and that for $^{90}$Y-DOTA chelated (2IT-BAD) was 11.4 MBq (309 μCi). Whole-body autoradiography of mice revealed substantially greater uptake of $^{90}$Y in the skeleton when MX-DTPA was used as the chelator. (Graphics generated from data in DeNardo et al.44)

**Figure 7** Cumulated activities ($\mu$Ci-h/g/μCi/g) for $^{111}$In-2IT-BAD and peptide-linked Lym-1 for liver (□) and tumor (●). When compared with 2IT-BAD, liver cumulated activity for DOTA-peptide Lym-1 was consistently reduced, whereas the tumor cumulated activities were maintained. The tumor-to-liver therapeutic index for DOTA-peptide-Lym-1 (cathepsin B degradable) was much higher than that for 2IT-BAD nondegradable linked Lym-1. Cumulated activities were obtained using nonlinear regression to analyze monoequponential activity concentrations obtained from the pharmacokinetic studies (error bars represent SE). (Graphics generated from data in DeNardo et al.45)
Does the Linker Make Any Difference?

In myeloablative strategies, the liver usually is dose-limiting for radiometal labeled mAbs. The radiation dose to the liver can be reduced by attaching the chelated radiometal to the mAb using a degradable peptide linker. Cathepsin cleavage in hepatocytes leads to a radioactive moiety that can be rapidly excreted by the kidney. DOTA-peptide-Lym-1 has a tetra peptide linker that is susceptible to cathepsin B, an enzyme in hepatocytes. The pharmacokinetic and dosimetric properties of 111In- and 90Y-labeled DOTA-peptide-Lym-1 were compared with those for the same radiolabeled mAbs with a nondegradable linker (2-iminothiolane (2-IT)-2-[p-(bromoacetamido)benzyl]-DOTA) in athymic mice bearing human NHL xenografts (Fig. 7). Liver concentration, cumulated activity, and radiation dose of the DOTA-peptide-Lym-1 were less than one half those of the corresponding 2-iminothiolane (2-IT)-2-[p-(bromoacetamido)-benzyl]-DOTA drugs.

Do Dosing, Imaging, and Dosimetry Make a Difference?

The amount of radionuclide administered and the manner in which it is administered affect RIT. There are 2 broad algorithms for selecting the dose of radionuclide. 90Y-ibritumomab and 131I-tositumomab use different algorithms. A fixed-dose approach, based on body weight and bone marrow status, is used for 90Y-ibritumomab whereas the dose of 131I-tositumomab varies, depending on the body pharmacokinetics and weight of individual patients. 90Y-ibritumomab is administered using an empirically selected radionuclide dose, based on observations of dose-limiting toxicity in a similar population of patients. It is assumed that a little pharmacokinetic variability exists between patients. The individualized approach to dosing used for 131I-tositumomab requires predictive dosimetry and is preferred in cases in which there is interpatient variability. In other forms of radiation therapy, treatment planning for an individual patient has been shown to be important for optimal response and morbidity.

Radioimmunoimaging demonstrates mAb distribution in the patient. To image, a gamma-emitter is necessary. Dosing analyses are useful in the course of drug development to evaluate safety, toxicity, and efficacy. Radiation dosimetry also can be used to assess the results of treatment and for treatment planning for individual patients. Treatment planning for an individual patient (“patient-specific dosimetry”) should be an ultimate goal.

Once the radionuclide dose has been determined, the dosing schedule must be considered. There are 2 approaches to administering RIT: a single large dose or multiple smaller doses of radionuclide can be given. Press and coworkers administered almost a Curie of 131I-anti-CD20 mAb to patients with NHL in a myeloablative strategy followed by autologous stem cell transplantation, showing remarkably good efficacy. A single dose of 131I- or 90Y-labeled mAb also has been shown to be effective in nonmyeloablative strategies. An alternative approach involves the administration of multiple doses, often referred to as “fractionation.” The rationale for fractionated RIT is based on evidence that the radiation dose to the tumor, and the dose tolerated by normal tissues, can be increased. Another advantage of fractionating the total radionuclide dose is better distribution of the microscopic radiation dose because of reduced heterogeneity of mAb targeting over several doses. Tumor control is less with nonuniform radiation because some regions of the tumor may be under-dosed. Fractionation also permits toxicity to be controlled by titration in the individual patient. Fractionation also can be used to reduce toxicity, when patients with bone marrow NHL are to be treated.

With external beam treatment, radiation is intermittently pulsed at a high, constant dose rate (about 60 Gy/h) to a limited region of the body. With RIT, radiation is continuously delivered at a low dose rate that decreases because of physical and biologic decay. Dose rates as low as 0.05 Gy/h can stop the growth of radiosensitive NHL cells. Additionally, mAbs themselves may, in some cases, exert anti NHL effects.

References

Treatment of NHL with mAbs