Since the development of monoclonal antibodies by Köhler and Milstein, their use as a targeted therapy for cancer has been explored widely. Depending on antigen specificity, antibodies have the potential to react preferentially with tumor cells while binding to a lesser degree with normal tissues. This is in great contrast to chemotherapy (and to some degree radiotherapy), where significant toxicity results from drug exposure to various organs. Naked (or unlabeled) antibodies may have direct antitumor effects through a variety of mechanisms or induce a secondary immune response against cancer cells. Immunotoxins additionally deliver a toxic drug or compound preferentially to malignant cells, whereas radiolabeled antibodies can administer targeted radiation to tumor tissue. Lymphomas may be a particularly good setting for this treatment modality as they are relatively vascular (with good antibody penetration), they respond to various drugs/toxins and radiotherapy, and they appear to respond to immune system manipulation. These modalities also offer the possibility of combination with chemotherapy or other biologics, which could potentially augment therapeutic activity, although toxicity may be worsened as well. This chapter reviews the available information on these active agents in non-Hodgkin’s lymphoma (NHL) and explores their demonstrated and potential roles in lymphoma therapy.

An ideal goal for any antitumor therapy is the delivery of specific toxicity to tumor cells while sparing normal cells. Nadler and colleagues conducted an initial trial of serotherapy with a monoclonal antibody directed against a lymphoma-associated antigen, which demonstrated some evidence of tumor cell killing. Similarly, Miller and colleagues generated patient-specific anti-idiotypic antibodies and demonstrated clear evidence of NHL regression. The immunoglobulin idiotype molecule on the surface of malignant B cells is an excellent example of a tumor-specific target for this strategy as it is expressed uniquely by the tumor cells of a given patient. These studies provided an important proof of the concept that monoclonal antibodies could provide an antitumor effect with acceptable toxicity. Significant issues impact the practicality of developing a unique monoclonal antibody for treatment of individual patient tumors against idiotypes, particularly with respect to effort and expense.

Efforts subsequently have turned primarily to development of antibodies that target antigens common to tumors in different patients so that an agent can be used more widely. More available antigens,
however, generally are also expressed to some degree on normal tissues, potentially providing toxicity if normal cells are damaged or eliminated and consequences result. Therefore, antigen target selection plays a major role in activity (depending on mechanism of action) and toxicity. Since 85 to 90 percent of lymphomas are of B cell origin, pan-B-cell antigens have been explored widely as a potential target for antibody therapy.

Another important issue is the antibody molecule itself. Most therapeutic antibodies were developed initially in the murine form; later, many were re-engineered as chimeric (roughly 60% human and 40% murine) and humanized (95% human with the murine component limited to the antigen binding site). Antibody structure and subclass may affect tumor penetration, complement activation, and antibody-dependent cellular cytotoxicity (ADCC). Murine antibodies can be immunogenic (although less so in patients who are immunosuppressed previously due to their malignancy or prior therapy) whereas chimeric and humanized antibodies are less immunogenic. Formation of a human antimouse antibody (HAMA), human antichimeric antibody (HACA), or human antihuman antibody (HAHA) may adversely impact antibody pharmacokinetics by increasing clearance from the plasma or may predispose to toxicities such as allergic-type reactions. Therefore, most treatments with murine antibodies have been limited to relatively few doses, with repeated dosing being more feasible with humanized or chimeric agents.

**Rituximab**

Rituximab, a chimeric monoclonal antibody directed against the CD20 antigen, was the first monoclonal antibody approved by the US Food and Drug Administration (FDA) for the treatment of cancer and has been administered, currently, to over 100,000 patients. This agent has been shown to mediate ADCC and complement-dependent cytotoxicity (CDC) and to cause induction of apoptosis of lymphoma cells in vitro.\(^3\)\(^-\)\(^5\) Several phase I/II clinical trials established the standard dosing regimen of 375 mg per m\(^2\) per week administered intravenously for 4 weeks and demonstrated an acceptable toxicity profile consisting primarily of infusion reactions including fever, rigors, and myalgias (Table 18–1).\(^6\)\(^-\)\(^8\) Dose-limiting toxicity (DLT) was not observed. Circulating B cells were depleted rapidly and recovered 3 to 6 months after the last infusion. No changes in circulating immunoglobulin levels were noted. Significant rituximab serum levels persisted several months after completion of therapy.

Several patients demonstrated evidence of clinical response, leading to the development of a multicenter phase II pivotal trial of rituximab in relapsed indolent NHL.\(^9\) One hundred sixty-six patients were treated with the standard dosing regimen over 4 consecutive weeks. Overall response rate was 48 percent, including 6 percent complete remissions. In contrast to many other agents, time to response with rituximab can be delayed and occurred at a median of 50 days in this study. Median time to progression was 13 months in responding patients. The majority of assessable patients were found to achieve molecular remissions in blood or bone marrow, as detected by polymerase chain reaction assays for \textit{bcl-2} gene rearrangements. Lower response rates were observed in the small lymphocytic lymphoma subtypes (12% overall) as well as in chemotherapy-resistant patients, whereas patients with prior high-dose chemotherapy and stem cell transplantation had higher response rates (78% versus 43%). A comparable toxicity profile to earlier studies was observed, and infusion reactions were managed by diphenhydramine and acetaminophen premedication, as well as slowing of the intravenous rate as required. Of particular importance, cytopenias were minimal, suggesting that overlapping toxicity with chemotherapy might be minimal. This trial led to the approval and widespread acceptance of rituximab as a single-agent therapy for relapsed and refractory low-grade NHL. Further studies have demonstrated efficacy in bulky disease\(^10\) and in the up-front setting.\(^11\)\(^,\)\(^12\) Extended schedules (eight weekly infusions) have been explored with acceptable toxicity, although additional benefit is unclear.\(^13\) Finally, retreatment with rituximab in previously responding patients has been shown to be safe (particularly given the lack of immunogenicity of this chimeric molecule), with 40 percent of patients responding with durations appearing to be longer than first responses in many individuals.\(^14\)
In other NHL histologies, rituximab generally has been less effective as a single agent. In aggressive NHL, Coiffier and colleagues treated 54 patients predominantly with diffuse large cell NHL (many with no prior therapy) with eight weekly infusions of rituximab, with an overall response rate of 31 percent (9% complete responses) with time to progression 246+ days in the responding group.\(^{15}\) In a mixture of previously untreated and relapsed patients with mantle cell lymphoma, rituximab achieved a response rate of 34 percent, with 14 percent complete responses and a median time to progression of 7 months.\(^{16}\) These limited results have led to its use as a single-agent therapy in these NHL subtypes predominantly as a palliative measure.

Other attempts have been made to change rituximab dose and schedule to improve on the modest response rates observed in small lymphocytic lymphoma (SLL)/chronic lymphocytic leukemia (CLL). Byrd and colleagues have turned to a thrice-weekly schedule,\(^{17}\) whereas O’Brien and colleagues have extended the dose escalation in a separate trial.\(^{18}\) Both have improved response rates to the point that roughly one-third of patients achieve objective tumor reductions; however, the practicalities and expense associated with these regimens may make them difficult to use in many settings. It is also notable that patients with extensive circulating tumor cell burden, such as patients with CLL and a high white blood cell count, may be at risk for life-threatening infusion-related complications associated with acute tumor lysis and cytokine release. These individuals should be observed carefully during therapy.\(^{19}\)

Other studies have evaluated the use of rituximab in plasma cell neoplasms such as Waldenström’s macroglobulinemia (WM) and multiple myeloma. Treon and colleagues reported a retrospective review of a multicenter experience of rituximab therapy of WM, demonstrating that roughly one-fourth of patients exhibited evidence of disease improvement, predominantly in reduction of M protein and improvement in hematologic parameters.\(^{20}\) A prospective multicenter trial is currently under way to examine the role of rituximab therapy in WM. Among the subset of multiple myeloma patients whose plasma cells express the CD20 antigen, occasional patients have been shown to have some evidence of response.\(^{21}\) This area is also under further evaluation at the present time.

A second generation of regimens has been developed that combines chemotherapy and rituximab to take advantage of the potential for synergy between agents. Given the limited hematologic toxicity of rituximab and the fact that cytotoxic agents remain the primary DLT for most NHL treatment regimens, combination regimens have been demonstrated to have

### Table 18–1. UNCONJUGATED MONOCLONAL ANTIBODIES IN NON-HODGKIN’S LYMPHOMA

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Target Antigen</th>
<th>Disease</th>
<th>Trial Phase</th>
<th>No. of Patients</th>
<th>Schedule</th>
<th>Response Rate (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab</td>
<td>CD20</td>
<td>Relapsed LG/F</td>
<td>I/II, II</td>
<td>232</td>
<td>375 mg/m²/wk × 4</td>
<td>46–48</td>
<td>6–9</td>
</tr>
<tr>
<td>Rituximab</td>
<td>CD20</td>
<td>Bulky relapsed LG/F</td>
<td>II</td>
<td>28</td>
<td>375 mg/m²/wk × 4</td>
<td>43</td>
<td>10</td>
</tr>
<tr>
<td>Rituximab</td>
<td>CD20</td>
<td>Untreated LG/F</td>
<td>II</td>
<td>89</td>
<td>375 mg/m²/wk × 4 (q6months)</td>
<td>64–69</td>
<td>11, 12</td>
</tr>
<tr>
<td>Rituximab</td>
<td>CD20</td>
<td>Relapsed LG/F</td>
<td>II</td>
<td>37</td>
<td>375 mg/m²/wk × 8</td>
<td>57</td>
<td>13</td>
</tr>
<tr>
<td>Rituximab</td>
<td>CD20</td>
<td>LG/F</td>
<td>II</td>
<td>57</td>
<td>375 mg/m²/wk × 4</td>
<td>40</td>
<td>14</td>
</tr>
<tr>
<td>Rituximab</td>
<td>CD20</td>
<td>Aggressive NHL</td>
<td>II</td>
<td>54</td>
<td>375 mg/m²/wk × 8</td>
<td>31</td>
<td>15</td>
</tr>
<tr>
<td>Rituximab</td>
<td>CD20</td>
<td>Mantle cell NHL</td>
<td>II</td>
<td>87</td>
<td>375 mg/m²/wk × 4</td>
<td>34</td>
<td>16</td>
</tr>
<tr>
<td>Rituximab</td>
<td>CD20</td>
<td>SLL/CLL</td>
<td>I/II</td>
<td>43</td>
<td>Dose escalation or 3 × weekly</td>
<td>35–50</td>
<td>17, 18</td>
</tr>
<tr>
<td>Rituximab</td>
<td>CD20</td>
<td>LG/F NHL</td>
<td>II</td>
<td>40</td>
<td>Rituximab + CHOP</td>
<td>95</td>
<td>22</td>
</tr>
<tr>
<td>Rituximab</td>
<td>CD20</td>
<td>DLBCL</td>
<td>II</td>
<td>31</td>
<td>Rituximab + CHO</td>
<td>96</td>
<td>24</td>
</tr>
<tr>
<td>Epratuzumab</td>
<td>CD22</td>
<td>Relapsed NHL</td>
<td>I/II</td>
<td>35</td>
<td>240 mg/m²/wk</td>
<td>46 FL;</td>
<td>30, 31</td>
</tr>
<tr>
<td>Hu1D10</td>
<td>HLA-DR B chain</td>
<td>Relapsed NHL</td>
<td>I</td>
<td>14</td>
<td>0.15–5.0 mg/kg/wk × 4</td>
<td>—</td>
<td>33</td>
</tr>
</tbody>
</table>

LG/F = low-grade or follicular NHL (non-Hodgkin’s lymphoma); DLBCL = diffuse large B-cell lymphoma; SLL = small lymphocytic lymphoma; CLL = chronic lymphocytic leukemia; CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone; FL = follicular lymphoma; HLA = human leukocyte antigen.
manageable safety profiles. Virtually every chemotherapy regimen has been explored in conjunction with rituximab, but available data are limited as to any added benefit. However, a number of phase II trials have suggested a potential augmentation of antitumor effect relative to historically treated groups. Czuczman and colleagues conducted a trial of 40 patients with low-grade NHL (31 of whom had received no prior therapy) with the combination of CHOP plus rituximab infusions scheduled before cycle 1, after cycle 6, and at several points during chemotherapy. Virtually all patients demonstrated objective responses (95%), with the majority (55%) being complete responses (CRs). Median time to progression was not reached at 36 months of follow-up, and several patients were negative following therapy for evidence of minimal residual disease by bcl-2 analysis of blood or bone marrow. Toxicity was comparable to that expected with CHOP alone. A randomized trial with this combination versus chemotherapy alone is now being planned. In mantle cell lymphoma, the combination of rituximab with CHOP was less promising. Although a high response rate was observed, disease-free survival did not appear to be extended.

In diffuse large B-cell lymphoma, Link and colleagues administered CHOP chemotherapy with rituximab (one dose per cycle) to 31 previously untreated patients. An overall response rate of 96 percent was observed (63% CR), and relatively few relapses have occurred during the initial assessment period. The majority of these patients, however, fell into lower-risk international prognostic index groups. Appropriately, randomized trials are under way to compare CHOP alone with CHOP plus rituximab. Preliminary results from Coiffier and colleagues from the GELA (Groupe d’Etude des Lymphomes de l’Adulte) suggest a benefit from the combination regimen (complete response rates of 76% versus 60% with 1-year overall survival of 83% versus 68%; both statistically significant differences), although further follow-up is necessary.

Ritiximab is an active and useful agent for the therapy of NHL in many different settings. Further study is needed to establish treatment schedules, such as maintenance therapy after remission induction, and to assess the role of combinations with chemotherapy. A further area of investigation is combination with other biologics. Initial evaluations have been conducted employing rituximab in conjunction with interferon, and ongoing trials are examining the potential for synergy with other monoclonals described in this chapter. These studies should ultimately define the optimal setting for the use of rituximab among available NHL treatments.

**Campath-1H**

Campath-1H is a humanized monoclonal antibody targeting the CD52 antigen, which is expressed broadly on mononuclear white blood cells. Initial trials were complicated by infusion reactions mediated by release of various cytokines, as well as prolonged immunosuppression resulting in opportunistic infections. More recent efforts incorporating prophylaxis with anti-infective and anti-inflammatory agents have reduced, but not eliminated, these complications. Activity has been significant in the blood and bone marrow compartments, with less activity against nodal disease, which generally is more prominent in NHL.

Thus, CLL has been targeted with this agent, with significant activity. The overall response rate is 33 percent, with virtually all patients being partial responders. The median time to progression in responders is greater than 9 months, but toxicity remains an issue. Campath-1H recently was approved by the FDA for the treatment of fludarabine-refractory CLL, and efforts are under way to explore its potential in combination with other agents, including rituximab, in NHL.

**Epratuzumab**

The CD22 antigen is expressed broadly in normal and malignant B cells, in a distribution similar to that of CD20. Antigen density is more variable, however, and the molecule is internalized and has been demonstrated to have both cell-surface and cytoplasmic expression. Epratuzumab is a humanized immunoglobulin (Ig) G1 version of the LL2 monoclonal antibody, which targets the CD22 antigen, and was evaluated initially as part of a radioimmunotherapeutic strategy for NHL (in conjugation with
iodine-131 or ⁹⁰yttrium). A phase I/II trial with this agent demonstrated no DLT with doses of 120 to 1,000 mg per m² per week for four treatments.³⁰,³¹ Infusion reactions (predominantly fevers, rigors, and hypotension) are unusual, allowing administration over 30 to 60 minutes. B cells are partially depleted in some patients, with no changes in hematologic parameters, serum immunoglobulins, or blood chemistries. Antibody is detected in the serum of patients 3 to 4 months after the completion of therapy, and immunogenicity appears to be rare. Significant antitumor activity has been detected in patients with follicular NHL and in diffuse large B-cell NHL. At optimal dose levels (240 mg per m² per week or greater), 6 of 13 patients with follicular NHL demonstrated objective responses, half of which are CRs and most of which are still ongoing (several over 1 to 2 years). In a heavily pretreated group of relapsed diffuse large B-cell lymphoma patients (median three prior regimens, 65% with elevated LDH, median age of 60 years), 5 of 22 (23%) achieved objective responses, 3 of which were complete. One complete remission is ongoing over 2 years after completion of therapy. Several patients have been retreated, some with evidence of another response. Epratuzumab is currently under evaluation in rituximab-refractory indolent NHL and in combination with rituximab in follicular NHL and diffuse large B-cell NHL. Further studies also are planned in relapsed aggressive NHL.

**Hu1D10**

Hu1D10 is a humanized IgG1 monoclonal antibody that binds to a variant of the human leukocyte antigen (HLA)-DR B chain (likely a post-translational modification).³² Roughly 70 percent of normal individuals express the target antigen in their peripheral blood (predominantly in the B-lymphocyte compartment), although expression has been seen in peripheral blood monocytes and dendritic cells. This antibody has been demonstrated to induce ADCC and CDC as well as signaling through tyrosine phosphorylation in lymphoma cell lines, and thus has been explored as a potential NHL therapy. By immunohistochemistry, approximately 70 percent of NHL specimens react with the antibody, and similar rates of binding have been observed in CLL and acute lymphocytic leukemia. Link and colleagues conducted a phase I study of Hu1D10 in NHL under the sponsorship of the National Cancer Institute (see Table 18–1). To date, patients have been treated at dose levels ranging from 0.15 mg per kg per week to 5 mg per kg per week × 4 with acceptable toxicity and evidence of antitumor activity.³³ This agent is under evaluation in a phase II trial in indolent NHL as well as in other disease settings.

**IMMUNOTOXINS IN TREATMENT OF NON-HODGKIN’S LYMPHOMA**

The introduction of unconjugated monoclonal antibodies (mAbs) into the clinical arena represents a major breakthrough in the treatment of lymphoid malignancies. Although several unconjugated monoclonal antibodies noted above have demonstrated promising results in patients with NHL, the limitations of unconjugated antibodies also have been recognized. Unconjugated mAbs have relatively low endogenous cytotoxicity and depend on the host immune system (ADCC and CDC) to mount an antitumor response. In an effort to enhance antitumor activity, investigators also have used antibodies to deliver a drug or potent cytotoxin directly to the tumor cell surface. Immunotoxins (ITs) refer to conjugates between a mAb and a toxin.

Although the concept itself is simple, the development of ITs is complex. An ideal IT molecule is comprised of three components: (1) an antibody that binds specifically to the targeted cell but does not bind to other tissues, (2) a toxin that is potent for the target cell but is minimally toxic while circulating in the blood stream, (3) a linker between the antibody and the toxin that is easily cleavable at the tumor cell surface but remains stable when the toxin circulates in the blood.

The choice of an appropriate target antigen is critical. Such a target antigen or receptor should be present on malignant cells but not on normal tissue. Ideally, it should be expressed on all malignant cells because direct contact of each individual tumor cell with the toxin molecule is needed for sufficient tumor cell kill. Antigen cannot shed or be secreted from malignant cells as circulating free antigen will cause rapid clearance of IT from serum.
Most currently used toxins are protein synthesis inhibitors that work in the cytosol but not on the cell surface. Thus, the target antigen must internalize on binding to the antibody. The toxin itself must be very potent and, at nanomolar concentrations, be able to kill five or more logarithms of malignant cells.34 Because all natural toxins have domains mediating nonspecific binding to cells, they must be modified before binding to carrier mAbs to attenuate their undesirable nonspecific toxicity.

**Ricin A Chain-Based Immunotoxins**

Ricin-based ITs are the most extensively studied ITs in clinical practice. Ricin, one of the most potent natural poisons, is a heterodimer comprised of an A chain and a B chain. The A chain is an N-glycosidase that inhibits protein synthesis. The B chain mediates nonspecific binding of the toxin to the cell surface and also assists in translocating the A chain across the cell membrane.

Before ricin can be conjugated to an antibody, the toxin domain responsible for nonspecific tissue binding must be deactivated. The simplest approach is to physically separate the A chain from the B chain and conjugate the A chain directly to an antibody. Initial clinical experience came from trials using conjugates of ricin A chain with antibodies targeting the CD5 antigen: T101 murine antibody in CLL patients35 and H65 antibody in cutaneous T-cell lymphoma (CTCL) patients.36 Toxicity was minimal, but only transient decreases in circulating leukemic cells in CLL patients and a few short partial lasting responses (PRs) in CTCL were observed. The low response rate could be attributed to rapid clearance of IT after binding to excess antigen circulating in serum.

Vitetta and colleagues altered the ricin A chain prior to antibody conjugation to reduce nonspecific toxicity. They used deglycosylated A chain, which decreased the nonspecific hepatic uptake of A chain. An initial trial in 15 refractory B-cell NHL patients used fragment antigen binding (Fab)-RFB4-dgA, in which deglycosylated A chain was attached to the Fab’ fraction of a murine anti-CD22 antibody (Table 18–2).37 The Fab fraction was used instead of a whole antibody molecule to decrease the potential immunogenicity of the murine antibody and to decrease the incidence of HAMA. Major toxicities included dose-limiting pulmonary edema, expressive aphasia, rhabdomyolysis, and acute renal failure. Other observed adverse reactions included hypoalbuminemia, weight gain, fever, tachycardia, myalgias, anorexia, and nausea. Four of 14 patients developed HAMA and/or human antiricin antibody (HARA). Clinical responses seen in 43 percent of patients were only partial and transient. Although the use of the Fab’ fragment led to a lower incidence of HAMA/HARA, the short half-

<table>
<thead>
<tr>
<th>Immunotoxin</th>
<th>Target Antigen</th>
<th>Disease</th>
<th>Trial Phase</th>
<th>No. of Patients</th>
<th>Schedule</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fab’-RFB4-dgA</td>
<td>CD22</td>
<td>Relapsed B NHL</td>
<td>I</td>
<td>15</td>
<td>IV over 4 h q48h</td>
<td>37</td>
</tr>
<tr>
<td>IgG-RFB4-dgA</td>
<td>CD22</td>
<td>Relapsed B NHL</td>
<td>I</td>
<td>26</td>
<td>IV over 4 h q48h</td>
<td>38</td>
</tr>
<tr>
<td>RFB4-SMPT-dgA</td>
<td>CD22</td>
<td>Relapsed B NHL</td>
<td>I</td>
<td>24</td>
<td>IV over 4 h q48h</td>
<td>39</td>
</tr>
<tr>
<td>RFB4-SMPT-dgA</td>
<td>CD22</td>
<td>Relapsed B NHL</td>
<td>I</td>
<td>18</td>
<td>IV continuous infusion × 8 d</td>
<td>40</td>
</tr>
<tr>
<td>IgG-HD37-dgA</td>
<td>CD19</td>
<td>Relapsed B NHL</td>
<td>I</td>
<td>23</td>
<td>IV over 4 h every other day × 8 d or continuous infusion × 8 d</td>
<td>41</td>
</tr>
<tr>
<td>Anti-B4-bR</td>
<td>CD19</td>
<td>Refractory B NHL</td>
<td>I</td>
<td>25</td>
<td>IV 1-hour infusion daily × 5 d</td>
<td>43</td>
</tr>
<tr>
<td>Anti-B4-bR</td>
<td>CD19</td>
<td>Refractory B NHL</td>
<td>I</td>
<td>34</td>
<td>IV continuous infusion × 7 d</td>
<td>44</td>
</tr>
<tr>
<td>Anti-B4-bR</td>
<td>CD19</td>
<td>Post-ABMT adjuvant B NHL</td>
<td>II</td>
<td>49</td>
<td>IV continuous infusion × 7 d</td>
<td>45</td>
</tr>
<tr>
<td>Anti-B4-bR</td>
<td>CD19</td>
<td>Post-ABMT adjuvant B NHL</td>
<td>III</td>
<td>82</td>
<td>IV continuous infusion × 7 d</td>
<td>46</td>
</tr>
<tr>
<td>DAB466L-2</td>
<td>CD25</td>
<td>Refractory NHL, HD, CLL, CTCL</td>
<td>I</td>
<td>18</td>
<td>IV bolus daily × 11 doses over 18 d</td>
<td>48</td>
</tr>
<tr>
<td>DAB388L-2</td>
<td>CD25</td>
<td>Refractory NHL, HD, CTCL</td>
<td>I</td>
<td>73</td>
<td>IV bolus × 5 d every 21 d</td>
<td>49</td>
</tr>
</tbody>
</table>

Fab = fragment antigen binding; dgA = deglycosylated ricin A chain; NHL = non-Hodgkin’s lymphoma; IV = intravenously; Ig = immunoglobulin; bR = blocked ricin; ABMT = autologous bone marrow transplantation; IL = interleukin; HD = Hodgkin’s disease; CLL = chronic lymphocytic leukemia; CTCL = cutaneous T-cell lymphoma.
life of this small immunotoxin may have contributed to an inferior response rate. In a subsequent phase I trial, IgG-RFB4-dgA immunotoxin was used, employing the whole antibody instead of the Fab fragment. Although the half-life of this IT was longer, the response rate was not improved, and the toxicities and incidence of HAMA/HARA were comparable to Fab'-RFB4-dgA.

Another modification to improve the pharmacokinetics and biodistribution of an IT used a hindered disulphide bond (SMPT) as a linker between an antibody and a toxin. RFB4-SMPT-dgA immunotoxin consisted of an IgG1 mouse anti-CD22 monoclonal antibody coupled to chemically deglycosylated ricin A chain (dgA). Twenty-four patients with refractory B-cell NHL received 2 to 12 doses of IT infused via central venous catheter every 48 hours. The main DLT was vascular leak syndrome (VLS), characterized by a decrease in serum albumin with a progressive increase in body weight due to fluid retention. Two patients died from pulmonary edema as a manifestation of severe VLS. Other adverse reactions included myalgias, nausea, anorexia, transient aphasia, mild neutropenia, and thrombocytopenia. No hepatotoxicity and minimal bone marrow toxicity were observed. At 1 month after IT treatment there was 1 CR and 5 PRs. Responses were observed in lymph nodes and spleen and occurred rapidly within the first week of treatment, but they were not durable. There was a significant association between clinical response, peak levels of immunotoxin, serum half-life, AUC, and low tumor burden. In an attempt to maintain constant blood concentrations of IT and to improve the therapeutic index, IgG-RFB4-SMPT-dgA was administered by continuous infusion over 8 days to 18 patients with refractory B-cell lymphoma. The trial did not show any advantage to continuous infusion over intermitent bolus administration, with similar clinical responses, toxicity, and immunogenicity.

The IgG-HD37-dgA IT is comprised of deglycosylated A chain conjugated to a murine anti-CD19 antibody, HD37. In a phase I trial, 23 patients received intermittent bolus infusion and 9 patients were treated with continuous infusion over the same 8-day period. There was no obvious advantage to either schedule. As before, the DLT was VLS. A novel toxicity of delayed acrocyanosis with reversible superficial distal digital skin necrosis was described. One patient achieved a CR sustained for more than 40 months, whereas 2 additional patients had PRs.

Expression of a given target antigen can be focal within a tumor or can change during the natural history of a tumor. To overcome this phenotypic heterogeneity of lymphomas, investigators have used a cocktail of ITs directed against different determinants. Combotox is a 1:1 mixture of two ITs prepared from deglycosylated ricin A chain conjugated to mAbs directed against CD22 (RFB4-dgA) and CD19 (HD37-dgA). In a phase I trial, 22 patients with refractory B-cell malignancies expressing CD19 and CD22 were treated with an 8-day continuous infusion of Combotox. Patients with even small numbers of circulating tumor cells (> 50 per mm³) tolerated Combotox well. In contrast, patients without circulating tumor cells (< 50 per mm³) had unpredictable clinical courses with significant toxicity and 2 treatment-related deaths from hemolytic-uremic syndrome. Possibly, the formation of IT aggregates could cause occlusion of small vessels with resulting toxicity. Clinical responses observed with Combotox were mostly partial and transient. Results of the study did not support the use of a mixture of antibodies since it increased toxicity but not efficacy as compared to the separate use of ITs.

**Blocked Ricin Immunotoxin**

Although A chain–based toxins do not undergo nonspecific binding, the ability of the toxin to translocate across the cell membrane is diminished in the absence of B chain, thereby decreasing toxin potency. To minimize nonspecificity but to retain cytotoxicity of ricin-based ITs, a modified ricin molecule was designed with the binding domain of the B chain blocked by the attachment of natural ligands. In anti-B4-bR, blocked ricin is linked to the anti-B4 (CD19) antibody. The CD19 antigen is a highly B-cell lineage–restricted antigen and is not present on hematopoietic stem cells.

In a phase I trial, 25 patients with refractory B-cell malignancies (23 NHL, 1 CLL, 1 ALL) received anti-B4-bR by daily 1-hour infusions for 5 consecutive days. Therapeutic serum levels of anti-B4-bR, as
determined from in vitro cytotoxicity studies, were transiently obtained at doses greater than 20 µg per kg per day. The maximal tolerated dose (MTD) was reached at 50 µg per kg per day. The DLT was defined by reversible grade 3 elevations in hepatic transaminases without impaired hepatic synthetic function. Minor toxicities included transient hypoalbuminemia, anemia, thrombocytopenia, and fevers. Notably, capillary leak syndrome, which was the DLT in ricin A chain immunotoxin trials, occurred in only 1 patient treated with a dose above the DLT. In 9 patients HAMA and HARA were detected. One CR, maintained for over 21 months, and 2 PRs were observed.

To overcome the suboptimal pharmacokinetics of the bolus schedule, a second phase I dose-escalation trial was undertaken with anti-B4-bR administered by 7-day continuous infusion.44 Thirty-four patients (26 NHL, 4 ALL, 4 CLL) were enrolled. Within 96 hours, cytotoxic serum levels of anti-B4-bR greater than 1.0 nmol per L were achieved, and these were sustained for the duration of the 7-day infusion. The MTD was reached at 50 µg per kg per day × 7 days. The DLT again was defined by reversible grade 4 increases in hepatic transaminases and grade 4 thrombocytopenia. The nonspecific toxicities of therapy including fevers and myalgias were enhanced by continuous infusion. Manifestations of capillary leak syndrome were observed including edema, weight gain, and dyspnea without pulmonary edema or pleural effusions. Twenty-five patients developed HAMA and/or HARA after a first course of anti-B4-bR therapy. Unfortunately, the improved pharmacokinetic profile with continuous infusion as compared with bolus administration did not translate into a higher response rate. Responses included 2 CRs in patients with the lowest tumor bulk and five PRs of short duration.

Because of the relatively low response rates achieved with immunotoxins in patients with bulky tumors, investigators have explored the role of ITs in the minimal residual disease state. Anti-B4-bR was investigated as an adjuvant treatment for patients in CR after autologous bone marrow transplantation (ABMT) for relapsed NHL. Phase I and II trials demonstrated the safety and feasibility of this treatment approach as well as potential efficacy.45 In the phase II trial, 49 patients in CR after ABMT for B-cell NHL received anti-B4-bR as a 7-day continuous infusion every 2 weeks for up to three courses. Although 75 percent of the patients remained in CR after a median follow-up of 15 months, there was no control group of patients who did not receive adjuvant therapy.46 Thus, a large phase III study of anti-B4-bR post-ABMT was conducted.47 The promising efficacy of anti-B4-bR in the phase II setting was not confirmed in the larger randomized trial. After a median follow-up of 2 years, the estimated 2-year disease-free survival was 42 percent for patients treated with anti-B4-bR compared with 62 percent for patients in the observation-alone group. The trial was terminated early when it was determined that further follow-up would be highly unlikely to demonstrate an advantage of anti-B4-bR therapy.

**Diphtheria Toxin-Based Fusion Protein**

Another group of clinical studies employed a fusion toxin directed against interleukin (IL)-2 receptor (CD25)-bearing lymphoid neoplasms (see Table 18–2). The high-affinity form of the IL-2 receptor is expressed in a variety of hematologic malignancies, including Hodgkin’s disease (HD), non-Hodgkin’s lymphoma, and hairy cell leukemia, but not on normal tissues. DAB486IL-2 is a genetically engineered fusion protein isolated from the extract of *Escherichia coli*. It is a product of the recombinant gene created by replacing the diphtheria toxin-receptor binding domain with a synthetic gene encoding IL-2. In a phase I trial, 18 patients with chemotherapy-resistant IL-2 expressing hematologic malignancies (9 NHL, 3 HD, 5 CLL, 1 CTCL) were treated with DAB486IL-2 by daily intravenous bolus for 11 doses over 18 days.48 The MTD was reached at a total dose of 1 mg per kg and was defined by reversible elevations of hepatic transaminases. Other mild side effects included nausea, rash, transient increases in serum creatinine, proteinuria, chest tightness, and fever. Capillary leak syndrome was not observed. The serum half-life of DAB486IL-2 was short. After therapy, 50 percent of patients developed an antibody to diphtheria toxin or DAB486 IL-2. One CR, lasting more than 18 months, and 2 PRs, lasting 5 and longer than 12 months, respectively, were observed.
To overcome some of the drawbacks of DAB486IL-2, a newer version of fusion protein, DAB389IL-2, was designed. This fusion toxin has a lower molecular weight, improved affinity for the IL-2 receptor, a 10-fold increase in potency, and an extended half-life. In a phase I trial, 73 patients (17 NHL, 35 CTCL, 21 HD) received up to eight courses of DAB389IL-2 given as a short intravenous infusion daily for 5 days, repeated every 21 days. The DLT was asthenia. Other new toxicities included VLS, rashes, hypersensitivity reactions, and hypotension. Antibodies to DAB389IL-2, diphtheria toxin, and IL-2 were measured prior to treatment and after subsequent courses of therapy. Thirty-eight percent of patients had detectable titers of antibody at baseline and 92 percent had detectable titers after two courses. The presence of antibodies did not preclude a response, suggesting that some of the antibodies might not be neutralizing. One patient with intermediate-grade NHL achieved a durable CR (39+ months), and there were 5 CRs and 8 PRs in the CTCL group, with 2 more PRs in NHL patients.

Conclusion

Despite the potent in vitro cytotoxicity of ITs and their theoretic advantages over unconjugated antibodies for the therapy of NHL, these agents have limited use in vivo. The foremost obstacle to their successful administration has been toxin-related DLT. In addition, tumor cells may have some degree of intrinsic resistance to the chosen toxins. Overall, efficacy has been unimpressive when compared with that of the most effective unconjugated antibodies, and the toxicity and cumbersome administration schedules have been troublesome. In the absence of newly identified less immunogenic toxins and reduced nonspecific toxicities of the conjugates, the role of ITs in the therapy of NHL is limited.

RADIOIMMUNOTHERAPY OF NON-HODGKIN’S LYMPHOMA

Conjugation of an antibody with a radioisotope offers the potential for an additional therapeutic effect. Radiolabeled antibodies generally possess all of the antitumor potential of an unlabeled antibody and also deliver radioactivity to cells expressing the targeted antigen in a fairly specific fashion. This approach has been evaluated in NHL using several antibodies and primarily two isotopes: iodine-131 and 90yttrium. The DLT of radioimmunotherapy is myelosuppression, and patients with limited bone marrow reserve may have more toxicity. The potential for long-term side effects, including secondary malignancies, has been a concern for some clinicians regarding the use of this treatment modality. Nonetheless, recent information has suggested that the potential added complications involved in using a radiolabeled antibody over an unlabeled antibody may be justified due to higher response rates and demonstrated efficacy of radiolabeled products in some patients, in which similar unlabeled antibodies were ineffective. Some promising efforts have been undertaken using high-dose radioimmunotherapy with stem cell support (to allow for dose escalation and potential augmented antitumor effect) or in combinations of chemotherapy with radioimmunotherapy.

Most trials of radioimmunotherapy of lymphoma have used murine antibodies. The logistic issues and technical effort required to construct a chimeric or humanized agent can be substantial, and the widespread use of single- or few-dose regimens (rather than extended, multidose schedules) in radioimmunotherapy makes immunogenicity less of a problem. Some level of immunogenicity (and resultant rapid clearance of unbound, circulating drug) may be desirable to minimize nonspecific toxicity. Potential problems with murine antibodies include the occurrence of HAMA. The appearance of HAMA could preclude retreatment due to associated infusion reactions or other toxicity and may render the agent ineffective due to unfavorable pharmacokinetics. This issue has been not evaluated systematically, and the clinical significance of a HAMA response is not clearly established for most agents.

The choice of isotope in radioimmunotherapy also potentially impacts efficacy, toxicity, and convenience. Iodine-131 and 90yttrium have been the most commonly explored isotopes in this setting. Iodine-131 has been used for many years as part of therapy for thyroid disorders and is more familiar to those working in nuclear medicine. It is relatively inexpensive and available and can be readily conjugated to...
an antibody. Its emissions (γ and β) allow for imaging and dosimetry, while emitting a lower β energy across a path length of 0.8 to 1.0 mm. Uptake in the thyroid gland results in the potential for hypothyroidism, which can usually be blocked by potassium iodide oral supplementation around the time of therapy. Although it can be administered on an outpatient basis in most areas of the United States, radiation safety guidelines in some areas require that patients are treated in the hospital and observed for 2 to 3 days until levels decline. 

90Yttrium is being used with increasing frequency and can be administered on an outpatient basis in all settings. It emits β energy that is roughly five times greater than that of iodine-131, with a resultant path length of about 5 mm. This could be beneficial in larger tumors if impaired vascularity is present, but it also might be more toxic to normal tissues that may be affected by this energy. This isotope cannot be assessed directly by imaging as it does not possess a γ emission. Thus, indium-111 must be used as a surrogate if dosimetry is required. 90Yttrium possesses a shorter half-life than does iodine-131 (64 hours versus 8 days), but free isotope may be taken up and retained by bone. Yttrium is not covalently bound to an antibody but must be attached via a chelating agent. The relative merits of these two isotopes for NHL therapy are a subject of much debate, with theoretic and practical advantages to both, but the clinical data have not established clearly the superiority of one or the other for most situations. Copper-67 and several other isotopes also are under investigation as radioimmunoconjugates for lymphoma therapy.

Initial efforts with radioimmunotherapy for lymphoma were undertaken by Rosen and colleagues using an iodine-131-labeled murine mAb (T101) in CTCL.50 Transient responses were observed with minimal toxicity, although plasmapheresis was required to reduce HAMA titers. This agent also has been evaluated in a 90yttrium-labeled form.51 Subsequent work has focused primarily on B-cell lymphomas. Similar to the approach with unlabeled antibodies, 90yttrium conjugated anti-idiotype antibodies have been assessed, although this target required development of a specific agent for each individual patient.52,53 Hence, further attention has turned toward pan-B-cell antibodies. CD37 (MB-1) has been targeted in two studies that demonstrated evidence of tumor targeting and response, while establishing DLT of bone marrow suppression and cytopenias.54,55 Czuczman and colleagues evaluated the CD21 antigen as a target, using the iodine-131-labeled antibody OKB7, also suggesting acceptable toxicity and reduction of disease burden in some patients.56 Efforts in HD have included 90yttrium-labeled antiferritin, studied both as a single agent57,58 and in combination with high-dose chemotherapy and stem cell transplantation,59 with some evidence of therapeutic effectiveness.

**Lym-1- and LL2-Radiolabeled Antibodies**

The Lym-1 murine antibody targets the HLA-DR antigen60 and has been extensively studied as an iodine-131-labeled agent in a number of trials led by DeNardo and colleagues61,62 that predominantly evaluate patients with aggressive NHL (Table 18–3). Acceptable toxicity has been observed, and about 50 percent of patients receiving therapy have demonstrated clinical response. The relatively short-duration (several months) responses may reflect the growth rate of these aggressive tumors. Nonetheless, this agent warrants further evaluation, and modifications in the dosing regimen are currently under study. Lym-1 also is being studied with a copper-67 label, allowing for better imaging and potentially improved tumor-radiation doses, although the limited availability of this isotope ultimately may interfere with its widespread use.63

Goldenberg and colleagues have led in the development of radiolabeled versions of the LL2 antibody, which targets CD22, in both iodine-131 and 90yttrium forms, also demonstrating clear activity in a refractory group of NHL patients (see Table 18–3).64,65 This differs from other agents in two important ways. The humanized version (epratuzumab) of this radiolabeled agent is available, making repeated dosing regimens more practical, given the lower risk of immunogenicity. Also, LL2 is internalized on binding to CD22, allowing internalization of the antibody and its radioconjugate into the cell. This may allow 90yttrium, in particular, which is not dehalogenated by cells as is iodine-131, to reside in the tumor tissue for
a more extended period of time, potentially providing greater radiation doses. Studies have employed this radioimmunoconjugate in nonmyeloablative doses as well as higher doses requiring stem cell support, both with promising results.

Radiolabeled Antibodies Targeting CD20

CD20-directed compounds have been the most successfully developed radioimmunotherapeutic agents to date. This target is highly expressed on B-cell lymphomas and is not generally modulated or shed. Kaminski and colleagues have led in the development of iodine-131 anti-B1 (CD20) antibody (tositumomab), demonstrating safety and effectiveness primarily in relapsed low-grade and transformed low-grade NHL (see Table 18–3). They have established a dosing regimen employing two infusions of unlabeled antibody to saturate binding sites in the spleen and preferentially target tumor tissue, followed by radiolabeled antibody. The first (dosimetric) injection uses 5 mCi of iodine-131 to obtain gamma camera images and total-body counts at three time points over 1 week. This allows calculation of radiation clearance and a determination of the mCi dose to be administered in the second (therapeutic) injection, given 1 week later. Patient-specific characteristics such as tumor burden and spleen size affect total-body residence time, so dosimetry is required to determine the injected dose necessary to achieve the total-body radiation exposure desired (65 or 75 cGy, depending on platelet count) with this agent. The long-term follow-up includes 59 patients with refractory or relapsed NHL, predominantly with low-grade or transformed histologies, demonstrating an overall response rate of 71 percent, with 34 percent CRs. For the 42 responders, progression-free survival was 12 months, with CRs lasting a median of 20.3 months and as long as 5.7+ years. Toxicity has included reversible myelosuppression, with low rates of infection or transfusion support, as well as fatigue, fever, and nausea in a minority of patients. These results have been confirmed in a multicenter phase II study with comparable response rates and durations. Responses of HAMA occur in 5 to 10 percent of relapsed NHL patients receiving this agent. A pivotal trial has been conducted in 60 patients, comparing the response obtained in each patient from the immediate prior chemotherapy with the response obtained to the iodine-131 anti-B1 antibody. This demonstrated that higher response rates and longer durations were observed more commonly with radioimmunotherapy compared with the preceding chemotherapy.

In a parallel fashion, Press and colleagues have explored high-dose radioimmunotherapy, using myeloablative doses of iodine-131 anti-B1 antibody, either alone or in combination with chemotherapy, followed by autologous stem cell rescue. Their experience suggests that this type of regimen may be administered safely and can result in remission durations that compare favorably with historic controls. The high doses of radioactivity employed (up to 785 mCi of iodine-131) make this approach impractical for widespread use due to logistic issues with radiation safety; however, high-dose radioimmunotherapy may prove to be a valuable adjunct to standard high-dose chemotherapy approaches.

The murine parent antibody of rituximab has been employed in a yttrium-radiolabeled version...
(Y2B8 or $^{90}$yttrium ibritumomab tiuxetan) in studies led by Witzig and colleagues. These studies were conducted predominantly in patients with low-grade NHL. This agent also is administered in a two-dose fashion, employing two injections of unlabeled antibody (rituximab) followed by labeled antibody administered 1 week later. The use of $^{90}$yttrium allows for outpatient administration in all settings; any dosimetry requires the use of indium-111 as a surrogate. Thus, a dosing regimen has been established without dosimetry, allowing for administration on a millicurie-per-kilogram basis. Initial results showed an overall response rate of 67 percent in 34 patients with low-grade NHL (26% CRs), with comparable response rates in a smaller number of aggressive NHL patients. Immunogenicity is rare with this agent, with only 1 patient developing an anti-antibody response, presumably due to the fact that the cold infusions consist of a chimeric rather than a murine antibody. Otherwise, toxicities include infusion reactions and myelosuppression. Further studies have evaluated Y2B8 in a randomized trial with rituximab, as well as in patients with disease that is refractory to rituximab, suggesting improved efficacy relative to the unlabeled antibody.

Despite these potential problems, radioimmunotherapy remains a promising modality for NHL therapy, and these compounds have resulted in the highest single-agent response rates among lymphoma treatments. Iodine-131 anti-B1 antibody also has been explored as initial therapy for low-grade NHL. Although HAMA responses are frequent, this 1-week therapy has resulted in a 97 percent response rate, with 71 percent complete remissions and a median progression-free survival not yet reached at 3 years. Sequential combination with fludarabine monophosphate can reduce immunogenicity, with the potential for added therapeutic benefit and with manageable initial toxicity.

A number of sequential combinations are in clinical trials, both in indolent and aggressive NHL. Although concurrent administration of radiolabeled antibodies with chemotherapy is more complicated than with naked antibodies, due to overlapping myelotoxicity, this approach warrants evaluation to take advantage of possible synergy observed in vitro. However, as in the development of unlabeled antibodies and immunotoxins, it is only through randomized trials that we can establish fully the role of these promising new agents for NHL.

**Conclusion**

At the present time, there are certain limitations to the use of radioimmunotherapy in patients with NHL. Patients with cytopenias may not be candidates due to the toxicity profile, which has correlated generally with pretherapy platelet counts. For similar reasons, concerns remain in patients with prior high-dose chemotherapy and stem cell rescue due to their limited marrow reserve; most trials have excluded these patients. Extensive bone marrow involvement may result in higher delivery of the radioimmunoconjugate to the marrow, leading to a higher radiation dose and more toxicity. Studies are under way to evaluate this issue and the potential need for dose modifications in this setting. The possibility of long-term complications remains, such as secondary malignancies, although the limited information currently available does not demonstrate higher rates to date relative to similar patient populations with lymphoma and prior chemotherapy.

**REFERENCES**


