The late 20th century saw developments in hybridoma and recombinant DNA technology that enabled a new era of monoclonal antibody therapy for non–Hodgkin’s lymphoma (NHL). Over the past decade, the success of the anti-CD20 antibody rituximab has resulted in its use in virtually all patients with B cell NHL and has heralded an explosion in research and development. In addition to targeting novel antigens, antibodies are being engineered to alter host immune responses and carry payloads of radiation, drugs, or toxins. We review some of the challenges in monoclonal antibody therapy and explore their use in clinical trials to date.

**Target Antigens**

The success of antibody therapy depends both on the properties of the antibody and those of the antigen. The ideal target antigen should be expressed preferentially on neoplastic cells, it should not be modulated from the cell surface or secreted into the circulation, and it should have a critical biological function. Although numerous potential targets have been identified, it is rare that they meet all of these goals. The considerable success of some monoclonal antibodies, however, reminds us that although it is important to have a firm understanding of the ideal target, less than perfect agents often exceed expectations. A selected list of targets is discussed below.

**Antitumor Activity of Monoclonal Antibodies**

Four main mechanisms have been implicated in the elimination of cancer cells by monoclonal antibodies: complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), direct cytotoxicity via antigen signaling, and delivery of toxin or radiation to the target cells. The relative importance of these pathways may depend on the tumor cell type, the properties of the antibody and the target, and the specific clinical setting (e.g., single agent versus combination with chemotherapy or cytokines).

Both CDC and ADCC depend on the interaction of the host immune system with antibody on targeted cells. Multiple preclinical studies have shown that alterations in the protein sequence and carbohydrate make-up of the immunoglobulin constant region (Fc) may alter this interaction and new antibodies have incorporated some of these concepts (1–4).

The ability of a monoclonal antibody to directly induce a cell to undergo apoptosis or stop proliferating depends in part on the properties of the target antigen. Unlike CDC and ADCC, direct cytotoxicity is independent of the host immune system.

Finally, monoclonal antibodies may be used to deliver payloads of drugs, toxins, or radiation directly to targeted cells. To date, this approach has been most successful using anti-CD20 radioimmunoconjugates. By carrying toxic payloads directly to malignant cells, immunoconjugates may be capable of overcoming issues such as antigen modulation or resistance to CDC or ADCC.

**Enhancing Response to Antibody Therapy**

Several attempts have been made to improve on the current successes of monoclonal antibodies. In some cases, this has involved the engineering of antibodies that are better at initiating ADCC or CDC. Another approach has been to optimize the host response by administering immunostimulatory cytokines.
The CD20 antigen is expressed exclusively on normal and malignant B cells (5). Although the absence of CD20 does not seem to have any deleterious effects on B cell function, it is a good target antigen because it is tightly bound to the membrane with little modulation, it is present in low concentrations in the serum, and it likely has an important role in B cell activation and regulation of cell cycle (5–8).

**Anti-CD20 Antibodies**

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**Rituximab.** Rituximab is a chimeric IgG1 κ monoclonal antibody that recognizes a discontinuous epitope on the extracellular loop of CD20 with high affinity (9, 10). It was approved by the Food and Drug Administration in 1997 for the treatment of relapsed or refractory CD20-positive indolent or follicular B cell NHL. As discussed above, rituximab induces target cell death through ADCC, CDC, as well as by direct cytotoxicity. Several clinical trials have shown the efficacy of rituximab in multiple clinical settings. Responses to single agent rituximab have ranged from 72% in previously untreated patients with follicular lymphoma, to 48% in patients with relapsed indolent lymphoma, to 40% in patients with relapsed lymphoma who had previously received rituximab (11–13).

**Chemotherapy.** The combination of rituximab plus chemotherapy has produced impressive results. With 9 years of follow-up in the first phase II trial to evaluate the safety and efficacy of rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) in patients with indolent B cell NHL, Czuczman et al. reported a median time to progression of 82.3 months with no long-term sequelae (14). These impressive results have been confirmed in phase III trials comparing rituximab plus chemotherapy to chemotherapy alone (Table 1; refs. 15–17). It is important to note that despite significant improvements in response rate and...
remission duration, the addition of rituximab to chemotherapy in patients with indolent lymphoma still has not shown an overall survival benefit. Although additional follow-up may show an effect on overall survival, a potential explanation for this phenomenon is that those patients randomized to non-rituximab-containing regimens in phase III trials almost certainly received rituximab later in the course of their therapy.

The results of phase III trials evaluating chemoimmunotherapy in high-grade lymphoma have been equally encouraging. The Groupe d’Etude des Lymphomes de l’Adulte evaluated R-CHOP versus CHOP in previously untreated patients, ages 60 to 80 years, with diffuse large B cell lymphoma (DLBCL; ref. 18). With a median follow-up of 5 years, progression-free survival (PFS) rate (54% versus 30%; P < 0.0001), and overall survival rate (58% versus 45%, P < 0.0073) were significantly higher in the R-CHOP group (19). The superiority of R-CHOP in younger patients was also shown in the MabThera International trial (20). With a median follow-up of 34 months, the primary end point (3-year event-free survival), and the secondary end point (3-year overall survival) were both significantly improved by the addition of rituximab to chemotherapy (79% versus 59%, P < 0.0001, and 93% versus 84%, P = 0.0001, respectively). On the basis of these two trials, the addition of rituximab to CHOP has become the standard of care for all patients with untreated DLBCL.

**Rituximab maintenance.** Given the limited success and poor tolerability of IFN, the concept of maintenance therapy for indolent NHL temporarily fell out of favor. The success of rituximab both at prolonging remissions and at reproducing responses at the time of relapse, as well as its tolerability, reintroduced the possibility that maintenance therapy could be beneficial. Four phase III trials have now supported this theory (Table 2; refs. 21–24). It is clear, based on these trials, that prolonged therapy with rituximab has the potential to increase responses and prolong remissions in patients with indolent lymphoma. Remaining questions include the optimal duration and dosing schedule, and importantly, the potential for long-term adverse effects. Answers to these questions may come from longer follow-up of these important trials. To date, no study has shown a benefit for rituximab maintenance in aggressive lymphomas.

**Rituximab toxicity.** Despite overwhelming data in its favor, rituximab is not without toxicity. Although anaphylaxis is rare, infusion-related symptoms are common and can vary from mild nuisance to life-threatening. These side effects are partially related to the number of tumor cells circulating in the peripheral blood and can usually be avoided by premedication with acetaminophen, diphenhydramine, or corticosteroids. Similarly, rituximab-induced destruction of a high volume of tumor cells has been reported to cause tumor lysis syndrome such that prophylaxis with allopurinol or rasburicase may be indicated (25, 26).

The other major category of side effects related to rituximab is immunosuppression. Two types of neutropenia have been noted following rituximab administration: an early onset neutropenia of little clinical significance and a delayed neutropenia occurring 2 to 4 months following rituximab administration (27). More relevant to the majority of patients receiving rituximab is the prolonged B-lymphopenia following rituximab. In an early phase II trial, patients treated with rituximab had a rapid and profound reduction in circulating B lymphocytes that lasted for ~6 months (28). Although mean immunoglobulin levels remained stable, owing to the fact that plasma cells are CD20-negative, more than one-third of patients had a >20% decrease in immunoglobulins. Somewhat surprisingly, serious viral infections including cytomegalovirus, hepatitis B, Varizella-Zoster virus, and JC virus among others have been described—the Food and Drug Administration recently issued a warning regarding the risk of progressive multifocal leukoencephalopathy in patients with autoimmune disorders being treated with rituximab (29–31). Although rituximab seems safe for the majority of patients, it is a relatively new drug and it is important to remain vigilant for rare and previously unnoticed complications.

**Novel anti-CD20 antibodies.** Despite the positive results seen in the rituximab trials, there remains room for improvement. Rituximab is a chimeric human-mouse antibody: it possesses murine segments for antigen binding but a human Fc segment capable of binding to host effector cells. Humanized

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**Table 3. Radioimmunotherapy trials**

<table>
<thead>
<tr>
<th>Study author</th>
<th>Lymphoma subtype</th>
<th>Nature of trial</th>
<th>Prior treatment</th>
<th>Treatment</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fisher et al. (74)</td>
<td>Low-grade, follicular, or transformed</td>
<td>Phases I-III*</td>
<td>Yes</td>
<td>131I tositumomab</td>
<td>RR, 56%; CR, 30%; 5-y PFS, 17%</td>
</tr>
<tr>
<td>Kaminski et al. (75)</td>
<td>Follicular</td>
<td>Phase II</td>
<td>No</td>
<td>131I tositumomab</td>
<td>RR, 95%; CR, 75%; 5-y PFS, 59%</td>
</tr>
<tr>
<td>Kaminski et al. (76)</td>
<td>Low-grade, follicular, or transformed</td>
<td>Phase II</td>
<td>Previous response to 131I tositumomab</td>
<td>RR, 58%; CR, 25%; mPFS, 11.8 mo.</td>
<td></td>
</tr>
<tr>
<td>Witzig et al. (77)</td>
<td>Low-grade, follicular, or transformed</td>
<td>Phase III</td>
<td>Yes</td>
<td>90Y ibritumomab</td>
<td>RR, 80% vs. 56% (P = 0.002)</td>
</tr>
<tr>
<td>Leonard et al. (34)</td>
<td>Follicular</td>
<td>Phase II</td>
<td>No</td>
<td>Fludarabine 1/131I tositumomab</td>
<td>RR, 100%; CR, 86%; 5-y PFS, 60%</td>
</tr>
<tr>
<td>Press et al. (35)</td>
<td>Follicular</td>
<td>Phase II</td>
<td>No</td>
<td>CHOP 1/131I tositumomab</td>
<td>RR, 91%; CR, 69%; 5-y PFS, 67%</td>
</tr>
</tbody>
</table>

Abbreviations: mPFS, median progression-free survival (time from dosimetric dose to progression or death); R-4, rituximab (375 mg/m²) weekly for 4 wk.

*Integrated analysis of the five clinical trials resulting in Food and Drug Administration approval of 131I tositumomab.
† Fludarabine (25 mg/m²) given thrice for 5 d every 5 wk followed by 131I tositumomab 6 to 8 wk later in responders.
‡ CHOP given six times for 21 d followed by 131I tositumomab in responders.
Radioimmunotherapy. Radioimmunotherapy takes advantage of the specificity of monoclonal antibodies to deliver radiation directly to tumor cells. In so doing, normal tissues are spared the toxicity associated with external beam radiation while cancer cells are exposed to a cross-fire of α or β particles. Radioimmunotherapy produces very few side effects except in those situations in which bound target cells lie in close proximity to normal cells, as in the bone marrow. Indeed, myelosuppression is the principal significant side effect of radioimmunotherapy, and is often prolonged in heavily pretreated patients or those with extensive bone marrow involvement. To date, two agents have been approved by the Food and Drug Administration for the treatment of relapsed, rituximab-refractory follicular lymphoma: 90Y ibritumomab tiuxetan and 131I tositumomab. Several phase II trials which show the safety and efficacy of both agents have been published over the past decade (Table 3). As would be expected, patients treated with radioimmunotherapy earlier in the course of their disease had higher response rates.

In an attempt to capitalize on the higher response rates of early treatment, and spare patients the toxicity of aggressive chemotherapy, radioimmunotherapy consolidation has been administered to patients with previously untreated follicular lymphoma following chemotherapy in two phase II trials (34, 35). This approach was not only safe and efficacious, it also reduced the tumor burden within the marrow, thereby allowing patients who would not otherwise have been candidates for radioimmunotherapy to benefit from its effects. Based on these results, a Southwest Oncology Group/Cancer and Leukemia Group B intergroup phase III trial to compare CHOP-131I tositumomab to R-CHOP in previously untreated follicular lymphoma was designed and is currently under way.

The most significant concern related to the use of radioimmunotherapy is the potential for secondary malignancies, specifically acute myeloid leukemia and myelodysplastic syndrome. Because lymphoma commonly involves the bone marrow, hematopoietic precursor cells are likely to receive some cross-fire radiation from antibody bound to neighboring cells. The result is reversible myelosuppression in the short-term but may potentially result in myelodysplastic syndrome/acute myeloid leukemia several years later, as can be seen with external beam radiation. Combined analyses from seven phase I and II trials with 131I tositumomab reported an overall annual incidence of myelodysplastic syndrome/acute myeloid leukemia of 1.4% per year (36). Although this is in keeping with the expected rates in patients receiving chemotherapy, the follow-up reported may be too short to adequately capture the true complication rate. Longer follow-up of studies with strict criteria for reporting myelodysplastic syndrome/acute myeloid leukemia will be needed before the true incidence of secondary malignancies is known, particularly in studies in which radioimmunotherapy is incorporated into an initial treatment strategy.

Anti-CD22 antibodies. CD22 is a member of the immunoglobulin superfamily and is exclusively expressed on the surface of mature and malignant B cells. By virtue of its immunoreceptor tyrosine-based inhibitory motifs, CD22 negatively regulates B cell receptor signaling (37, 38). Unlike CD20, CD22 is rapidly modulated from the cell surface but is not present in soluble form. Epratuzumab is a humanized IgG1 capable of eliciting ADCC and direct cytotoxicity (39). In two phase I/II studies, it has shown both safety and moderate efficacy (40, 41). In an attempt to capitalize on the tolerability and potentially different mechanisms of action of rituximab and epratuzumab, two phase II trials were conducted to evaluate the safety and efficacy of the simultaneous full-dose administration of both monoclonal antibodies (42, 43). Again, therapy was well-tolerated and produced responses in patients with relapsed indolent lymphoma as well as relapsed DLBCL. Although encouraging, without a phase III trial comparing the combination to single agent rituximab, no definitive conclusions can be drawn. Furthermore, the proportion of rituximab-naive patients, who made up the bulk of patients in these two trials, is rapidly shrinking.

Table 4. Trials of antibodies against other targets

<table>
<thead>
<tr>
<th>Study author</th>
<th>Target antigen</th>
<th>Lymphoma subtype</th>
<th>Nature of trial</th>
<th>Prior treatment</th>
<th>Treatment</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leonard et al. (40)</td>
<td>CD22</td>
<td>Follicular lymphoma</td>
<td>Phase I/II</td>
<td>Yes</td>
<td>Epratuzumab</td>
<td>RR, 43%</td>
</tr>
<tr>
<td>Leonard et al. (41)</td>
<td>CD22</td>
<td>Aggressive NHL</td>
<td>Phase I/II</td>
<td>Yes</td>
<td>Epratuzumab</td>
<td>RR, 10%</td>
</tr>
<tr>
<td>Leonard et al. (42)</td>
<td>CD20 + CD22</td>
<td>B cell NHL</td>
<td>Phase II</td>
<td>Yes</td>
<td>Epratuzumab + rituximab</td>
<td>Indolent RR, 63%; aggressive RR, 67%</td>
</tr>
<tr>
<td>Strauss et al. (43)</td>
<td>CD20 + CD22</td>
<td>B cell NHL</td>
<td>Phase II</td>
<td>Yes</td>
<td>Epratuzumab + rituximab</td>
<td>RR, 47%</td>
</tr>
<tr>
<td>Forero-Torres et al. (54)</td>
<td>CD40</td>
<td>B cell NHL</td>
<td>Phase I</td>
<td>Yes</td>
<td>SGN-40</td>
<td>RR, 19%</td>
</tr>
<tr>
<td>Czuczman et al. (60)</td>
<td>CD80</td>
<td>Follicular</td>
<td>Phase I/II</td>
<td>Yes</td>
<td>Galiximab</td>
<td>RR, 11%</td>
</tr>
<tr>
<td>Friedberg et al. (61)</td>
<td>CD20 + CD80</td>
<td>Follicular</td>
<td>Phase II</td>
<td>Yes</td>
<td>Galiximab + rituximab</td>
<td>RR, 64%; CR, 17%</td>
</tr>
<tr>
<td>Link et al. (66)</td>
<td>HLA-DR</td>
<td>B cell NHL</td>
<td>Phase I</td>
<td>Yes</td>
<td>Apolizumab</td>
<td>Five patients responded</td>
</tr>
</tbody>
</table>
hypothesized that sequential therapy would be feasible given that the humanized structure of epratuzumab is rarely associated with human anti-human antibody formation. Some subjects in these studies had diseases resistant to other treatments and/or histologies in which anti-CD20 radioimmunotherapy is generally less effective (e.g., aggressive NHL), which suggests the potential utility of this approach.

An additional strategy to improve the efficacy of anti-CD22 antibodies has been to develop a bispecific anti-CD20, anti-CD22 antibody (50). TF3 is a trivalent antibody composed of two hA20 anti-CD20 F(ab) fragments fused to one epratuzumab anti-CD22 F(ab) fragment. Preclinical studies with bispecific antibodies have shown promise and clinical evaluation is awaited.

**Anti-CD40 Antibodies**

CD40 belongs to the tumor necrosis factor receptor family. It is expressed by antigen-presenting cells including normal B cells, B and T cell lymphomas, multiple myeloma, and Reed-Sternberg cells (51). The CD40-CD40 ligand interaction is thought to regulate B cell proliferation, differentiation, and possibly, survival (52). SGN-40 is a humanized IgG1 with high affinity for CD40. In vitro data suggested that it could potentially inhibit proliferation and directly induce apoptosis in B cell lymphoma lines (53). In a recent phase I testing, SGN-40 was well tolerated and produced responses in 3 of 16 patients with NHL, including 2 patients with extensively treated aggressive lymphoma (54, 55).

**Anti-CD80 Antibodies**

CD80 is a surface protein that is transiently expressed on activated B cells and has a role in regulating T cell activity (56–58). CD80 is a good target antigen because it is constitutively expressed in a variety of malignant B cells, including follicular lymphoma, and it has a postulated role in B cell activation (59). Galiximab is a chimeric IgG1 anti-CD80 antibody with immunomodulatory properties that has shown a favorable toxicity profile in >200 patients with psoriasis (Table 4). In a phase I/II trial in patients with relapsed or refractory follicular lymphoma, single-agent galiximab produced no dose-limiting toxicity but showed limited activity (60). A recent phase II trial using the combination of rituximab plus galiximab in patients was significantly more efficacious, but without a control group, it is difficult to draw any conclusions (61).

**Anti–HLA-DR Antibodies**

HLA class II antigens are interesting targets for monoclonal antibodies because they are expressed on B cells throughout differentiation and they play an important role in cell cycling and proliferation. There is significant in vitro evidence that anti-class II antibodies inhibit B cell proliferation and trigger apoptosis, possibly via induction of the Fas/Fas ligand pathway (62–65). Apolizumab (Hu1D10) is a humanized anti–HLA-DR antibody capable of inducing CDC, ADCC, and programmed cell death. Reported toxicities include significant infusion reactions and hemolytic uremic syndrome (66–68). Other anti–HLA-DR antibodies are currently under development (69–71).

**Conclusion**

The development of monoclonal antibodies has dramatically altered cancer care. As a first line agent and in patients with relapsed disease, by itself or in combination with chemotherapy, as a single course or as maintenance therapy over 2 years, rituximab has shown the potential to improve outcomes with manageable toxicity. New antibodies have been engineered to induce stronger CDC and ADCC whereas avoiding production of neutralizing antibodies. Antibodies to novel target antigens have shown tolerability and single-agent activity. New mechanisms of delivering toxins and radiation are in development. The major challenge over the next decade will be to design strategies to systematically evaluate these agents. What is the optimal duration of therapy? Which patients derive the most benefit? Can combinations based on biologically plausible mechanisms and empirical observations improve on current results? A coordinated effort among basic scientists, clinicians, and research groups will be required to realize the full potential of this exciting therapeutic modality.

**References**

Phase I-III Trials of Anti–B Cell Therapy in NHL


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Phase I to III Trials of Anti–B Cell Therapy in Non–Hodgkin’s Lymphoma

Peter Martin, Richard R. Furman, Morton Coleman, et al.


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