Monoclonal antibody therapy with rituximab in combination with standard chemotherapy has improved the survival of patients with advanced-stage follicular lymphoma (FL). A series of next-generation anti-CD20 antibodies may be less immunogenic and have even greater antitumor activity through augmented interactions with host effector mechanisms responsible for tumor cell kill. Additional approaches with patient-specific immunoglobulin idiotype vaccines; novel monoclonal antibodies binding to biologically active cell-surface antigen are also demonstrating early clinical activity. Antibodies targeting radioisotopes, toxins or drugs are also slowly entering clinical trials and practice. Last, allogeneic stem cell transplantation following reduced-intensity conditioning provides graft-versus-tumor immune responses that may be able to control FL and allow this risky but potentially curative treatment option to older patients or those with comorbidities.

Introduction
The past 10 years have witnessed an explosion of immunologically based treatment options for patients with follicular lymphoma (FL), with many more agents poised to enter clinical practice. To date, the most important has been the targeting of CD20 on the surface of the B cell with rituximab. Early trials demonstrated single-agent activity, safety and activity combined with or following chemotherapy, while later phase 3 trials have demonstrated that chemoimmunotherapy has improved progression-free and overall survival of patients with advanced-stage FL. This has spurred interest in the development of new antibodies and related agents, including next-generation anti-CD20 monoclonal antibodies (mAbs) with augmented activity through genetic manipulation of the immunoglobulin constant and variable regions. Novel mAbs targeting other cell-surface antigens are being evaluated as single agents or in combination with rituximab. The use of antibodies to target radioisotopes, despite impressive single-agent antitumor responses, has thus far had limited success in clinical practice, often due to complexities with the delivery of treatment or concerns regarding potential for late toxicity. However, the phase 2 results from Southwest Oncology Group (SWOG) 9911 are very encouraging and form the basis for the ongoing phase 3 trial of R-CHOP versus CHOP^{131}I-tositumomab (SWOG 016).1

Additional approaches include patient-specific vaccines based on the clonally derived immunoglobulin idiotype protein used to induce an endogenous humoral and/or cellular anti-idiotype response in the patient. In most cases, these have been used following cytoreductive chemotherapy or rituximab to prevent or delay relapse or progression. The results of ongoing phase 3 trials will soon be reported.

Last, the ultimate immunotherapy is allogeneic stem cell transplantation, where transplantation of a new immune system results in T cells from the allogeneic donor that can recognize either tumor-specific antigens or minor hematologic histocompatible antigen polymorphisms that differ between the patient and the donor and ultimately mediate graft-versus-lymphoma (GVL) activity. These transplantations are now more widely available to even elderly patients through the use of reduced-intensity or nonmyeloablative conditioning. FL appears to be very sensitive to GVL, and this approach may be associated with long-term disease-free survival and possibly cure. Significant morbidity and mortality from infection and graft-versus-host disease (GVHD) require prudent discussions with the patient regarding optimal timing of this approach, and more research on optimal conditioning regimens and better ability to separate GVL from GVHD.

The focus of this manuscript is to review these new treatment options and provide a basis for understanding the risks and benefits of these new approaches both for selecting patients for clinical trials and to help physicians counsel patients regarding a myriad of treatment opportunities.

Monoclonal Antibodies
The success of rituximab demonstrated that the addition of a relatively nontoxic therapy to conventional chemotherapy could improve survival in patients with FL and has spurred the development of next generation agents or novel antibodies aimed at capturing a portion of this market share. This is good news for clinicians and patients, as it has provided access to new agents, but it also will present an increasing challenge to choose among these many treatments.

Retrospective analysis of many failures and a few successes of antibody therapy suggest that the characteristics of the cell surface antigen play an important role in the efficacy of a new therapy. For example, characteristics of an ideal antigen to target with a mAb-based treatment would be tumor specificity, or at least minimal expression on critical host cells, having an essential biologic activity that may be triggered or blocked by the antibody, no possibility of mutation, and minimal shedding or extracellular se-
Next-Generation Anti-CD20 Antibodies

Multiple new antibodies targeting CD20 are in development and are now beginning to enter clinical trials (Table 1). In most cases, these antibodies are engineered to have augmented antitumor activity due to increased complement or increased ADCC activity. Most antibodies are either fully human or humanized IgG constructs to further decrease immunogenicity. While this has rarely been a problem using rituximab in patients with non-Hodgkin lymphoma (NHL), it has emerged as a significant issue in the treatment of patients with auto-immune diseases. While the exact mechanism of anti-CD20–mediated cell killing in vivo is still unknown, most believe that in patients with NHL, increasing ADCC has the most potential for improved clinical activity. Several of the new antibodies have genetically altered sequences so that they bind with greater affinity to the FcγR3a on ADCC effector cells. Prior studies have demonstrated that polymorphisms in the FcγR3a at position 158 encoding for valine (higher affinity) or phenoalanine (lower affinity) are associated with differential activity of rituximab in patients with follicular NHL. Several of the next-generation antibodies bind with increased activity to the lower-affinity polymorphism FcγR3a receptor. It remains to be seen if they will be associated with increased clinical activity.

Three new anti-CD20 antibodies have completed early clinical trials. Ofatumumab (HuMax-CD20) is a human IgG1 that binds to a different epitope of the CD20 antigen and exhibits greater complement binding and is more efficient in lysing tumor cells with lower levels of CD20 in vitro. Early results using doses from 300 mg to 1000 mg weekly × 4 have been reported in 40 patients with recurring FL, demonstrating significant response rate. This included a 57% response rate in 14 patients who had been treated with prior rituximab but who were not refractory to rituximab (defined as no response to or progression within 6 months of rituximab). Current studies include evaluation in fludarabine- and alemtuzumab-refractory chronic lymphocytic leukemia (CLL) and in rituximab-refractory NHL.

Ocrelizumab (Innu-106, ha20) is a humanized antibody based on the IgG1 construct used in the anti-CD22 antibody epratuzumab. It may be associated with fewer infusion-related adverse events but seems to have similar activity to that expected with rituximab alone. Phase 1/2 studies have reported encouraging overall and complete remission rates. Ongoing studies are evaluating lower doses of the antibody (80 mg/m²) and have demonstrated clinical activity.

Ocrelizumab is a humanized version of the murine 2H7 antibody with more potent ADCC ability in vitro. It has been evaluated in Europe in recurring low-grade NHL. Because of the humanized IgG1 construct, it will likely induce a lower incidence of immune response (than the chimeric antibody rituximab), which may be beneficial in the treatment of patients with autoimmune diseases.

Several genetically enhanced antibodies have been developed and are just entering early-phase clinical trials. This includes AME-133, a humanized antibody with better binding to the low-affinity polymorphism FcγR3a and PRO131921, an engineered, humanized 2H7 with greater binding to FcγR3a and augmented ADCC. Clinical trials with these new antibodies are under way. Last, GA-101 is a mAb that binds to the CD20 antigen in a similar fashion to the murine antibody B1 (a type II binding antibody) that has greater direct growth arrest and induction of apoptosis compared with rituximab. GA-101 has a glyco-engineered FC region with a modified elbow hinge with a 50-fold augmented binding to FcγR3a and increased ADCC in vivo and increased activity in xenograft NHL murine models.

It will be interesting to see if any of the next-generation anti-CD20 antibodies will have significant activity in pa-

<table>
<thead>
<tr>
<th>Antibody name</th>
<th>Type</th>
<th>ADCC</th>
<th>CDC</th>
<th>Direct effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab</td>
<td>Chimeric IgG1</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>Cragg et al⁸</td>
</tr>
<tr>
<td>Ocrelizumab</td>
<td>Humanized IgG1</td>
<td>++++</td>
<td>++</td>
<td>+</td>
<td>Vugmeyster et al⁷</td>
</tr>
<tr>
<td>PRO131921</td>
<td>Engineered ocrelizumab</td>
<td>++++</td>
<td>+/−</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Veltuzumab</td>
<td>Humanized IgG1</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>Stein et al³⁹</td>
</tr>
<tr>
<td>Ofatumumab</td>
<td>Human IgG1</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>Hagenbeek et al⁴</td>
</tr>
<tr>
<td>AME-133</td>
<td>Humanized IgG1</td>
<td>++++</td>
<td>++</td>
<td>+</td>
<td>Weiner et al⁸</td>
</tr>
<tr>
<td>GA-101</td>
<td>Humanized IgG1</td>
<td>++++</td>
<td>−</td>
<td>++++</td>
<td>Umana et al¹⁰</td>
</tr>
</tbody>
</table>

Abbreviations: ADCC, antibody-dependent cell–mediated cytotoxicity; CDC, complement-dependent cytotoxicity.
tients with rituximab-refractory NHL. The cause of either innate or acquired resistance to rituximab-mediated killing is poorly understood. In a few cases, the loss of surface expression of the CD20 antigen has been described, which would lead to resistance to all therapy targeted against CD20. However, in most cases, tumor cells continue to express the CD20 antigen, despite being resistant to rituximab, leading to the possibility that antibodies with augmented effector function may have activity in this resistant population.

**New Monoclonal Antibodies for NHL**

A large number of mAbs are currently being evaluated in FL, and a number are in phase 3 clinical trials (Table 2). These include the anti-CD80 antibody galiximab, which blocks the CD80/CD28 pathway and results in decreased tumor proliferation, ADCC and apoptosis. While the single-agent activity in patients with recurring NHL was only 11%, several of the responses occurred late, suggesting alterations in immune regulation. A phase 1/2 trial of galiximab (500 mg/m² dose) in combination with rituximab (no rituximab-refractory patients) demonstrated a 66% response rate with a median progression-free survival of 12.1 months. The combination is now being compared with rituximab alone.

Two anti-CD40 antibodies are in clinical trials. SGN-40 is a mild agonist that does not block CD40 ligand from binding to CD40. It is effective against tumor cell lines and in xenograft models. Early clinical trials suggest significant activity in relapsed aggressive NHL, with less activity in relapsed FL. HCD122 (CHIR-12.12) is a fully human anti-CD40 antibody that completely blocks CD40L interaction with CD40. It also has demonstrated preclinical activity in xenograft models and is being evaluated in CLL, myeloma and lymphoma.

Antibodies directed against the rapidly modulating antigen CD22 have also been evaluated in clinical trials. Epratuzumab is a humanized antibody based on the murine antibody mLL2. It has been given to patients in doses from 120 mg to 1000 mg/m² weekly × 4 weeks with minimal adverse reactions despite short infusion times. Responses were observed in both indolent and aggressive NHL. Combination trials with rituximab suggested a higher response rate with more complete remissions than might be expected with rituximab alone. Because the CD22 antigen is rapidly internalized, a drug- or toxin-conjugated antibody may have greater activity. Inotuzumab ozogamicin (CMC-544) is an anti-CD22 antibody linked to calicheamicin. In vitro, it exhibits very potent cell killing and in a phase 1/2 trial in patients relapsed NHL demonstrated encouraging clinical activity, including a 69% response rate in FL. Dose-limiting toxicity was thrombocytopenia, and the maximum tolerated dose was determined to be 1.8 mg/m² every 4 weeks. Studies in combination with rituximab are ongoing.

**Lymphoma Idiotype Vaccines**

Results from the next year will be critical to the success or failure of this approach. Several ongoing phase 3 randomized clinical trials of the efficacy of patient-specific vaccination with tumor-derived immunoglobulin idiotype sequences are nearing completion. This approach requires the isolation and production of either the immunoglobulin protein or the DNA encoding the idiotype protein and subsequent immunization of the patient (usually following some cytoreductive therapy) to induce a host antitumor response and delay or prevent tumor progression/relapse. Phase 1 and 2 trials have demonstrated that these approaches can generate both humoral and cellular anti-idiotype immune responses, and that patients who generate these immune responses have longer duration of remissions than patients who do not successfully mount an immune response to the vaccine. Whether a humoral or cellular immune response is the most important in the antitumor effects is controversial, with data supporting the value of both immune responses.

Currently, three large phase 3 clinical trials, based on smaller phase 2 trials demonstrating the induction of immune responses, have either fully accrued or are ongoing. Investigators at several centers, including the MD Anderson Cancer Center, are evaluating vaccination with the tumor-derived immunoglobulin produced through “rescue” hybridization (Biovest International). Trials from Genitope and Favrille are using idiotype protein isolated

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**Table 2. Selected monoclonal antibodies in clinical trials in follicular non-Hodgkin lymphoma.**

<table>
<thead>
<tr>
<th>Antibody name</th>
<th>Type</th>
<th>DLT</th>
<th>OR rel FL, %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab, anti-CD20</td>
<td>Chimeric IgG1</td>
<td>None</td>
<td>48</td>
<td>McLaughlin et al¹⁰⁵</td>
</tr>
<tr>
<td>Epratuzumab, anti-CD22</td>
<td>Humanized IgG1</td>
<td>None</td>
<td>43 at 360 mg/m² dose</td>
<td>Leonard et al¹⁷</td>
</tr>
<tr>
<td>Epratuzumab + rituximab</td>
<td>Combination</td>
<td>None</td>
<td>67</td>
<td>Leonard et al¹⁹</td>
</tr>
<tr>
<td>Inotuzumab ozogamicin</td>
<td>Humanized IgG4</td>
<td>Decreased platelets</td>
<td>69</td>
<td>Fayad et al²⁰</td>
</tr>
<tr>
<td>Anti-CD22-calicheamicin</td>
<td>Humanized IgG1</td>
<td>None</td>
<td>11</td>
<td>Czuczeman et al¹¹</td>
</tr>
<tr>
<td>Galiximab, anti-CD80</td>
<td>Humanized IgG1</td>
<td>None</td>
<td>66</td>
<td>Leonard et al¹²</td>
</tr>
<tr>
<td>Galiximab + rituximab</td>
<td>Combination</td>
<td>None</td>
<td>0</td>
<td>Advani et al¹³</td>
</tr>
<tr>
<td>SGN-40, anti-CD40</td>
<td>Humanized IgG1</td>
<td>Cytokine release</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: DLT; OR rel FL
by the cloning of the Ig variable regions from tumor biopsies and production of protein in mammalian or insect cell lines. The Biovest International and the Genitope trials vaccinate patients following cytoreductive chemotherapy and require a complete or partial remission, respectively. Patients were randomized in a double-blinded fashion to receive vaccination with their specific idiotypic vaccine coupled to the carrier protein KLH versus carrier protein, along with local injection of the cytokine GM-CSF. In contrast, Farville has conducted a similar phase 3 trial of FavId vaccination for patients with at least stable disease following treatment with rituximab (4 weekly infusions of 375 mg/m²). As the use of rituximab has been shown to delay the generation of humoral immune responses, the vaccinations continue until disease progression. Rituximab use would not be expected to interfere with the generation of anti-idiotypic cell–mediated immunity. The results of these trials are eagerly awaited. However, as rituximab alone or combined with chemotherapy with or without “maintenance” therapy has increasingly become a standard treatment for patients with follicular NHL, identifying the optimal use of these new treatments, if successful, will be challenging.

Allogeneic Stem Cell Transplantation
Transplantation of an allogeneic donor immune system represents a radical form of immunotherapy. Recent advances in the use of nonmyeloablative or reduced-intensity conditioning and allogeneic transplantation have decreased the acute nonrelapse mortality of this procedure and allows for the treatment of older patients and patients with significant comorbidities. The use of minimal conditioning requires that the majority of the antitumor effects must come from immunologic graft-versus-tumor (GVT) activity. Follicular NHL appears to be particularly sensitive to immunologic attack, and several groups have reported high complete response (CR) rates and low risk of relapse, even in patients with advanced FL who have failed multiple other treatment regimens. Unfortunately, it has been difficult to obtain GVT responses in the absence of GVHD; thus, this approach is not without significant risk. While the risk of early death from conditioning toxicity has markedly decreased, there continues to be risk from both acute and chronic GVHD or resulting immunosuppression from GVHD treatment leading to late nonrelapse deaths. One of the major challenges to physicians and patients alike is to determine the optimal timing to use such a high-risk but potentially curative therapy.

Myeloablative Allogeneic Transplantation for FL
Myeloablative conditioning is usually reserved for younger patients without medical comorbidities. In most series, median age is in the mid 40s. Analysis of multiple trials and registry data suggest that there is a low risk of relapse in patients who survive the 2 years after transplantation with nearly a flat progression-free survival curve. This is thought to be due to several factors, including the use of a tumor-free graft, the myeloablative conditioning, and GVL antitumor activity of the allogenic donor. Comparison of allogeneic versus high-dose therapy and autologous transplantation is difficult, as the former is usually done as a last option in refractory patients, while the later is optimal at the time of first or second chemotherapy-sensitive relapse. All comparisons, however, show a higher acute mortality from the conditioning regimen, but lower risk of relapse in surviving patients following allogeneic transplantation, leading to curves that may cross in long-term follow-up. Because of the risk of early mortality, and the early success observed with reduced-intensity or nonmyeloablative conditioning regimens, in many transplantation centers this approach is restricted to younger, higher-risk chemotherapy-refractory patients.

Nonmyeloablative or Reduced-Intensity Allogeneic Transplantation for FL
Multiple groups have now published small series of patients with FL treated with reduced-intensity allogeneic stem cell transplantation (Table 3). In Seattle, at the Fred Hutchinson Cancer Research Center (FHCRC), a nonmyeloablative conditioning regimen based on fludarabine (90 mg/m²) and low-dose total body irradiation (TBI) of 200 cGy was developed in a canine transplantation model and extended to clinical trials. Postgrafting immunosuppression with MMF and cyclosporine was used to prevent graft rejection and prophylaxis against GVHD. This regimen was found to reliably allow allogeneic engraftment from matched related and unrelated donors, and to allow the generation of potent GVT immune responses active in a number of malignancies. The regimen has been broadly used in more than 1,000 patients in a consortium of institutions in collaboration with the FHCRC.

Recently, we have updated our experience in the use of this regimen in 62 patients with indolent NHL or indolent NHL that had histologic transformation to an aggressive histology (n = 16) to better define the risks and toxicity of this approach. We analyzed the outcome based on histologic transformation and type of donor available (matched related, matched unrelated and mismatched unrelated). Fifty-four of the patients had an underlying FL. Patients received conditioning with the TBI with or without fludarabine regimen and received peripheral blood stem cell grafts from related (n = 34) or unrelated donors (n = 28). The patient median age was 54 years (range, 33-66 years), median number of prior regimens was 6, and 32% had failed a prior high-dose regimen and autologous stem cell transplantation. The regimen was well tolerated, with a median of 6 days of neutropenia. Acute grades II-IV GVHD was observed in 63% of patients, with 18% having serious grade III-IV GVHD, and extensive chronic GVHD was observed in 47% of patients.

Disease responses were observed in 59% of patients with measurable disease at the time of transplantation, including 21 CR and 6 partial response (PR). Of 16 patients...
in CR from salvage therapy at the time of transplantation, only 2 relapsed. Chemotherapy sensitivity predicted a higher likelihood of achieving a CR. The cumulative incidence of disease progression at 3 years after transplantation was only 14% for patients with indolent disease versus 38% for patients with transformed disease. The best outcome was observed for patients with non-transformed disease who received grafts from matched related donors (n = 26), who at 3 years had estimated rates of overall and progression-free survivals and nonrelapse mortality of 67%, 54% and 23%, respectively. Patients with transformed lymphoma had a much worse outcome with high rates of relapse/progression and estimated overall and progression-free survivals at 3 years of only 21% and 18%, respectively. Risk factors for nonrelapse mortality included unrelated donor grafts (especially mismatched grafts) and high number of comorbidities at the time of transplantation, as well as bulky disease. Risk factors for relapse included transformed or chemotherapy-refractory disease. For the entire indolent group, the 3-year estimated overall and progression-free survival were 52% and 43%, respectively.

Other studies have reported 2-year disease-free survivals ranging from 54% to 84% and nonrelapse mortality rates ranging from 10% to 31% for patients with indolent NHL. The best results have been reported from the M.D. Anderson Cancer center using a fludarabine and cyclophosphamide with or without rituximab conditioning regimen with 2-year survivals of 84%. Patients who did relapse were frequently treated with rituximab and donor lymphocyte infusions and could again obtain a remission. Compared with the Seattle results, these patients had less prior therapy (median of 2 prior regimens vs 6 in the Seattle study), and most patients were in remission at the time of transplantation. In another series, the inclusion of alemtuzumab in the conditioning regimen was associated with a low mortality, but relatively high rate of relapse (44%), likely due to T-cell depletion of the donor graft. Ongoing studies are evaluating the addition of targeted therapies such as rituximab or radiolabeled antibodies to the conditioning regimens to augment early tumor control.

From these studies, it is clear that allogeneic transplantation can result in potent GVT immune responses and prolonged progression-free survival, but at the risk of significant nonrelapse mortality from GVHD and/or infections and risks from ongoing chronic GVHD. These risks cannot be taken lightly, and identification of the optimal time in the course of FL to consider this treatment is controversial. Outcomes appear superior when the disease is still chemosensitive and nonbulky. A conservative approach is to use standard chemoimmunotherapy and to consider high-dose therapy and autologous stem cell transplantation for patients with short remissions. Patients who fail autologous transplantation can then be evaluated for nonmyeloablative or reduced-intensity allografts if an appropriate donor can be identified. A more aggressive strategy would be to consider allografting earlier in the course of the disease. These discussions with patients are difficult and need to be individualized based on patient goals, preferences, prognosis of the disease with conventional therapy, comorbidities and donor availability.

**Conclusions**

The development of immunotherapy has changed the face of therapy for patients with FL and is beginning to improve survival. The future is bright, with a large number of next-generation anti-CD20 antibodies and antibodies directed at new antigens entering clinical trials. Custom vaccination with the immunoglobulin idiomophy remains an appeal-
ing approach, but will depend on definitive phase 3 trial results. Allogeneic transplantation may provide a curative treatment option when many other treatments have failed but must be done with careful consideration of the risks and benefits. Charting a course through the large number of treatment options available to newly diagnosed patients is difficult and must be done with an eye toward the future.

Correspondence
David Maloney, MD, PhD, Fred Hutchinson Cancer Resch. Ctr., 1100 Fairview Ave. N, D1-100, Seattle, WA 98109; phone (206) 667-5616; fax (206) 667-6124; dmaloney@fhcrc.org

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