Anti-CD20 Antibody Therapy for B-Cell Lymphomas

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A 62-year-old man is evaluated for abdominal pain. Computed tomography (CT) shows a 7-cm mesenteric mass. An incisional biopsy reveals a diffuse large B-cell lymphoma, and immunohistochemical staining is positive for the B-cell antigen CD20. The patient is referred to an oncologist. A positron-emission tomographic–CT (PET-CT) scan reveals involvement of additional nodes in the lower chest and abdomen. The serum level of lactate dehydrogenase is twice the upper limit of the normal range. The results of bone marrow biopsy and aspiration are normal on pathological analysis and flow cytometry. The patient reports an unintentional weight loss of $11\frac{1}{2}$ kg (25 lb) during the previous 6 months but no unexplained fevers or night sweats. He has no history of cardiac disease, and an echocardiogram shows a normal left ventricular ejection fraction. The oncologist recommends treatment with the anti-CD20 monoclonal antibody rituximab in combination with a chemotherapy regimen of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP).

More than 25 histologic subtypes of B-cell lymphoma, with a wide range of biologic and clinical features, are recognized in the 2008 World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues. The American Cancer Society estimates that in 2012, a total of 70,130 new cases of B-cell lymphoma will be diagnosed in the United States, and 18,940 patients will die from the disease. B-cell lymphomas account for 4% of all cancers and 3% of cancer-related deaths among adults in the United States.

Diffuse large B-cell lymphoma is the most common type of non-Hodgkin’s lymphoma (accounting for approximately 30% of cases) in Europe and North America. It is considered to be an aggressive cancer that requires immediate treatment. The goal of therapy is induction of a complete remission and cure.

Follicular lymphoma is the second most common histologic form of non-Hodgkin’s lymphoma (accounting for approximately 25 to 30% of cases). Follicular lymphoma is often clinically indolent initially but with a tendency for relapse to occur after standard treatments. The goals of therapy are to prolong progression-free survival and to improve quality of life and overall survival.

All lymphocytes are derived from a common progenitor cell that originates in the bone marrow. The process of lymphoid differentiation includes a series of DNA
recombination steps to create the extensive repertoire of immunoglobulins (for B cells) or T-cell receptors (for T cells). Lymphoid cancers develop as a result of the acquisition of genetic abnormalities during differentiation. Most B-cell lymphomas are clonal descendants of distinct populations of B-cell precursors.

The process of lymphoid differentiation has traditionally been characterized on histologic analysis by the identification of cell-surface markers that are expressed at specific stages in lymphoid-cell development. CD20 is a B-cell-specific differentiation antigen that is expressed on mature B cells and in most B-cell non-Hodgkin's lymphomas but not on early B-cell progenitors or later mature plasma cells. The human protein has multiple membrane-spanning domains; there is no known ligand. CD20 is part of a multimeric cell-surface complex that regulates calcium transport and is involved in the regulation of B-cell activation and proliferation. However, studies involving disruption of the gene encoding CD20 in mice do not demonstrate a critical function for this molecule in B-cell development or the generation of immune responses.

Rituximab is a chimeric monoclonal antibody (incorporating human immunoglobulin G1 heavy-chain sequences and murine immunoglobulin variable regions) that recognizes the human CD20 antigen. It was the first antibody approved by the Food and Drug Administration for use in the treatment of lymphoma. The recognition that rituximab could have a substantial therapeutic effect in cases of relapsed, indolent non-Hodgkin's lymphoma ushered in the era of monoclonal antibody therapy for cancer.

Anti-CD20 antibodies cause the death of tumor cells through several mechanisms. However, the exact contribution of each mechanism to the observed clinical activity of these antibodies remains controversial. (Fig. 1). Binding of antibody to CD20 may activate the complement cascade through C1q, leading to cell death or deposition of complement proteins on the cell membrane, a phenomenon known as complement-dependent cytotoxicity. Antibody-coated cells may be killed by immune cells expressing Fcγ receptors through antibody-dependent cell-mediated cytotoxicity. Finally, antibody binding to CD20 may have direct antiproliferative effects or may actively induce cell death (apoptosis). These effects appear to be additive and possibly synergistic when anti-CD20 antibody therapy is combined with chemotherapy.

All clinically active anti-CD20 antibodies bind to a small loop of the CD20 molecule containing approximately 40 amino acids expressed on the cell surface (Fig. 1). In vitro, these antibodies can be separated into two types on the basis of cellular effects observed on binding to CD20-expressing cells. Type I antibodies (rituximab and ofatumumab) induce redistribution of CD20 into large lipid rafts in the plasma membrane and have strong complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity but have minimal direct antitumor effects. Ofatumumab, a human antibody, may bind to a small region of CD20 that is closer to the cell membrane than the region that rituximab binds to, explaining the increased ability of ofatumumab to bind C1q and mediate complement-dependent lysis. Type II antibodies (tosilumomab and obinutuzumab) do not induce redistribution of cell-surface CD20 and have minimal complement-dependent cytotoxicity, strong antibody-dependent cell-mediated cytotoxicity, and increased direct antitumor effects. To date, none of the newer anti-CD20 antibodies have been shown to be clinically more effective than rituximab in a direct comparison.

**CLINICAL EVIDENCE**

Rituximab has been investigated in both aggressive and indolent cases of non-Hodgkin's lymphomas, as either a single agent or in combination with standard chemotherapy regimens for lymphoma, such as CHOP (immunotherapy). On the basis of preliminary studies, only combination regimens were selected for evaluation in advanced-phase trials involving patients with aggressive lymphomas, whereas both single-agent and combination regimens have been evaluated in patients with indolent forms of disease.

Several trials have evaluated the effect of adding rituximab to chemotherapy in aggressive forms of non-Hodgkin's lymphoma, such as diffuse large B-cell lymphoma. The benefit of this approach is illustrated by the MabThera International Trial, in which 824 patients with diffuse large B-cell lymphoma were randomly assigned to receive either CHOP-like chemotherapy alone or...
CHOP-like chemotherapy plus rituximab. Radiotherapy was administered to sites of bulky disease and elsewhere at the physician's discretion, and growth factors were administered for neutropenia. The 3-year rate of progression-free survival was 68% with chemotherapy alone versus 85% with the addition of rituximab. Overall survival rates at 3 years were 84% and 93%, respectively.

In indolent non-Hodgkin's lymphoma (e.g., follicular lymphoma), both single-agent and combination rituximab regimens have been investigated.\textsuperscript{14,16,22-25} The efficacy of single-agent therapy was shown in a nonrandomized study involving 166 patients with low-grade or follicular lymphomas.\textsuperscript{14} The initial overall response rate (rate of complete or partial response) was

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**Figure 1. Mechanisms of Action of Anti-CD20 Antibodies.**

Anti-CD20 antibodies bind to the CD20 molecule on the surface of the malignant B cell in non-Hodgkin's lymphoma (NHL), leading to cell death. Three mechanisms of action of anti-CD20 antibodies have been proposed. In complement-dependent cytotoxicity, the first component of complement (C1) binds to the Fc portion of the anti-CD20 molecule, resulting in the activation of the complement cascade and cell lysis through the formation of membrane attack complexes (MAC). In antibody-dependent cell-mediated cytotoxicity (ADCC), effector cells, such as natural killer cells or macrophages, bind to the Fc portion of the anti-CD20 molecule through Fcγ receptors; the effector cells then release effector molecules such as perforin, which cause cell lysis. In direct cytotoxicity, the anti-CD20 antibody induces internal signaling within the tumor cell, causing antiproliferative effects or cell death, which may involve apoptosis or other cell-death pathways. In the top inset, anti-CD20 antibodies bind to an extracellular portion of the CD20 molecule. Most anti-CD20 antibodies, including rituximab, tositumomab, and obinutuzumab, bind to the larger of two extracellular loops within the CD20 molecule; this loop includes the alanine-N-proline (ANP) residues at positions 170 to 172. Ofatumumab binds to two sites on the CD20 molecule: the smaller extracellular loop and positions 159 to 166 on the larger loop. This unique binding pattern of ofatumumab, which results in increased proximity of the antibody to the cell membrane, may account for the greater potency of the drug in inducing complement-mediated lysis. In the lower inset, the structure of chimeric and human antibodies is shown.
48%, which is similar to that for single-agent cytotoxic chemotherapy.

In one of the larger trials of rituximab combined with chemotherapy for indolent lymphoma, 428 patients with previously untreated, advanced-stage follicular lymphoma were randomly assigned to receive either CHOP or CHOP plus rituximab (R-CHOP). The rates of overall response were 90% with CHOP and 96% with R-CHOP. The estimated survival rates at 2 years were 90% and 95%, respectively.

**Clinical Use**

The most important procedure in confirming the diagnosis of non-Hodgkin’s lymphoma is a tissue biopsy, with the sample submitted for analysis by a hematopathologist, including immunohistochemical analysis and often flow cytometry and molecular studies. In general, samples obtained by means of fine-needle aspiration are insufficient. After the diagnosis has been confirmed, a thorough staging of the disease is required, including physical examination, assessment of performance status, radiography (including CT and PET scanning), bone marrow biopsy, and laboratory studies to determine the lactate dehydrogenase level as well as renal, hepatic, and marrow function. Patients should be screened for hepatitis B, and those with evidence of previous exposure need to be monitored closely (with serial transaminase and those with evidence of previous exposure by means of echocardiography or radionuclide ventriculography. Those with significantly impaired cardiac function (ejection fraction, <45%) should be treated with alternative regimens that do not include bolus anthracyclines.

For patients with aggressive non-Hodgkin’s lymphoma, the use of the international prognostic index (IPI) provides valuable information on the likelihood of survival with the use of conventional chemotherapy. This index has been shown to maintain its prognostic usefulness in the rituximab era. In patients with localized aggressive non-Hodgkin’s lymphoma (stage I or II), three cycles of R-CHOP followed by involved-field irradiation or six to eight cycles of R-CHOP alone may be used. A regimen of six to eight cycles of R-CHOP is considered the current standard of care for patients with advanced disease (stage III or IV). R-CHOP is usually administered every 21 days, and rituximab is typically given on the first day of each cycle (Table 1).

Rituximab is administered at a dose of 375 mg per square meter of body-surface area by intravenous infusion after premedication with acetaminophen and diphenhydramine. Because of the risk of infusion-related symptoms, the first dose is typically administered slowly, at an initial infusion rate of 50 mg per hour, with incremental increases every 30 minutes thereafter. In subsequent cycles, rituximab can usually be administered faster with minimal symptoms, and premedication may not be necessary.

The goal of therapy is a complete remission, and it is critical to try to deliver all agents on schedule and at full doses. Growth-factor support is used for patients with neutropenic fever or a delay in the recovery of white cells between cycles and is recommended for patients over 65 years of age in order to prevent febrile neutropenia and infection. Patients who are treated with chemoimmunotherapy are at increased risk for infection and need to be monitored and treated as necessary.

At 6 to 8 weeks after completion of therapy, PET scanning of all areas of previous disease is performed to confirm complete remission. Patients with a confirmed, PET-negative remission after a full course of therapy are monitored for evidence of relapse by means of physical examination, laboratory studies, and periodic imaging studies. Patients with aggressive non-Hodgkin’s lymphoma do not appear to benefit from maintenance rituximab therapy after R-CHOP induction therapy. Relapse is most common in the first 3 years, but late relapses may occur.

**Table 1. A Typical Regimen of Rituximab with Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone (R-CHOP).**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
</tr>
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<tbody>
<tr>
<td>Rituximab</td>
<td>Intravenous infusion (375 mg/m² of body-surface area) on day 1</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Intravenous bolus (750 mg/m²) on day 1</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Intravenous bolus (50 mg/m²) on day 1</td>
</tr>
<tr>
<td>Vincristine</td>
<td>Intravenous bolus (1.4 mg/m²) on day 1</td>
</tr>
<tr>
<td>Prednisone</td>
<td>Oral administration (100 mg) on days 1 to 5</td>
</tr>
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Patients who do not have a complete remission and those who have a relapse should be considered for salvage therapy. Patients who have a response to salvage therapy should then be considered for high-dose therapy with hematologic stem-cell support. Appropriate candidates for transplantation are generally patients without prohibitive coexisting illnesses who are under the age of 75 years. In most patients, rituximab has been added to salvage regimens, although there is no proof that it provides an additional benefit.

Patients with indolent, asymptomatic non-Hodgkin’s lymphoma may be monitored, with treatment administered at the time of disease progression or the development of symptoms. Antibody therapy has been used alone and in combination or in sequence with conventional chemotherapy. In a study involving patients with follicular lymphoma and a low tumor burden, treatment with a single course of rituximab alone led to a response rate of 73%, with a complete-response rate of 20%. Extending the treatment course for 1 year led to a rate of event-free survival of 45% at 8 years. For patients requiring chemotherapy (typically, those with symptomatic or bulky disease), rituximab is combined with regimens such as CHOP, CVP (cyclophosphamide, vincristine, and prednisone), FMC (fludarabine, mitoxantrone, and cyclophosphamide), or bendamustine.

In contrast to the treatment approach in patients with aggressive non-Hodgkin’s lymphoma, achievement of a complete response is not yet considered a mandatory goal of treatment in patients with indolent lymphoma, although those with a complete response to initial therapy have improved progression-free survival. Most patients with indolent non-Hodgkin’s lymphoma remain at risk for relapse after initial therapy. In one study, patients who received maintenance therapy with rituximab (one dose every 2 months for up to 2 years) had an improvement in progression-free survival (74.9%, vs. 57.6% among patients who did not receive rituximab as maintenance therapy), but at a median follow-up of 36 months, there was no significant difference in overall survival between the two groups. Maintenance therapy is associated with increased risks of neutropenia and infection requiring treatment.

Patients who do not have a response to antibody therapy or who have a relapse or disease progression within 6 months after the discontinuation of treatment are said to have rituximab resistance. Currently, the molecular mechanism of this resistance is unclear. Among patients who have a relapse, immunotherapy is frequently administered again, with or without subsequent maintenance rituximab therapy.

In patients with indolent non-Hodgkin’s lymphoma, there have been studies of radiolabeled anti-CD20 antibodies, including ibritumomab tiuxetan (an yttrium-90–conjugated monoclonal antibody) and tositumomab (an iodine-131–conjugated antibody). The use of radiolabeled anti-CD20 antibodies is associated with substantial delayed hematologic toxicity related to non-specific targeting of the bone marrow. Because of such side effects, these antibodies should be used as single agents or in sequence with conventional chemotherapy. Despite high response rates with these agents, concern about the risks of late myelodysplasia and acute myelogenous leukemia, as well as the cumbersome logistics of coordinating with nuclear medicine for administration, have limited their widespread use.

A study of treatment costs that was based on 2003 data showed that the cost of rituximab therapy was $2,871 per infusion in the United States. Similar studies have shown cost estimates of £1,170 (about $2,150 in 2004 dollars) per infusion in the United Kingdom and €2,088 (about $2,700 in 2005 dollars) per infusion in the Netherlands.

ADVERSE EFFECTS

Most of the clinical experience with anti-CD20 antibodies has been with rituximab. The initial dose is associated with infusion-related symptoms (e.g., urticaria, fever, and chills) in more than half of patients. Although these symptoms are usually modest, more serious reactions can occur, including hypotension, rigors, bronchospasm, and angioedema, in as many as 10% of patients. Cardiac arrhythmias and acute coronary syndromes have been reported with the first dose. Rituximab has also been associated with the development of acute interstitial lung disease, which can be fatal. The infusion should
be slowed or stopped if acute symptoms occur and then resumed when the symptoms abate. Subsequent infusions are usually associated with fewer symptoms and can generally be administered at a faster rate. In patients with clinically significant type 1 hypersensitivity reactions and a positive skin test for rituximab, a 12-step rapid desensitization protocol can be considered for subsequent infusions.44

Bowel perforations have been reported with rituximab treatment; this may be of particular concern in patients with lymphomatous involvement of the gastrointestinal tract.45 Mucocutaneous reactions such as the Stevens–Johnson syndrome have been described.46

Rituximab causes expected B-cell depletion and some degree of immunodeficiency, and infectious complications are therefore not uncommon. In an analysis of pooled data from 356 patients, 30% of patients had infectious events: 19% with bacterial infections, 10% with viral infections, and 1% with fungal infections.47 Fulminant hepatitis B reactivation has been observed after therapy; patients should be screened for hepatitis B, and carriers of the virus should be monitored closely.48 Progressive multifocal leukoencephalopathy from the JC virus is a rare but devastating complication that has been observed in patients receiving rituximab, most of whom received rituximab in combination with chemotherapy or hematopoietic stem-cell transplantation.49

Delayed-onset neutropenia (typically occurring 3 to 4 weeks after treatment) has been reported in 3 to 27% of patients.50 Several analyses have suggested an association with a genetic polymorphism that results in higher-affinity FcγRIIIa receptors.51,52 Delayed-onset neutropenia usually resolves with the administration of growth factors.

**Areas of Uncertainty**

For patients with aggressive non-Hodgkin’s lymphoma, R-CHOP is currently the standard therapy. However, it is not clear that the optimal chemotherapy regimen has yet been defined. Many of the initial trials used eight cycles of therapy on a 21-day cycle, whereas subsequent studies using R-CHOP on a 14-day cycle showed that a regimen of six cycles was sufficient.18,20 Other combination chemotherapy regimens are also under investigation. For example, a regimen in which etoposide is added to the R-CHOP regimen, with infusion of etoposide, vincristine, and doxorubicin during a 96-hour period rather than bolus administration (designated DA-EPOCH-R), is being compared with R-CHOP in an ongoing clinical trial.

Molecular profiling has shown that morphologically identical cases of diffuse large B-cell lymphoma actually comprise several disease subtypes with different clinical behavior on the basis of the cell of origin. The two main subtypes are germinal-center B-cell–like and activated B-cell–like diffuse large B-cell lymphomas.53 Clinically, patients with the activated B-cell–like molecular profile have had inferior outcomes with most treatments, including R-CHOP and DA-EPOCH-R, although the addition of antibody therapy has improved the outcomes for both groups of patients.54,55 To date, no accepted standard treatment has proved to have greater efficacy on the basis of molecular subtype or surrogate immunohistochemical markers.

For patients with indolent non-Hodgkin’s lymphoma requiring therapy, the major areas of uncertainty include the choice of initial chemotherapy to combine with rituximab, the role of radioimmunotherapy, and the role of maintenance therapy in inducing rituximab resistance. In patients with rituximab resistance, the role of additional therapy with anti-CD20 antibodies alone or in combination with chemotherapy is unknown.

For patients with indolent disease who have a low tumor burden, there is uncertainty about the role of observation as compared with initial therapy. A recent randomized trial showed high rates of response to single-agent rituximab, with or without maintenance therapy, as compared with observation, and a prolonged interval before subsequent therapy was required.56 Longer follow-up will be necessary to assess the response to subsequent therapy and overall survival.

Multiple next-generation anti-CD20 antibodies have been designed with augmented effector function, as described above. The clinical role of such antibodies remains undefined. Two of these antibodies (ofatumumab and obinutuzumab) are being evaluated in phase 3 trials in comparison with rituximab or rituximab-containing regimens.
in both patients with indolent and those with aggressive non-Hodgkin’s lymphoma.

GUIDELINES

For patients of all ages who have advanced-stage, aggressive diffuse large B-cell non-Hodgkin’s lymphoma, the National Comprehensive Cancer Network (NCCN) guidelines recommend treatment with R-CHOP every 21 days for six cycles. For patients with early-stage disease, the guidelines recommend R-CHOP for three cycles with involved-field radiation or R-CHOP for six cycles. The European Society for Medical Oncology (ESMO) guidelines are slightly different. They recommend chemotherapy with R-CHOP every 21 days for six to eight cycles for patients under the age of 60 years who have low age-adjusted IPI scores; for patients with higher-risk IPI scores, they recommend enrollment in clinical trials. For patients between the ages of 60 and 80 years, the ESMO guidelines recommend R-CHOP every 21 days for eight cycles or R-CHOP every 14 days for six cycles. There is no recommendation for consolidation with involved-field irradiation.

For patients with indolent non-Hodgkin’s lymphoma requiring therapy, the NCCN guidelines recommend treatment with rituximab in combination with any one of various chemotherapeutic regimens, including bendamustine, CVP, or CHOP, or radioimmunotherapy. Patients in remission may be offered consolidation treatment with radioimmunotherapy or maintenance rituximab therapy. Elderly or infirm patients, in whom the above regimens may have unacceptable adverse effects, single-agent rituximab therapy or radioimmunotherapy may be administered. The recommendations in the ESMO guidelines are generally similar.

RECOMMENDATIONS

The patient described in the vignette is an appropriate candidate for a rituximab-containing regimen. He has stage III disease (involvement on both sides of the diaphragm without extralymphatic involvement) and an elevated lactate dehydrogenase level, and he has no contraindication to bolus anthracycline therapy. He has a high to high-intermediate risk score. I would recommend that he receive R-CHOP every 21 days for six cycles. I would expect his likelihood of 3-year survival to be approximately 65%. I would obtain a PET-CT scan at the completion of therapy to confirm remission of disease. If a complete remission is confirmed, I would not recommend maintenance therapy but would instead monitor the patient for relapse. For persistent or relapsed disease, I would assess his ability to tolerate aggressive salvage therapy and high-dose treatment.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

REFERENCES


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