CLINICAL PRACTICE

Monoclonal Anti-CD 20 Antibody Used in Non-Hodgkin's Lymphoma: A Case Study

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ABBREVIATIONS: CD = cluster designation antigen; CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy; CT = computed axial tomography; NCI = National Cancer Institute; NHL = non-Hodgkin's lymphoma.

INDEX TERMS: Non-Hodgkin's lymphoma; lymphoproliferative disorder; immunophenotyping; Rituxan® therapy.

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A 26-year old gentleman with a history of peptic ulcer disease was admitted in March of 2001 with a two-month history of abdominal pain and a 13 pound weight loss. CT of the abdomen showed a 6 cm x 7 cm pelvic mass and a second 6 cm x 7 cm periaortic mass in the post-hepatic area. The patient underwent exploratory laparotomy and pathology was consistent with diffuse, large-cell lymphoma with immunoblastic features. The operative report and the surgeon described an intra-abdominal cavity consisting of multiple bulky lymphadenopathy in the retroperitoneum, involving the small bowel. Flow cytometry showed a monoclonal population of cells positive for CD19, CD20, and HLA-DR. The patient underwent six cycles of chemotherapy that included Rituxan®. After three cycles of chemotherapy, he had complete resolution of the pelvic and periaortic masses. He still had residual tumor in the post-hepatic area, 5 cm x 6 cm in size. He received three more cycles of chemotherapy with Rituxan® and CT scans were repeated. The CT neck, chest, and pelvis scans were negative; however the post-hepatic mass remained.

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MEDICAL HISTORY: Otherwise unremarkable.

LABORATORY FINDINGS: See Table 1.

Immunophenotypic analysis revealed a monoclonal B lymphoid population characterized as CD19+, CD20+, CD5-, CD10+/-, CD23-/+, FM C7-/+, HLA-DR+, and restricted to kappa light chain immunoglobulin with intermediate intensity (Table 1). DNA content analysis of all cells showed a diploid pattern with an increased proliferative rate. The common morphologic features of a B-cell lymphoma are the large cell size, usually four to five times that of a small lymphocyte and a diffuse pattern of growth. The described findings indicated B-cell lymphoma of mixed cell size; an intermediate proliferative rate indicated an aggressive process.

The cells associated with B-cell lymphomas express surface immunoglobulin. All immunoglobulin molecules contain a light chain and a heavy chain. In a random, benign collection of lymphoid cells, the kappa light chains are present on roughly two-thirds of the cells and lambda light chains in one-third. Monoclonal antibody directed at kappa light chain could be used to mark two-thirds of the cells; an antibody to lambda would mark one-third. A malignant collection of lymphoid cells is monoclonal. The cells bear identical surface immunoglobulin molecules with either kappa or lambda light chains, but not a mixture of the two. Monoclonal antibody directed towards a specific kind of light chain would mark either all or none of the cells.

Immunophenotyping refers to the technique of identifying molecules that are associated with lymphoma cells and which help to characterize them. It is helpful because in many instances different kinds of benign and malignant lymphoid cells resemble each other in routinely stained tissue sections and smears. It is especially useful in the case of B-cell lymphomas that express surface immunoglobulin. A lymphoma panel is designed to characterize lymphoproliferative disorders, which are usually comprised of mature B or T cells. The routine lymphoma panel is performed using three-color flow cytometry with the B cell markers CD19, CD20, kappa, and lambda. For B cell malignancies, demonstration of the presence of a monoclonal population by restricted kappa or lambda immunoglobulin light chain expression is diagnostic.

DISCUSSION

Non-Hodgkin's lymphoma (NHL) is the most common cancer involving the lymphatic system. Lymph nodes are found in the underarm, pelvis, neck, and abdomen. Because there are lymph tissues in many parts of the body, NHL can start in almost any part of the body and can spread to almost any organ or tissue in the body. Since the early 1970s, incidence rates for non-Hodgkin's lymphoma have nearly doubled. The overall five-year survival rate is only 55%. Of the 500,000 Americans with lymphoma, 66% have this form and each year approximately 23,000 Americans will die from the disease.[3]

NHL has shown the greatest increase in incidence in the past 25 years, surpassed only by lung cancer and melanoma.[4] Although recent studies have provided some intriguing clues, the cause of what some experts call the 'NHL epidemic' is not known.

According to the National Cancer Institute working formulation, NHLs are classified as low, intermediate, and high grade. This classification scheme accurately predicts the survival of untreated patients, but is not as reliable in predicting the outcomes following treatment. Low grade lymphomas are slow-growing tumors, and some patients can survive for more than a decade without treatment. Although chemotherapy can often shrink low-grade lymphomas, the cancer usually recurs within five years. In contrast, intermediate-grade and high grade lymphomas are fast growing tumors that, without treatment, generally

Table 1. Immunophenotyping results

Marker	Cells expressing[5]	Function[5]	% Positive
	B-cell Panel		
HLA DR	B cell, monocytes, myeloid progenitors, activated T cells	Class II MHC, antigen presentation	86
CD 19	B cells	B cell activation	83
CD 20	B cells	Ca^{++} channel B cell activation	85
CD 10	Early B cells	Neutral endopeptidase	15
CD 5	T cells, B cell subset, CLL, Mantle cell lymphoma	T cell activation CD 72 ligand	0
s Kappa	B cells	Antigen recognition, B cell activation	81
s Lambda	B cells	Antigen Recognition, B cell activation	1
CD 23	Activated B cells, CLL	Fc epsilon RII (IgE receptor)	21
FM C7	The incidence at which FMC7 is positive is >30% of cells in intermediate lymphocytic lymphoma. With mantle cell lymphoma it is usually 40% to 80%; in CLL, it is 10% to 40%.[4]		22
	Other Markers		
CD 45	Panhematopoietic	Signal transduction: tyrosine phosphatase	98

are fatal within a year or two of diagnosis. Chemotherapy may cure many types of these lymphomas.

Research is also under way to evaluate the safety and effectiveness of monoclonal antibody therapies in NHL patients. Researchers have designed monoclonal antibodies that bind specific epitopes unique to lymphoma cells. The antibodies may be attached to radioactive compounds or toxins that kill the cells. Monoclonal antibody therapy is designed to more selectively target cancer cells, often resulting in less severe side effects than standard therapy. Researchers are also testing the anti-cancer potential of a number of compounds produced by immune cells. These compounds, which include interleukin-2 and alpha interferon, are usually given in addition to standard chemotherapy or radiation therapies.

Presentations at the American Society of Hematology 2003 meeting in San Francisco provided new insights into the diagnosis and treatment of aggressive NHL including the most common subtype, diffuse large B-cell lymphoma. Combining the monoclonal antibody Rituxan with standard chemotherapy is being touted as the first new drug combination in 20 years to improve overall survival in this group of patients.[4] The human chimeric monoclonal anti-CD20 antibody became available under the name Rituxan as the first monoclonal antibody approved by the United States Food and Drug Administration for treating malignancies, including NHL. Rituxan induced apoptosis in target cells with antibody and complement-dependent cellular cytotoxicity. It was less effective in relapsed and advanced disease; however, its specific targeting of B-cells has been further exploited to aim radionuclides at tumor cells while limiting toxicity to normal cells. Rituxan will bind to non-specific CD20 sites and decrease the number of circulating and competing normal B-cells, allowing the infused radionuclides to bind effectively to CD20 sites on the malignant cells. The overall response rate was 87% for low grade and 43% for intermediate NHL.[4] Clearly, the addition of a radionuclide to the specific monoclonal antibody improves the response rate to this treatment, which can also be integrated with chemotherapy and improve the cure rate of NHL.[5]

Rituxan is genetically engineered from portions of mouse and human antibodies and is produced through recombinant DNA technology. Animals, e.g., mice, rabbits, and goats, are immunized with the antigen for which a corresponding antibody is desired. Serum from the immunized animals is collected and purified. The antibodies obtained are polyclonal antibodies. Because they are made in vivo, they consist of many different kinds of antibodies with varying degrees of specificity for the antigen. Individual cells that secrete the desired antibody can be isolated from immunized animals. The selected cell is then fused with a neoplastic plasma cell that also has a natural propensity to make antibodies. This fused hybrid cell, called a "hybridoma", secretes only one kind of antibody. The serum produced by a culture of the hybridoma cells thus is rich in a 'monoclonal antibody'.

The addition of the monoclonal antibody Rituxan to the standard four drug combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy leads to significant prolongation of event-free survival in patients without significant additional toxicity. There are fewer adverse additional treatment-related side effects among CHOP-plus-Rituxan patients than CHOP alone. The majority of patients experienced infusion-related symptoms with their first Rituxan infusion. These symptoms include, but are not limited to flu-like fever, chills, nausea, urticaria, headache, bronchospasm, angioedema, and hypertension. These symptoms varied in severity and generally are reversible with medical intervention.[5]

Patients respond much quicker to the combination than to CHOP alone.[4] These results are compelling because standard chemotherapy has shown only a 30% to 40% cure rate in a disease that can be rapidly fatal.

CASE FOLLOW-UP

The patient presented with physical and laboratory findings consistent with an active stage of NHL. Following exploratory surgery and treatment, the patient entered remission. The patient has since returned to the physician for a routine checkup, where laboratory results indicated more abnormal cells in the abdomen. Upon further testing the cells were also found to have entered the lungs. Therapy was resumed, including radiation cycles as well as Rituxan. The patient continues to be treated in this manner.

REFERENCES

1. Department of Laboratory Medicine Hematopathology Laboratory. June 2000 University of Washington. Available at: http://depts.washington.edu/labweb/dept/div/heme/cds.html. Accessed March 10, 2004.
2. Bennett JM, and others. Proposals for the classification of chronic (mature) B and T lymphoid leukaemias. French-American-British (FAB) Cooperative Group. J Clin Pathol 1989;42:567-84.
3. The Lymphoma Research Foundation. March 2003. Lymphoma Research Foundation. January 2004. Available at: http://www.lymphoma.org/site/PageServer?pagename=learning. Accessed March 10, 2004.
4. Hindmarsh T. The use of monoclonal antibodies. Eur J Hematol 2002;69:21-3.
5. Witzig T. Monoclonal antibody Rituxan. Internat Soc Analyt Cytology Cytometry 2002;50:10-1.