OVERVIEW
Lymphomas represent about 4% of the new cases of cancer diagnosed in the U.S. each year. They constitute the fifth most common cancer diagnosis and the fifth leading cause of cancer deaths. In fact, although the incidence of most cancers is decreasing, lymphoma is one of only two tumors that are increasing in frequency. The cause of this increase is unknown.

B-cell lymphomas, a type of non-Hodgkin’s lymphoma (NHL), account for approximately 85% of the approximately 56,000 new cases of NHL diagnosed annually in the U.S. These lymphomas are specifically characterized in subtypes by numerous chemical and genetic attributes. They are generally classified into two subsets used to define outcomes: indolent (slowly growing) and aggressive (rapidly growing).

NON-HODGKIN’S LYMPHOMA
Classification
NHL comprises a heterogeneous collection of lymphoproliferative malignancies. These are most common in people older than 55 years of age.

- According to the World Health Organization (WHO) classification, diffuse large B-cell lymphoma (DLBCL) is the most common subtype of NHL, accounting for approximately 31% of all new patients.
- Follicular lymphoma (FL) is the second most common NHL subtype, accounting for another 22% of cases.
- Mantle-cell lymphoma (MCL), another subtype, represents 6% of cases.
- Small lymphocytic lymphoma with chronic lymphocytic leukemia (SLL/CLL) represents another 6% of NHL cases.

Demographics and Etiology
Over the past 30 years, there has been a steady increase in the incidence of NHL; more cases have been diagnosed in men than in women, at a median age above 50 years. Although the exact causes of B-cell lymphomas are unknown, several environmental factors and genetic abnormalities are believed to play a role.

Aggressive B-cell lymphomas are typically treated with radiotherapy, chemotherapy regimens, biologic agents, or a combination of treatments. The overall five-year survival rate for patients with NHL is approximately 60%, but response rates and survival vary greatly according to the grade and type of B-cell lymphoma being treated.

Genetic Factors
Recently, there has been great interest in a new molecular technique that has enabled clinicians to distinguish genetically between lymphomas. The Lymphochip is essentially a small piece of glass on which are contained, in a grid-like pattern, thousands of genes expressed by normal B cells. Using this gene chip, researchers can study the genetic expression of a particular malignant lymphoid cell.

Through this technique of gene-expression profiling, it has been discovered that DLBCL consists of two different diseases: germinal-center B-like DLBCL and activated B-like DLBCL.

More than 75% of patients with germinal-center B-like DLBCL were alive five years after treatment, compared with fewer than 25% of patients with the latter type. It is hoped that such information will help physicians understand the molecular basis of differences in treatment outcomes. Armed with this information, physicians may be able to identify patients who are not likely to respond to current treatment so that more targeted or experimental treatments may be offered.

The molecular classification of tumors according to gene expression can thus identify previously undetected and clini-
cally significant subtypes of cancer. Measurement of the expression of six genes is sufficient to predict overall survival in DLBCL. The genes that were the strongest predictors were LMO2, BCL6, FN1, CCND2, SCYA3, and BCL2. New therapeutic agents may be directed against one or several of these genes.

**TREATMENT**

**Standard Therapy**

At its most fundamental level, cancer has emerged as a disease of genes and DNA, and the entire message can now be interpreted with the aid of sophisticated techniques in molecular and cellular biology.

**Rituximab–CHOP Chemotherapy**

Aggressive chemotherapy may cure 50% of DLBCL cases; bone marrow transplantation might be able to rescue another 25%. Over the last eight years, the introduction of monoclonal antibodies—specifically the anti-CD20 monoclonal antibody rituximab (Rituxan, Genentech/Biogen Idec; MabThera, Roche)—has radically changed therapy for B-cell NHL.

Rituximab, a genetically engineered chimeric mouse/human monoclonal antibody, binds to the transmembrane antigen, CD20, a non-glycosylated phosphoprotein, located on pre-B and mature B lymphocytes. This antigen is expressed on more than 95% of all B-cell NHLs and on normal B cells; however, it is not expressed on hematopoietic stem cells, normal cells, or malignant plasma cells. The Fc domain of rituximab recruits immune effector functions to mediate B-cell lysis.

Possible mechanisms include complement-dependent cytotoxicity (CDC), resulting from C1q binding, and antibody-dependent cellular cytotoxicity (ADCC), mediated by one or more of the Fc gamma receptors on the surface of granulocytes, macrophages, and natural killer cells. It is also possible that the binding of rituximab to the CD20 antigen on the cell surface directly induces apoptosis.

For patients with both follicular and diffuse large B-cell NHL, several large-scale, prospective randomized trials have demonstrated prolongation of remission when rituximab is incorporated into first-line treatment. For patients with follicular lymphoma, rituximab can be a component of salvage therapy.

An Italian study was conducted from June 2002 to June 2004 in 26 patients with stage III/IV DLBCL. The patients ranged in age from 60 to 76 years. They received a regimen of rituximab, followed on the next day by CHOP chemotherapy—cyclophosphamide, doxorubicin (Adriamycin), and vincristine (Oncovin)—accompanied by prednisone, given for five days.

The patients also received granulocyte–colony-stimulating factor (G-CSF) on days 7 to 11 of each treatment cycle to minimize treatment-induced reductions in neutrophil counts and the accompanying risk of infection. The regimen was repeated every two weeks, for a total of six full cycles.

The intensity of this R-CHOP regimen, on the basis of its dose sizes and frequency of repetition at two-week intervals, was twice that originally reported for the regimen in 1984, when it had been used to treat metastatic breast cancer. As part of this study, patients in whom DLBCL had spread beyond the lymph nodes and patients with large or bulky sites of local disease were referred for radiotherapy when this was feasible.

Of the 26 patients in the study, 24 received all six planned cycles of treatment with the intensified R-CHOP regimen. Twenty of the 26 patients in the study (77%) achieved complete remissions, and six patients (23%) achieved partial remissions, for an overall response rate of 100%.

At a mean follow-up of 23 months, 79% of the patients were still alive. Despite the increase in treatment intensity from the original CHOP protocol, only one patient in the study died as the result of a stroke. Although a significant reduction in the cardiac left ventricular ejection fraction was among the potentially serious toxic effects of the intensified regimen, clinically evident heart failure developed in only one patient, and it was successfully managed with medication.

Six patients experienced severe grade 3/4 adverse hematological effects from the treatment regimen in the form of neutropenia or anemia; three other patients developed severe infections or diarrhea. All patients in the study experienced alopecia.

Although a larger multicenter trial is needed to determine the effects of dose-intensive R-CHOP therapy for DLBCL, this study was an excellent example of the poor prognosis of the patients at the time of their diagnosis. The results with the intensified R-CHOP regimen were encouraging and confirmed the safety and efficacy of a dose-dense regimen of CHOP for treating older patients with an aggressive, high-risk NHL such as DLBCL. Furthermore, the addition of rituximab appeared to increase the rate of response from the regimen without increasing toxicity.

As a result of these and other studies, rituximab may be included for previously untreated patients with stage III/IV follicular lymphoma in combination with cyclophosphamide, vincristine, and prednisone (CVP) chemotherapy.

For maintenance therapy, rituximab may be used in patients with relapsed follicular lymphoma who respond to induction therapy with chemotherapy or immunochemotherapy. Six courses of rituximab, perhaps as the gold standard, are effective in the treatment of patients with DLBCL in combination with CHOP chemotherapy.

**Emerging New Targets**

**Aggressive Non-Hodgkin’s Lymphoma**

(Open Large B-Cell Lymphoma)

The practice of treating patients with relatively nonspecific cytotoxic compounds, such as chemotherapy, is now being complemented by a new era of targeted lymphoma drug development. Researchers are now able to identify a wide array of molecular targets and agents. The new agents include several broad classes of drugs that:

- modulate or change the expression of important genes in the cancer cell.
- intercept the messages generated by detrimental gene expression.
- represent repackaged or reformulated derivatives of tried-and-true favorites.

Several newly available drugs have a unique effect on DNA. In normal cells, DNA exists in two states: condensed and open.
A simple way to envision a molecule of DNA is to think of a long piece of thread upon which an important message is encrypted. If that piece of thread were rolled up into a tight ball, it would be in a condensed state and we would be unable to read the encrypted message. If that piece of thread were uncurled, it would exist in an open state, at which point it would be possible to read the encoded message.

In the cell, the balance between these two DNA states is tightly maintained by two sets of opposing enzymes: the histone acetyl-transferases (HATs) and the histone deacetylases (HDACs). Some theories suggest that cancer cells behave abnormally because the cells cannot read certain important messages that instruct them how to behave, although this is admittedly an overly simplistic interpretation.

**HDAC Inhibitors.** Recently, a class of drugs known as HDAC inhibitors has been developed. Examples include:

- the anticonvulsant agent valproic acid (e.g., Depakene, Abbott).
- phenylbutyrate (trIButyrate, Triple Crown America/Eurohealth), originally developed for urea cycle disorders.
- depsipeptide (Romidepsin, Gloucester Pharmaceuticals).
- suberoylanilide hydroxamic acid (SAHA, Aton Pharma; Zolinza, Merck).
- LAQ-824 (Novartis Oncology).

These drugs are being studied in various phase 1 and 2 clinical trials, and they have shown intriguing activity in the treatment of many kinds of lymphoma.

For example, in patients with T-cell lymphomas of the skin that are refractory to standard chemotherapy, depsipeptide has brought about significant regression of disease. Vorinostat has produced significant tumor shrinkage in patients who have experienced a relapse of disease even after stem-cell transplantation. One patient with a difficult-to-treat transformed lymphoma, in fact, achieved a complete remission.

Several studies have suggested that these agents might work by turning on genes that instruct lymphoma cells to stop dividing and even to die. Although the precise mechanism of action is probably more complicated than this, HDAC inhibitors will undoubtedly emerge as valuable adjuncts to future lymphoma therapies.

**Oblimersen.** If HDAC inhibitors can play a role in determining gene expression, drugs like oblimersen (G3139, Gensense, Genta Corp.) help to prevent information in the DNA that has already been “turned on” (transcribed) from becoming a frank messenger (protein). Oblimersen belongs to a new class of agents known as antisense oligonucleotide molecules; they are against the “sense” (the normal message); the prefix oligo- represents “a short piece of;” and nucleotide represents “nucleic acid.” These antisense molecules are short pieces of nucleic acid that bind to and destroy specific messages (messenger RNA) produced by cancer cells.

Oblimersen binds to a specific message in the cell known as Bcl-2. Bcl-2 is overexpressed in many kinds of lymphoma, including DLBCL and follicular lymphoma, and it helps to prevent cells from undergoing apoptosis. Bcl-2 is often referred to as being anti-apoptotic, because it promotes tumor cell survival and it can compromise the effects of chemotherapy.

Many laboratory studies have validated the potent activity of oblimersen, and many clinical studies are now under way to determine its importance in the treatment of several cancers. Oblimersen probably works best when it is integrated into existing chemotherapy programs; in theory, it should sensitize lymphoma cells to the toxic effects of chemotherapy.

**Epratuzumab.** Epratuzumab (AMG 412, LymphoCide, Angen/Immunomedics), a monoclonal antibody, targets the CD22 antigen, a protein found on the surface of B lymphocytes, particularly on B-cell cancers. The combination of rituximab and epratuzumab appears to be well tolerated and potentially effective in the treatment of patients with relapsed or refractory B-cell NHL. Even though only six patients of the 23 in this study had aggressive NHL (the rest had low-grade disease), detectable cancer completely disappeared in 50% of these patients after the combination treatment. The most common side effects of treatment were fever, shivering, and fatigue.

**Bevacizumab.** Bevacizumab (Avastin, Genentech) interferes with the cancer cell’s blood supply, which is needed for cell survival and growth. The FDA has approved this drug for patients with advanced colon cancer in combination with chemotherapy. Bevacizumab has demonstrated modest anti-cancer activity as a single agent in the treatment of aggressive, relapsed NHL. Future studies are planned to evaluate bevacizumab in combination with chemotherapy for patients with advanced NHL.

**Gemcitabine.** In combination with rituximab, gemcitabine (Gemzar, Eli Lilly) may offer the advantage of fewer adverse events than other therapies for relapsed NHL. In seven patients with aggressive NHL that had relapsed after CHOP treatment with gemcitabine and rituximab resulted in two complete remissions and three partial remissions.

**DICE.** A phase 2 study of dexamethasone, ifosfamide, cisplatin (Platinol, Bristol-Myers Squibb), and etoposide (VePesid, Cipla) (DICE) as salvage chemotherapy for patients with relapsed and refractory lymphoma resulted in responses in the treatment of patients with relapsed NHL who had not responded to an autologous transplant. Overall, 40% of patients lived three years or more after treatment.

**Epothilone B.** A phase 2 study of the epothilone B analogue BMS-247550 (NSC 710428, Bristol-Myers Squibb) in patients with relapsed aggressive NHL is currently in progress. The epothilones are a new class of cytotoxic molecules, including epothilone A, B, and D, and are identified as potential chemotherapy drugs. Their mechanism of action is similar to that of the taxanes, but their chemical structure is simpler and they are more soluble in water.

Early studies in cancer cell lines and in human cancer patients indicate superior efficacy to the taxanes. Epothilones were originally identified as metabolites produced by the myxobacterium *Sorangium cellulosum*.

**Indolent Lymphoma**

Indolent NHL is a slow-growing form of lymphoma. It encompasses what had been called low-grade NHL and some categories of intermediate-grade NHL. Depending on the type of cancer, patients today often live years without a lot of treatment. The usual strategy had been to wait until symptoms were manifested to a large degree before treatment was ordered.
However, with newer immunotherapies now available, the era of watching and waiting may be over, because effective treatment may begin as soon as the lymphoma is detected. Immunotherapies include rituximab; alemtuzumab (Campath, Genzyme); and the conjugated monoclonal antibodies ibritumomab tiuxetan (Zevalin, Biogen Idec) and 131-I tositumomab (Bexxar, GlaxoSmithKline). These last two agents are linked to a radioactive isotope to target and kill specific cancer cells.

**Follicular Lymphoma**

**Rituximab Combinations.** In a large study by the European Organization for Research and Treatment of Cancer (EORTC), rituximab maintenance therapy improved progression-free and overall survival in patients with relapsed or refractory follicular lymphoma following induction with CHOP or R-CHOP when compared with placebo maintenance. The investigators concluded that R-CHOP and R-fludarabine phosphate (Fludara, Berlex) combinations were superior to rituximab alone or rituximab plus R-CVP in producing complete remissions. In addition, rituximab/fludarabine combinations produced complete remissions in 85% of patients; by contrast, R-CHOP led to complete remissions in 65% of patients.

Researchers from the M.D. Anderson Cancer Center in Houston, Texas, reported that the addition of rituximab to CHOP provided a high response rate and excellent early survival in a group of 45 patients with newly diagnosed follicular lymphoma who were considered to have a good prognosis. These patients had a complete response rate of 96%. With a median follow-up of 33 months, a three-year failure-free survival of 73% was reported, with an overall survival of 97%.

**Cytotoxic Agents.** Newer cytotoxic agents for follicular lymphoma include:

- bendamustine HCl (Treanda, Cephalon) (alkylating and nucleoside analogue moieties).
- proteasome inhibitors (Millennium; A. G. Scientific).
- bortezomib (Velcade, Millennium) alone or in combination with rituximab.
- targets of apoptosis.
- antisense (Genasense).
- pan–Bcl-2 inhibitors (obatoclax mesylate, GX15-070MS, Boehringer Mannheim).
- BH1 (Bcl-2 homology 3) domain–only small molecules and cell death receptors (tumor necrosis factor-related apoptosis-inducing ligand [TRAIL monoclonal antibody]).
- immunotoxins.
- calicheamicin, a natural product derived from Microcospora echinospora.
- gemtuzumab ozogamicin (Mylotarg, Wyeth), an antileukemia drug based on calicheamicin.
- inotuzumab ozogamicin (CMC-544, Wyeth), a compound similar to calicheamicin.

**Immune-Based Agents.** Other immune-based therapies include:

- cell-based vaccines (autologous tumor cells plus GM-CD40L plus low-dose interleukin-2).
- pulsed dendritic cells (Life Technologies, Inc.; Boehringer Mannheim)

Bendamustine does not interfere with other commonly used chemotherapy alkylating agents. It has been used in Germany for many years for the treatment NHL, CLL, multiple myeloma, breast cancer, and other solid tumors such as lung cancer. When bendamustine was administered to patients who had stopped responding to prior therapy, 74% experienced an anticancer response and more than one-third (39%) experienced a complete disappearance of detectable cancer.

The outcome in patients with follicular lymphoma has changed, showing clear benefits in progression-free survival and overall survival, as with the use of monoclonal antibodies along with chemotherapy. The long-term impact is still unclear, but this is the first time that such treatment demonstrated improvement in overall survival in follicular lymphoma.

**SUGGESTED READINGS**