A New Approach to Non-Hodgkin's Lymphoma

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Abstract

The incidence of non-Hodgkin's lymphomas continues to rise and the number of deaths attributable to these malignancies currently rank in the top five causes of cancer-related deaths in the United States. Recent insights into the pathogenesis and molecular characterization of these disorders have led to improvements in a clinicopathologically relevant classification system, as well as in the ability to accurately diagnose specific entities. Several new treatment modalities have been developed which may improve the outcome for selected patients. A better understanding of the biology of non-Hodgkin's lymphomas will hopefully translate into different therapies with improved outcomes. This article describes a new system for classifying the non-Hodgkin's lymphomas, highlights key aspects of the pathogenesis of non-Hodgkin's lymphomas and briefly reviews clinical and therapeutic approaches to the most common of these lymphomas.

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Introduction

Non-Hodgkin's lymphomas (NHL) are a group of malignancies of lymphoid origin that can be distinguished based on their clinicopathologic characteristics. As a group, NHL are the most frequently diagnosed malignancy of the immune system and the fifth most common cause of cancer-related deaths in the United States (1, 2). In the U.S. alone, over 55,000 new cases were expected in 1998 (2).

The incidence of NHL continues to show a steady increase. The reason for this increase in incidence is not clear, although various factors have been implicated, such as an increase in the aging population and a rise in immunosuppressive states (e.g., acquired immunodeficiency syndrome (AIDS), organ transplants). The combined influence of these and almost certainly other factors represents the explanation for this trend (3).

Classification of Non-Hodgkin's Lymphoma

The diversity in biological characteristics among the subtypes of NHL is reflected in differences in clinical characteristics, survival and response to treatments. For this reason, multiple efforts at classifying these disorders have been undertaken with the hope of providing common terminology that will allow for appropriate comparisons of treatment and prognosis between patients. This will, in turn, allow clinicians to be better informed about tumor behavior in order to tailor treatment to patient and tumor characteristics.

Early in this century, the distinction between Hodgkin's and non-Hodgkin's lymphoma became possible through identification of the Reed-Sternberg cell (4). The classification systems for NHL that ensued were based initially on morphologic characteristics. Perhaps the most widely accepted of the morphologic classification systems was the Rappaport classification which divided lymphomas into those with large or small cells with or without a follicular (nodular) growth pattern (5). Advances in immunology led to the ability to differentiate B-lymphocytes from T-lymphocytes. Armed with this information, new classification schemes were proposed that were based on the cell source and the grade of malignancy. In this area, the Kiel classification gained acceptance in Europe while the Lukes and Collins classification was more common in North America (6). A National Cancer Institute-directed effort resulted in the Working Formulation which divided the lymphomas primarily according to clinical outcome using the treatment protocols from that period. It used similar descriptions as in the Rappaport classification with updated terminology from the Lukes and Collins classification and grading from the Kiel classification (7). However, several flaws were inherent to this classification system: 1) it was based on clinical outcome as opposed to individual entities, thus, ignoring important characteristics such as cell of origin; 2) it separated large cell lymphomas into intermediate and high grade categories, a division which has not been supported in subsequent analysis (7).

Continued advances in laboratory technology gave way to the demonstration of consistent differences, at a molecular level, among morphologically similar tumors. This inevitably has led not only to the description of "new" entities, many of which relied heavily on cytogenetic studies for recognition, but also

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an increased understanding of the biology of lymphoid malignancies. Perhaps more importantly in a practical sense, the ability to more accurately diagnose lymphomas has also been strengthened with this new technology (8).

This new data on the biology of lymphomas led to a broader consensus on many topics. Thus, the International Lymphoma Study Group published the Revised European-American Lymphoma (REAL) classification in 1994 (9). The REAL classification focused on each disease as a distinct entity with its own clinical and laboratory features. It also emphasized the importance of distinguishing the histologic grade in addition to underscoring the biologic importance of the site of involvement (i.e., nodal vs. extranodal) (7).

The ability to reproduce the REAL classification scheme was one of the goals of the Non-Hodgkin's Lymphoma Classification Project. This group of expert clinicians and hematopathologists sought to determine the inter- and intraobserver reproducibility in the diagnosis of the various entities as well as to determine the importance of immunophenotyping in establishing the diagnosis. The Non-Hodgkin's Lymphoma Classification Project also evaluated the clinical characteristics of the various entities providing valuable insight as to the importance of these characteristics in determining prognosis and treatment.

Some important conclusions from this study are:

1. The reproducibility among the expert hematopathologists was equal to or greater than 85% for most histologic subtypes. Agreement was less than 85% for lymphoplasmacytoid lymphoma, nodal marginal zone B-cell lymphoma, high-grade B-cell Burkitt's-like lymphoma and among the various grades of follicular lymphoma.

phoma.

2. Although there was some overlap between histologic types for a particular characteristic, the new entities appeared to have distinct clinical characteristics.

3. Significant variability in survival rates within specific histologic types was observed and correlated with the number of adverse risk factors for a given patient (10).

Based on the REAL concept and classification, an international panel of experts is developing a classification that will be adopted by the World Health Organization, culminating an effort to achieve worldwide consensus on the classification of hematologic malignancies (Table 1) (7).

Biology of Non-Hodgkin's Lymphomas

Significant insight has been made in elucidating the pathogenesis of NHL through the identification of chromosomal translocations/mutations and the discovery of various biological agents that play a role in tumorigenesis. Many of these alterations in genetic material have been shown to play a role in tumorigenesis through the use of transgenic animal models. Furthermore, the repeated association of certain alterations with specific NHL subtypes makes it possible to use them as diagnostic markers (4, 8, 11).

Viruses play a role in initiating a malignant lymphoproliferation by introducing viral genetic material (4, 12). The mechanisms by which these gene alterations, whether by chromosomal translocations or virus-mediated changes, cause tumors are two-fold: a) activation/overexpression of oncogenes, or b) inactivation of tumor suppressor genes (4).

Table 1. The Proposed World Health Organization Classification of Non-Hodgkin's Lymphomas

<u>Precursor Cell Disorders</u>	
<u>B-cell</u>	<u>T-cell</u>
Lymphoblastic lymphoma	Lymphoblastic lymphoma
<u>Mature or Peripheral Cell Disorders</u>	
<u>B-cell</u>	<u>T-cell</u>
Small lymphocytic/ chronic lymphocytic leukemia	Mycosis fungoides
Follicular	Peripheral T-cell (unspecified)
Marginal zone	Angioimmunoblastic
Nodal	Angiocentric (nasal-type)
Mucosa-associated lymphoid tissue	Enteropathy-type intestinal
Splenic	Anaplastic large-cell
Mantle cell	Adult T-cell leukemia/lymphoma
Diffuse large B-cell	
Primary mediastinal large B-cell	
Burkitt	

Activation/overexpression of oncogenes

The reason these chromosomal translocations in NHL occur is not clear but most commonly seems to involve errors in antigen receptor gene rearrangements. In the B-lymphocytes, this would occur in the immunoglobulin gene and, in the T-lymphocytes, in the T-cell receptor genes (4, 8). The pattern of abnormal expression of an associated oncogene can be either through homotopic deregulation, in which the pattern of expression of a protooncogene normally expressed in the lymphocyte is altered, or heterotopic deregulation, in which a protooncogene normally *not* expressed is expressed. An alternative mechanism is the production of a new protein through the formation of a chimeric gene. This mechanism is more common in acute leukemias but within NHL is seen in anaplastic T-cell lymphoma (4).

Inactivation of tumor suppressor genes

The most common genetic alteration in human neoplasms is thought to be deletions or mutations of the p53 suppressor gene resulting in underexpression of p53 (4, 8). This alteration occurs in NHL and is seen in certain subtypes such as Burkitt's lymphoma and transformed diffuse large cell lymphoma. The mechanism of this alteration is also by point deletion or mutation (4, 8).

The frequent occurrence of specific chromosomal deletions has raised the suspicion of previously unidentified tumor suppressor genes. A common deletion encountered in NHL involves 6 (6q). Fifty percent of cases of small lymphocytic lymphoma/B-cell chronic lymphocytic leukemia contain deletions of chromosome 13q14, suggesting the presence of a tumor suppressor gene at that site as well (4).

Viral oncogenesis and chronic antigenic stimulation

Numerous viruses have been associated with the pathogenesis of NHL (12). The most widely recognized association is between Epstein-Barr virus (EBV) and various lymphoproliferative disorders, including Burkitt's lymphomas, human immunodeficiency virus (HIV)-associated lymphomas and lymphomas associated with other immunodeficiency states (12). Epstein-Barr virus can significantly alter B-cells, causing both immortalization and proliferation. The terminal repeats of the viral genome vary greatly in their sequence. This variability between circular episomes of EBV can serve as a marker of clonality. Frequently, EBV-associated lymphomas demonstrate the same terminal episomal sequence within the clonal lymphocyte population, suggesting origination from a single infected cell (13).

HTLV-I has been linked to adult T-cell leukemia/lymphoma. HTLV-I infection of the clonal cell population has been detected in nearly all cases. In contrast to EBV which alters the lymphocyte genome, HTLV-I produces a protein that activates host genes such as interleukin-2, *c-sis*, *c-fos* and granulocyte-macrophage-colony-stimulating factor (4, 12).

Human herpes virus 8 has been associated with acquired immune deficiency syndrome (AIDS)-associated body-cavity-based lymphomas in virtually all cases. The viral genome can

be found within the tumor cells. However, the exact mechanism of pathogenesis has yet to be elucidated. Furthermore, EBV can be detected in a high proportion of AIDS-body cavity-based lymphoma. Whether the presence of two viruses indicates a synergistic effect between the two has yet to be determined (4, 12).

An association between hepatitis C virus and NHL has been proposed due to the role of hepatitis C virus in mixed cryoglobulinemia, a disorder which can progress to NHL (14, 15). However, several studies looking at the incidence of hepatitis C virus in patients with NHL have shown a low prevalence of hepatitis C virus infection in these patients (16, 17). In one study, the prevalence of infection with hepatitis G virus in patients with NHL was higher than hepatitis C virus (17). Insufficient data is available at this time to be certain of an etiological association between hepatitis C virus infection and NHL.

Chronic antigenic stimulation has been proposed as one of the causal mechanisms in the development of some lymphomas (4). A fine example of this hypothesis is the relationship between mucosa-associated lymphoid tissue lymphomas and *Helicobacter pylori* (18). Zucca et al elegantly documented the progression from *H. pylori*-associated chronic gastritis to mucosa-associated lymphoid tissue lymphoma of the stomach (19). Eradication of *H. pylori* with antimicrobial medications has resulted in regression of mucosa-associated lymphoid tissue lymphomas in some patients (18).

Similar ideas have been proposed to explain, for instance, the relationship between BCL-2 [t(14;18) (q32;q21)] and follicular lymphomas. Transgenic mice with BCL-2 present in their genome develop polyclonal hyperplasia of mature, long-lived B-cells. The polyclonal nature of this hyperplasia contrasts sharply with the monoclonal consistency of human follicular lymphomas. Chronic antigenic stimulation would be synergistic with BCL-2 activation in driving a monoclonal expansion of B-cells (4, 8).

Immunophenotypic and Genotypic Findings in NHL

Advances in immunophenotypic analysis have led to improvements in the ability to accurately identify and diagnose NHL. If the cell composition of the neoplasm is monomorphic, determining B-cell or T-cell lineage is relatively straightforward (e.g., one or more B-cell markers and no T-cell markers).

However, if the cellular composition of the tumor specimen is polymorphic or includes many reactive cells, the determination may be much more difficult. An example of this would be "T-cell-rich B-cell lymphoma" (8).

The primary markers used to detect B-cell lineage are CD19, CD20 and CD22. These have the greatest sensitivity and specificity in frozen tissue samples. In paraffin sections, the most useful is L26, a monoclonal antibody that recognizes an intracellular epitope of CD20. In some cases of polymorphic composition, the diagnosis of B-cell lymphoma requires the demonstration of monoclonality using immunohistochemistry for

the detection of light chain restriction or by detection of an immunoglobulin gene rearrangement (8).

T-cell lymphoma immunophenotypic identification is more complex. The detection of one or more pan-T-cell markers (CD2, CD3, CD5 and CD7), in the absence of B-cell markers, confirms T-cell lineage. Most T-cell lymphomas express at least one of these markers. For example, 73% of lymphoblastic T-cell lymphomas express all 4 antigens and 81% of peripheral T-cell lymphomas express 2 or more antigens. However, T-cells lack mutually exclusive antigens (i.e., kappa or lambda light chains) making it difficult to establish monoclonality. To accomplish this, subset antigens (i.e., TdT, CD1a, CD4, CD8, CD99, cdk6) must be detected. Besides assisting in the diagnosis of T-cell lymphomas, subset antigens allow for the subclassification of these lymphomas (8).

The identification of genetic anomalies in NHL has assisted the field of lymphoma medicine in two significant ways: 1) it has aided in elucidating the molecular basis for lymphomatous transformation, and 2) it allows for the identification of neoplasms by the association with specific genetic alterations (Table 2) (4, 8).

The B-cell NHL known or postulated to have an association with specific genetic alterations include small lymphocytic lymphoma/B-cell chronic lymphocytic leukemia, lymphoplasmacytoid lymphoma, follicular lymphoma, mantle cell lymphoma, diffuse large B-cell lymphoma, mucosa-associated lymphoid tissue lymphoma, multiple myeloma and Burkitt's lymphoma. Although the molecular biology of T-cell NHL is not as well understood as that of the B-cell NHL, some data is available for anaplastic large T-cell lymphoma, cutaneous T-cell lymphoma and adult T-cell leukemia/lymphoma (4, 8).

Small lymphocytic lymphoma/B-cell chronic lymphocytic leukemia

This is a clonal B-cell disorder (100% have heavy or light chain rearrangement). Approximately 50% carry genetic ab-

normalities although no one unique alteration has been reported. The most common aberrations include gains of chromosomal material at 8q and 12, and the loss of genetic material at 6q, 11q, 13q and 17p. Deletions of 13q14 have been noted in close to 50% of cases, suggesting this may be the location of a tumor suppressor gene. Aberrations of the p53 tumor suppressor gene have been detected on approximately 10% of these neoplasms and its frequency is increased in those tumors that transform to a more aggressive histology (Richter's syndrome). The latter finding certainly provides evidence of a potential role for this gene in the development towards a more aggressive histology (4, 8, 9).

Trisomy 12 is reported in a third of the cases but may be seen in other normal CD 34+ hematopoietic precursor cells of the same patients. The finding of trisomy 12 correlates with poor survival in patients with small lymphocytic lymphoma/chronic lymphocytic leukemia (4). Initial reports suggested BCL-1, BCL-2 and BCL-3 may have been related to small lymphocytic lymphoma/chronic lymphocytic leukemia but recent studies have demonstrated no association with BCL-1 or BCL-2 (8).

Lymphoplasmacytoid lymphoma

About half of lymphoplasmacytoid lymphomas are associated with the translocation t(9;14) (p13;q32) (8, 9). The PAX-5 gene encodes for a B-cell specific transcription factor involved in B-cell proliferation and differentiation. PAX-5 is located at 9p13 whereas 14q32 contains an immunoglobulin heavy chain locus. Thus, the approximation of these two genes could result in the deregulation of PAX-5 with subsequent tumor development (4).

Mantle cell lymphoma

These lymphomas are typically associated with t(11;14) (q13;q32) which juxtaposes the BCL-1 locus at 11q13 with the immunoglobulin heavy chain locus (8, 9). This alteration leads

Table 2. Major Genetic Lesions and Their Distribution Among Non-Hodgkin's Lymphoma Categories

Gene	Translocation	Type and % of Lymphomas Associated with Gene
BCL-1	t (11;14) (q13;q32)	Mantle cell lymphoma (70%)
BCL-2	t (14;18) (q32;q21)	Follicular lymphoma (90%) Diffuse large cell lymphoma, "de novo" (20%) Diffuse large cell lymphoma, transformed (100%)
BCL-6	t (3;-) (q27;-) ^a	Diffuse large cell lymphoma (40%)
C-MYC	t (8;-) (q24;-) ^a	Burkitt's lymphoma (100%)
NPM/ALK	t (2;5) (p23;q35)	CD30+ Anaplastic large cell lymphoma (80%)
PAX-5	t (9;14) (p13;q32)	Lymphoplasmacytoid lymphoma (50%)
p53	t (17;-) (p13;-) ^a	Small lymphocytic lymphoma/B-cell chronic lymphocytic leukemia (10%) Diffuse large cell lymphoma, transformed (90%) AIDS-Burkitt's lymphoma (60%) Burkitt's lymphoma (30%)

^a Numerous sites of translocation may be involved with these genes.

to homotopic deregulation of BCL-1. BCL-1 encodes for cyclin D1 which is involved in early cell cycle regulation (4). The frequent occurrence of this genotype in mantle cell lymphoma (70% of cases) makes it a very useful marker for the diagnosis of this malignancy (8, 11). Point mutations, deletions or rearrangements of cyclin dependent inhibitors p15, p16 and p18 genes may be associated with a more aggressive clinical course and extranodal dissemination in a subset of mantle cell lymphomas (4).

Follicular lymphomas

Most follicular lymphomas contain rearrangements of BCL-2 with a break at 18q21 (8, 11). The most common translocation of lymphoid malignancies is t(14;18) (q32;q21) although other biological equivalents can be encountered, including t(2;18) (p11;q21) and t(18;22) (q21;q21) (8). This translocation involves the BCL-2 locus and the immunoglobulin heavy chain J segment, resulting in homotopic deregulation of BCL-2 (4).

BCL-2 is a mitochondrial protein with antiapoptotic properties. As noted earlier, the presence of BCL-2 in transgenic mice produces a polyclonal hyperplasia of B-cells. The occurrence of a monoclonal cell population as seen in human follicular lymphoma suggests a concomitant mechanism such as chronic antigenic stimulation. A fraction of follicular lymphomas will undergo transformation into an aggressive lymphoma usually with a diffuse large-cell histology. This transformation generally is accompanied by mutations and/or deletions of the p53 suppressor gene (4). BCL-2 protein expression is useful in distinguishing reactive from neoplastic follicles because it is usually absent from reactive follicles and present in most follicular lymphomas. It is important to note that normal lymphoid tissue containing BCL-2-immunoglobulin gene rearrangement has been documented (8).

Diffuse large B-cell lymphoma

The molecular basis for neoplastic transformation in diffuse large B-cell lymphoma involves genetic lesions specific for this disease as well as chromosomal alterations seen in other types of NHL. The BCL-6 translocation can be found in 40% of "de novo" diffuse large B-cell lymphoma and involves the 3q27 band (4, 8, 9). Numerous translocation sites have been identified for this promiscuous gene including juxtaposition with 14q32 (immunoglobulin heavy chain), 2p11 (immunoglobulin light chain kappa) and 22q11 (immunoglobulin light chain lambda). The usual consequence of these translocations is deregulated expression of BCL-6 by a mechanism called promoter substitution (i.e., the promoter sequence is either truncated or completely removed) (4).

The role of BCL-6 in the development of diffuse large B-cell lymphoma is still unclear. It is found expressed in the germinal center B-cells but not in the differentiated plasma cells derived from this group, thus suggesting a role in the differentiation of B-cells (8).

The multiple changes noted in the BCL-6 gene within diffuse large B-cell lymphoma certainly supports the theory that

other mechanisms besides chromosomal rearrangement may be at work. Nearly all diffuse large B-cell lymphomas will have a mutation or rearrangement of this gene (4). Furthermore, up to 70% of diffuse large B-cell lymphomas have multiple alterations of the BCL-6 gene. This multiplicity of genotypic findings in diffuse large B-cell lymphoma provides support to the perceived heterogeneity of diffuse large B-cell lymphoma, although the REAL classification study highlighted the impracticality of subclassifying diffuse large B-cell lymphomas from the standpoint of diagnostic reproducibility and clinical significance (4, 9). There is little reason not to believe that a more precise subdivision of these lymphomas will eventually be achieved. A recent study noting the prognostic significance of the histologic subtype of immunoblastic B-cell NHL is an example of this trend (20).

Other genetic alterations seen in diffuse large B-cell lymphomas include mutations and/or deletions of the p53 tumor suppressor gene and rearrangements of BCL-2. As mentioned earlier, p53 alterations can be found in diffuse large B-cell lymphomas arising after transformation from a follicular histology (4). The significance of BCL-2 gene rearrangement has been controversial. The presence of BCL-2 gene rearrangement in de novo diffuse large B-cell lymphoma was thought to suggest a follicle center cell origin with a clinical behavior similar to low-grade NHL. However, Gascoyne et al reviewed BCL-2 gene rearrangement and protein expression in 139 patients with diffuse large B-cell lymphoma and found that the presence of gene rearrangement had no significant influence on survival whereas BCL-2 protein expression was a strong significant predictor of overall survival, disease-free survival and relapse-free survival (21).

Burkitt's lymphoma

Burkitt's lymphoma can be classified as two lymphoma variants (sporadic or endemic) or as a leukemic variant containing Burkitt's tumor cells (L3 acute lymphoblastic leukemia) (4). All cases of Burkitt's lymphoma are associated with alterations involving 8q24 (8). There is also a variable association with other genetic lesions.

Alterations of 8q24 involve the C-MYC locus in conjunction with either an immunoglobulin heavy chain or immunoglobulin light chain (kappa or lambda) locus. The final result of any of these translocations is overexpression of C-MYC which encodes for a transcription regulator of cell proliferation, differentiation and apoptosis. Overexpression of C-MYC conceivably results in the predominance of a heterodimer complex which induces B-cell growth (4).

Other genetic lesions in Burkitt's lymphoma include inactivation of p53 in up to 30% of sporadic and endemic Burkitt's lymphoma, deletion of 6q and infection with EBV, the latter present in all cases of endemic Burkitt's lymphoma and a third of sporadic Burkitt's lymphoma cases (4, 8).

Lymphomas in immunocompromised patients

Patients with acquired or congenital immunodeficiency states are at an increased risk for developing lymphoprolife-

rative disorders (12). Of particular interest is the association of EBV with these disorders. Primary EBV infection in patients with severe combined immunodeficiency or Wiskott-Aldrich syndrome among others can lead to the development of a lymphomatous disorder. The same can occur to patients with an acquired immunodeficiency state such as AIDS or iatrogenic immunosuppression to prevent rejection of solid or hematopoietic organs. AIDS-related NHL can be classified in AIDS-Burkitt's lymphoma, AIDS-diffuse large B-cell lymphoma or AIDS-related body cavity-based lymphoma (4). C-MYC activation can be found in 100% of AIDS-Burkitt's lymphoma and EBV infection in 30% of those same patients. This frequency of EBV parallels that seen in sporadic Burkitt's lymphoma. Furthermore, p53 mutations can be found in up to 60% of patients (4, 11).

AIDS-diffuse large B-cell lymphoma expresses EBV infection in 70% of cases (compared with virtually none in immunocompetent patients) (11, 12). Also, BCL-2 is hardly encountered in AIDS-diffuse large B-cell lymphoma and BCL-6 is found in a minority (20%) of patients. Additionally, C-MYC alterations are detected in about a fifth of AIDS-diffuse large B-cell lymphomas. AIDS-related body cavity-based lymphomas have not shown alterations of protooncogenes or tumor suppressant genes but consistently demonstrate infection with human herpes virus 8 and EBV (4, 11, 12).

Posttransplant lymphoproliferative disorders occur in patients who receive immunosuppression due to organ transplantation. This group of disorders are generally associated with EBV infection although "late-onset" posttransplant lymphoproliferative disorders (i.e., presenting approximately five years after transplant) do not seem to be associated with EBV infection (22). Although oncogene alterations are found in posttransplant lymphoproliferative disorders, none occur on a consistent basis (11). However, BCL-6 was found to be present in approximately 45% of one patient series and correlated with a poor response to withdrawal of immunosuppression (23).

Anaplastic large T/null-cell lymphoma

This malignancy is characterized by a strong expression of CD30 (Ki-1). The majority of these lymphomas will express a T-cell phenotype. Those lymphomas with anaplastic features that express neither B- or T-cell phenotype are designated as "null" (24). The REAL classification grouped anaplastic lymphomas with B-cell phenotype within the category of diffuse large B-cell lymphoma, citing a relative absence of differences that make them clinically distinct from other diffuse large B-cell lymphomas (6, 24).

A recurrent cytogenetic abnormality has been detected which involves the translocation t(2;5) (p23;q35). This translocation affects two genes, NPM and ALK, resulting in heterotopic deregulation of ALK (4). Activation of the ALK receptor tyrosine kinase results in an unregulated mitogenic signal. Gene transfer of NPM-ALK in mice produces a B-cell lymphoid malignancy (24). It is notable that NPM-ALK selectively associates with anaplastic large cell lymphoma of T-cell origin (24). As with mantle cell lymphoma, this genetic finding has diagnos-

tic importance, particularly given the potential misdiagnosis of anaplastic large cell lymphoma with other non-lymphoid malignancies such as metastatic carcinoma, melanoma and sarcoma.

Anaplastic large T/null-cell lymphomas have two modes of presentation. One presentation is as a primary cutaneous disorder occurring predominantly in older adults, of an indolent nature with a regressing-relapsing course. The second type of presentation is more common, systemic in its involvement and affecting children and young adults. It resembles other large cell lymphomas in its aggressive behavior but responds favorably to cytotoxic chemotherapy (6, 24, 25).

Management of Non-Hodgkin's Lymphoma

Despite the progress that has been made in the treatment of non-Hodgkin's lymphomas, the majority of patients will not be cured of these diseases. Due to the increased understanding of the pathogenesis of lymphomas and the recognition of certain lymphomas as distinct entities, the approach to the patient with NHL must involve a comprehensive evaluation not only of the patient's characteristics and prognostic parameters, but also must take into account the characteristics of the specific lymphoma type. No longer should NHL be classified into the broad categories of low, intermediate or high grade lymphomas. The availability of laboratory technology mandates as precise a pathological identification of a tumor type as is possible and treatment directed to known responses for each lymphoma type taking into account the individual patient's situation.

General considerations in the diagnosis and treatment of patients with non-Hodgkin's lymphomas

The most common presentation of NHL is painless lymphadenopathy (26). Careful attention must be paid to detecting other sites of involvement (i.e., other areas of adenopathy, splenomegaly or central nervous system). Some patients present with constitutional symptoms such as weight loss, night sweats, fever or pruritus.

The diagnosis of NHL rests upon pathological examination of a biopsy specimen. Although less invasive techniques such as needle aspirates have been attempted, it is best to provide the hematopathologist with ample biopsy material, since an appropriate diagnosis will usually require histological, immunohistochemical and genotypical studies. The diagnosis of NHL can sometimes be difficult as noted in the clinical evaluation study of the REAL classification, where almost 2% of the initial cases were diagnosed as not having lymphoma (10).

Once the diagnosis is established, the patient must have a complete staging assessment. This "staging" has prognostic and therapeutic importance. Beyond an exhaustive physical examination, the ancillary studies should include laboratory tests, imaging studies and bone marrow aspirate and biopsy. The information obtained by this assessment will be used to determine the stage. The Ann Arbor Staging System is currently used for this purpose (Table 3) (27).

Table 3. Ann Arbor Staging Criteria

Stage	Criteria
IA or IB	One area of nodal or extranodal disease
IIA or IIB	Two or more nodal sites of disease or nodal and proximate extranodal sites on the same side of the diaphragm
IIIA or IIIB	Two or more nodal sites of disease or nodal and proximate extranodal sites of disease on both sides of the diaphragm
IVA or IVB	Disseminated extranodal with or without nodal sites of disease

A: No constitutional symptoms.

B: Constitutional symptoms: Weight loss >10% of baseline, Night sweats, Persistent fever

Besides determining the disease stage, it is important to consider other characteristics that have prognostic and therapeutic implications. Various clinical and biological prognostic parameters have been evaluated in relation to NHL. The International Lymphoma Study Group considered a number of these factors in a study which culminated in the development of the International Prognostic Index for Aggressive Non-Hodgkin's Lymphoma (Table 4) (28). This Index allows clinicians to stratify patients into risk groups based on age, performance status, serum lactate dehydrogenase (LDH) levels, stage and extranodal involvement. The risk groups correlate with five-year survival. Since its introduction, the International Prognostic Index has been validated in other types of NHL (29). The Non-Hodgkin's Lymphoma Classification Project found that within each lymphoma category, age was the single most important predictor of prognosis but also reconfirmed the validity of the International Prognostic Index for other lymphoma types (10). The patient's performance score is of importance since a low performance score is associated with decreased tolerance to aggressive treatment and worse outcome.

The choice of treatment will, thus, take into consideration the specific lymphoma type, patient characteristics, disease stage and the known efficacy of available treatment modalities. Despite the introduction of more aggressive or newer therapies, many patients can not be cured by standard therapy. However, some patients can be successfully treated and the quality of life improved in those that can not be cured.

Follicular lymphomas

Follicular lymphomas represent approximately one-fifth of all NHL and are the most frequently diagnosed lymphoma within the group of indolent NHL (10). Substantial controversy surrounds the subclassification of these lymphomas. Follicular lymphomas have usually been subdivided in three categories according to cell types and numbers: small cleaved, mixed small cleaved/large cell and large cell. However, the ability to accurately reproduce this subdivision has been plagued by a lack of uniform criteria and particularly by the subjectivity of the hematopathologist. The REAL classification subdivided follicular lymphomas in grades I, II and III with the first two generally considered indolent and grade III usually placed

Table 4. International Prognostic Index for Aggressive Non-Hodgkin's Lymphoma

Risk Group (all ages)	Risk Factors*	5-Year Survival Rate (%)
Low	0-1	73
Low-intermediate	2	51
High-intermediate	3	43
High	4-5	26

*Risk factors: Age over 60 years, Serum LDH above normal, Performance status 2-4, Stage III or IV, Extranodal involvement at more than one site.

within the aggressive category (9). However, the REAL classification did not provide criteria for determining each grade.

Part of the controversy regarding the subclassification of follicular lymphomas stems from arguments that such a division has little clinical utility. However, studies have shown that follicular lymphomas comprised primarily of small cleaved cells have a better survival than those with a predominantly large cell component but the difference between mixed cell follicular lymphoma and follicular large cell lymphoma is less clear (30, 31).

Most patients present with stage III or IV disease with up to 40% having bone marrow involvement (10). Histologic transformation towards a more aggressive NHL often occurs in these patients and is generally associated with abbreviated survival (32). Despite its indolent nature, follicular lymphomas remain a difficult disease to cure. Assessment of treatment efficacy is hampered due to the frequency of late relapses. Although various therapeutic agents have shown an improvement in disease-free survival, the average overall survival has remained little changed compared with the "watch and wait" approach (32, 33).

Thus, although most patients will present with advanced disease, the decision to treat rests on symptoms and patient preference (34). Treatment options include radiation therapy with or without cytotoxic chemotherapy, single or multiagent cytotoxic chemotherapy and, more recently, the use of immuno-

logical therapy (interferon, monoclonal antibodies and vaccines) (32, 34–36). Radiation therapy in limited stage disease (stage I or II) resulted in improved disease-free survival in some studies but not others (32, 37). However, overall survival was unchanged. Extended field radiation (e.g., both sides of the diaphragm) or total lymphoid irradiation improved disease-free survival but overall survival was again without change (32, 37). Advanced age (>60 years) has a particularly negative influence on survival (37). Interestingly, relapse within the radiation field occurred in less than 15% of patients suggesting a potential curative role for this modality (37).

Cytotoxic chemotherapy can be administered as single agent or combination therapy. Although patients treated with combination therapy achieve remission earlier, the remissions do not last longer than in patients treated with single agents and overall survival is unchanged (32, 33). The use of intensive cytotoxic chemotherapy with hematopoietic rescue has been evaluated in patients with relapsed follicular lymphoma but its use is still controversial. Despite higher complete remissions, disease-free survival curves have not plateaued in this group of

patients, although promising data exists to this regard (Table 5) (33, 38).

As mentioned earlier, controversy exists regarding the clinical utility of subclassifying follicular lymphomas. A Southwest Oncology Group retrospective analysis of 389 patients with follicular lymphoma treated uniformly with a doxorubicin-based regimen demonstrated no difference in survival between the various histologic types (31). The authors of this analysis speculate that the patients with large-cell follicular lymphoma may have done better because of the type of chemotherapy regimen. Other studies have demonstrated that, despite its more aggressive nature, patients with follicular large-cell lymphoma can obtain long term disease control with treatment similar to diffuse large B-cell lymphoma (45–47). Furthermore, good prognosis patients with follicular large-cell lymphoma treated with high dose cytotoxic therapy followed by autologous stem cell transplantation had improved survival compared with good prognosis patients with diffuse large-cell histology (45). Data from these studies indicate that anthracycline-based regimens may be more effective than oth-

Table 5. Status of High Dose Cytotoxic Chemotherapy with Hematopoietic Transplantation in Non-Hodgkin's Lymphoma

Histologic Diagnosis	Type of Transplant	Status at time of Transplant	Outcome (DFS ^c)	Ref.
Follicular lymphoma	Auto BMT ^a	1 st complete remission	66% @ 22 mo.	39
		1 st complete remission or chemosensitive relapse	53% @ 24 mo.	40
		Second or subsequent remission	55% @ 36 mo. ^d	41
	Allo BMT	Recurrent chemosensitive	49% @ 36 mo.	42
		Recurrent, chemoresistant	68% @ 24 mo.	43
	T-cell depleted Allo BMT	Relapsed, chemosensitive	53% @ 36 mo.	44
Diffuse large cell	Auto BMT	Chemosensitive first or Subsequent relapse	46% @ 5 yrs.	54
		High-risk 1 st complete Remission	57% @ 5 yrs.	56
		High-risk (as initial therapy) ^e	76% @ 36 mo.	57
		Poor-risk (as initial therapy)	87% @ 3 yrs.	58
Mantle cell	Auto SCT ^b	Untreated patients	72% @ 3 yrs.	60
		First relapse	34% @ 2 yrs.	61

^aBMT: bone marrow transplant, ^bSCT: stem cell transplant, ^cDFS: disease free survival, ^dexcluding treatment related deaths, ^eincluded lymphoblastic lymphoma.

ers in the treatment of follicular large-cell lymphoma (31, 45–47).

Promising new agents for therapy of follicular lymphomas include purine analogues such as fludarabine, pentostatin and 2-chlorodeoxyadenosine. The use of these agents has seen remissions as high as 89% depending on the type of agent and histologic grade. However, whether this response is long lasting is still to be determined (48, 49). The use of monoclonal antibodies, alone or radiolabeled, has shown responses ranging from 40% to 60% but long term outcome is yet to be determined (32). The response rate obtained when rituximab (anti-CD20 antibody) is utilized in relapsed indolent lymphoma is similar to that obtained from single alkylating agents (50).

As has been stressed, maintenance of remission is a significant obstacle in obtaining cure for these patients. Various studies have incorporated interferon therapy in conjunction with combination cytotoxic chemotherapy or used as maintenance therapy after chemotherapy. Although various cytotoxic treatment regimens were used and the dose of interferon alpha varied, the results of these studies support the use of interferon as initial therapy in combination with an anthracycline-containing regimen or as maintenance therapy in patients achieving complete remission after initial cytoreductive therapy (36).

Diffuse large B-cell lymphoma and peripheral T-cell lymphoma

Diffuse large B-cell lymphomas account for nearly 30% of NHL(10). These are aggressive lymphomas that produce “B” symptoms in 40% to 50% of patients. As opposed to follicular lymphomas, a greater number of patients (approximately 50%) will be diagnosed in stages I or II (10).

Peripheral T-cell lymphomas constitute 7% of newly diagnosed NHL. These are also aggressive lymphomas with slightly worse survival compared with diffuse large B-cell lymphomas (10).

Both of these histological groups tend to be responsive to cytotoxic chemotherapy and the treatment is similar for both groups of patients. Patients with stage I or nonbulky stage II disease can be treated with radiation therapy alone or in combination with cytotoxic chemotherapy (32, 38). Superior outcomes in patients treated with short course cytotoxic chemotherapy followed by involved field radiation were reported by both the Southwest Oncology Group and Eastern Cooperative Oncology Group (51, 52). Thus, the current recommendation for patients with stage I or nonbulky stage II disease is a short course (i.e., 3 or 4 cycles) of an anthracycline-based regimen such as CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) followed by radiation therapy (32).

Patients with bulky stage II, stage III or IV disease should be treated with cytotoxic chemotherapy. Thus far, no one regimen has proven superior to another. Fisher et al compared CHOP with three other regimens. Final analysis showed similar results from all four regimens but with less toxicity within the CHOP regimen (53).

The use of high-dose cytotoxic chemotherapy with hematopoietic rescue has been evaluated in high risk patients who are in first complete remission as well as in those with relapsed

disease. The PARMA trial randomized patients in first or subsequent relapse with chemosensitive NHL to therapy with high-dose cytotoxic chemotherapy plus autologous bone marrow transplantation versus continuation of conventional cytotoxic chemotherapy. Patients in the transplant arm had a significantly improved event-free survival of 53% compared with 12% in the conventional group (Table 5) (54).

The use of high-dose cytotoxic chemotherapy with autologous bone marrow transplantation in high-risk patients in first complete remission is somewhat more controversial. The Groupe d'Etude les Lymphomes compared high-dose cytotoxic chemotherapy followed by autologous bone marrow transplantation with consolidation chemotherapy in patients in first complete remission after induction therapy. Initially, no treatment related differences were found between the two arms (55). Subsequent analysis, though, showed that patients in the high-intermediate and high-risk groups had increased disease-free survival and overall survival when treated with high-dose therapy and transplant (56). Gianni et al randomized high and high-intermediate risk patients to either methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone and bleomycin versus high-dose sequential cytotoxic chemotherapy plus autologous stem cell rescue as initial or salvage therapy. High-dose sequential therapy with autologous bone marrow transplantation resulted in statistically significant higher rates of complete remission and event-free survival, although there was not any difference in overall survival (57). Santini et al randomized patients with advanced diffuse non-Hodgkin's lymphoma to either therapy with VACOP-B (etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone and bleomycin) or VACOP-B plus autologous bone marrow transplantation as front-line therapy. Patients with high-intermediate and high-risk groups had a favorable trend in terms of disease-free survival (58).

In general, patients with diffuse large B-cell lymphoma or peripheral T-cell lymphoma with bulky stage II, stage III or stage IV disease should receive an initial course of CHOP. High-dose cytotoxic chemotherapy with hematopoietic rescue should be offered to patients with relapsed disease. High risk patients in first complete remission may be considered for high-dose cytotoxic chemotherapy with hematopoietic rescue (32, 33, 38). The use of allogeneic hematopoietic transplant has yet to be validated for this group of patients but is currently under evaluation, particularly for the potential for graft vs. lymphoma effect.

Specific situations in patients with aggressive NHL need to be considered, such as the site of involvement. Patients with orbital or sinus involvement are at increased risk for central nervous system involvement and thus should receive central nervous system prophylaxis. Second, patients with testicular involvement are at increased risk for contralateral testicular involvement or central nervous system involvement at relapse. Therefore, these patients should receive central nervous system prophylaxis and radiation therapy to the testicular bed (32).

Marginal zone lymphomas

Within the REAL classification, this category includes mucosa-associated lymphoid tissue lymphomas and monocytoid B-cell lymphomas (9). These two lymphoma types share morphologic and immunophenotypic similarities which have been cited as a justification to group them together. However, immunohistochemical differences as well as prominent differences in site of involvement have been cited by some authors to maintain them as distinct entities (6).

Mucosa-associated lymphoid tissue lymphomas are B-cell malignancies that generally involve the glandular epithelium. The gastrointestinal tract is the most common site but the salivary glands, lungs or thyroid gland can also be involved. Within the gastrointestinal tract, the stomach is a frequent localization for mucosa-associated lymphoid tissue lymphoma and an association with *H. pylori* has been established. Patients usually present with nonspecific gastrointestinal complaints that, due to the vagueness of the symptoms, are present for years before a diagnosis is made (18, 32).

Patients with stage I gastric mucosa-associated lymphoid tissue lymphoma can be treated with a variety of antimicrobial and antiacid combinations aimed at eradicating *H. pylori*. Eliminating the chronic antigenic stimulation by *H. pylori* results in regression of the tumor in roughly 70% of patients. If complete remission of gastric mucosa-associated lymphoid tissue lymphoma is unsuccessful, or if a different site of involvement is present, radiation therapy can be useful (18, 32, 34).

Monocytoid B-cell lymphomas tend to be node-based malignancies and are more common in older, female patients (6, 10). Monocytoid B-cell lymphomas can also arise as a complication of Sjögren's syndrome (59). Monocytoid B-cell lymphomas tend to present with advanced stage disease and five-year failure-free survival in patients with monocytoid B-cell lymphoma tends to be inferior to that of patients with mucosa-associated lymphoid tissue lymphoma (29% vs. 60%) (10). Due to its indolent nature, treatment of monocytoid B-cell lymphomas can be viewed in similar fashion to follicular lymphomas, that is, a "watch and wait" strategy or, if the patient is symptomatic, single agent or combination cytotoxic therapy would be appropriate. Radiation therapy for localized disease is certainly an option for these patients as well (32).

Mantle cell lymphoma

The clinical, immunophenotypical and, particularly, genotypical characteristics of mantle cell lymphoma have contributed to its recognition as a distinct entity within the REAL classification. It represents approximately 6% of all NHL cases worldwide and is characterized by disseminated disease at diagnosis (80% of patients present with stage III or IV disease) (10). Further impacting this aggressive presentation is the advanced age of patients diagnosed with mantle cell lymphoma with a median age of 63 years. The Non-Hodgkin's Lymphoma Classification Project evaluation of the REAL classification found mantle cell lymphoma to have the worst survival among all NHL (11% five year failure-free survival) (10).

An optimal treatment for mantle cell lymphoma has yet to

be determined. Several studies evaluating high-dose cytotoxic therapy followed by hematopoietic rescue have shown a modest improvement in survival. One study using high-dose chemotherapy and total body irradiation followed by stem cell transplantation in previously untreated patients had an event-free survival of 72% at three years (60). Another study reported a 34% disease-free survival at two years in fourteen patients with previously treated mantle cell lymphoma using high-dose therapy with autologous stem cell transplantation (Table 5) (61). Monoclonal antibody therapy has thus far been found to have limited use in mantle cell lymphoma (62).

Future Directions

More detailed knowledge regarding the clinical and pathological characteristics of non-Hodgkin's lymphoma and of the patients affected by these disorders enables the treating physician to tailor therapy specifically for the patient, taking into account risk/prognostic factors and tumor behavior and response. Despite the progress made in the treatment of patients with NHL, an unacceptably large proportion of patients cannot be cured of these diseases. However, a better understanding as to the biology and pathogenesis of NHL, brought about by, and in conjunction with, advances in molecular medicine, have opened the door to potential new therapies in the treatment of these disorders. While the search for more effective drugs continues, attention is being turned to the use of biological techniques to widen the scope of available treatments. Examples of such interventions include immunotherapy (immune modulation through the use of cytokines, lymphokine-activated killer cell infusions, vaccine-based therapies, cell-based therapies or a combination of these) and gene manipulation (e.g., introduction of genes that either increase or decrease susceptibility to various drugs) (35).

The current environment surrounding all aspects of pathogenesis, diagnosis, classification and treatment of non-Hodgkin's lymphoma is a very dynamic one. Despite the significant improvement of present classification systems, better ones will surface in the not too distant future. The ability to identify and diagnose lymphoma entities with greater specificity, coupled with a concerted effort to use a common classification system in large-scale studies, will hopefully translate into the one objective that matters most: a cure for every patient.

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