Improving Outcomes for Patients with Diffuse Large B-Cell Lymphoma

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Abstract

Diffuse large B-cell lymphoma (DLBCL) is the most commonly occurring form of non-Hodgkin lymphoma in the western world. Until the mid 1990s the incidence of DLBCL increased in both sexes, across racial categories, and across all age groups except the very young, the etiology of most cases remains unknown. DLBCL is associated with an aggressive natural history, but it can be cured with combination chemotherapy regimens like cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP), which has been the mainstay of therapy for several decades. Remarkable progress has been made in understanding the biological heterogeneity of DLBCL and in improving survival for DLBCL patients with novel combinations of chemotherapy and immunotherapy. Gene expression profiling (GEP) has uncovered DLBCL subtypes that have distinct clinical behaviors and prognoses, and the addition of the monoclonal antibody, rituximab, to CHOP has markedly improved outcomes. Future approaches to DLBCL management will use molecular signatures identified through GEP to provide prognostic information and to isolate therapeutic targets that are being evaluated for DLBCL patients who relapse or those with high risk disease. CA Cancer J Clin 2010;60:393–408. © 2010 American Cancer Society, Inc.

General Overview

Approximately one-third of all adult lymphomas are diffuse large B-cell lymphoma (DLBCL), the most commonly occurring form of non-Hodgkin lymphoma (NHL) in the western world. DLBCL is associated with an aggressive natural history, with a median survival of less than one year in untreated patients.1 The cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy has been the mainstay of therapy for several decades, since more intensive chemotherapy were more toxic and failed to demonstrate additional benefits.2 In largely separate efforts, remarkable progress has been made during the past decade in understanding the biological heterogeneity of DLBCL and in improving survival for DLBCL patients with combinations of CHOP and immunotherapy. On one hand, gene expression profiling (GEP) has uncovered distinct molecular signatures for DLBCL subtypes that have distinct clinical behaviors and prognoses.3–5 On the other hand, the integration of antilymphoma monoclonal antibodies, notably rituximab (R), into combination therapies for DLBCL have markedly improved patient outcomes.6–9 Most recently, molecular signatures identified through GEP have not only contributed prognostic information, but also they have aided the identification of new therapeutic targets. These developments have led to agents that are currently being evaluated in patients who relapse. In addition, the newer agents are being incorporated into approaches for higher-risk patients.

Epidemiology

The incidence of NHL increased dramatically from the 1970s until the middle of the 1990s with an estimated 65,540 new cases expected in the United States in 2010, thus making NHL the seventh most common cancer
(excluding basal cell and squamous cell skin cancers). During this period, the steady increase of 3% to 4% per year in incident cases of lymphoma and DLBCL occurred in both sexes, across racial categories, and across all age groups except the very young. Incidence rates plateaued in the late 1990s, and from 1992 to 2006, the age-adjusted incidence rate for DLBCL increased approximately 1% per year, from 8.07 to 8.98 cases per 100,000 population. The increase in DLBCL incidence from the 1970s represents an unprecedented rise comparable only to the rise in skin cancers. Several factors have contributed to this increased incidence including: more sensitive methods for identifying diagnostic cases, improvements in cancer reporting for hematological malignancies, changes in the classification systems used for lymphoid malignancies, and the epidemic of human immunodeficiency virus (HIV) infections occurring during this period with an associated increase in HIV-associated lymphomas. However, in sum, the above-listed factors account for only approximately 50% of the additional cases of NHL. It has been well established that males have a 1.5-fold higher incidence of DLBCL and that there are significant racial differences in DLBCL incidence. Based on data from 37,009 cases of DLBCL in the Surveillance Epidemiology and End Results (SEER) registry, white Americans have an age-adjusted incidence of 7.36 per 100,000 population, black Americans have an incidence of 4.88 (black/white incidence rate ratio, 0.66; 95% confidence interval [CI], 0.64-0.69), and individuals in the mixed categories of American Indians, Alaska Natives, and Asian/Pacific Islanders have an incidence of 5.59 (incidence rate ratio compared with whites, 0.76; 95% CI, 0.73-0.79). For the majority of patients, the etiology of DLBCL remains unknown. Some factors that influence the risk of lymphoma include genetics, comorbid diseases or their treatments (notably immunosuppression), environmental factors such as ultraviolet radiation, pesticides, hair dyes, and diet.

**Dietary Factors That May Influence DLBCL Risk**

Overall, the role of dietary factors in DLBCL risk has yet to be well understood. Most studies have focused on particular dietary factors hypothesized to modify the risk of developing lymphoma. In particular, dietary flavonoids, which are found primarily in fruit and vegetables, have been proposed to be anticarcinogenic. In one case-control study, higher total flavonoid intake was associated with a 47% risk reduction in NHL when comparing the highest quartile of intake to the lowest, with similar risk reduction seen for DLBCL, odds ratio (OR), 0.39; 95% CI, 0.22-0.71. In another study, greater intake of total fruits and vegetables was associated with lower NHL risk but was not observed among patients with DLBCL. Conversely, severe obesity (defined as BMI of ≥40 kg/m²) was not associated with risk of NHL overall, or most NHL subtypes, but was associated with increased risk of DLBCL (pooled OR, 1.80), which may warrant further investigation.

**Genetic Factors That May Influence DLBCL Risk**

Whereas the etiologies for DLBCL and most other lymphomas remain unknown, the strongest known NHL risk factor is severe immunosuppression. As a result, case-control studies investigating the genetics of lymphomagenesis have focused on variation in genes regulating inflammatory pathways, lymphoid cell cycle, apoptosis, and development. Although no single DLBCL susceptibility gene has been identified, some evidence suggests that a genetic predisposition underlies the etiology of DLBCL and other NHLs: 1) NHL risk is increased in individuals with a family history of hematopoietic malignancy, 2) immigrants retain the NHL incidence rates and patterns of their country of origin, and 3) some common genetic variations have been identified in lymphoma patients. For instance, individuals who reported a first-degree relative with NHL (OR, 1.4; 95% CI, 1.1-2.0), Hodgkin lymphoma (OR 1.7; 95% CI, 1.1-2.7); leukemia (OR 1.3; 95% CI, 1.0-1.6), or any hematological malignancy (OR, 1.3; 95% CI, 1.1-1.6) had higher risks of DLBCL. Several studies indicate that genetic variants that promote B-cell survival and growth increase the risk of lymphoma. Results of case-control studies, including a large pooled study by InterLymph, an international consortium of NHL epidemiologists, found positive associations between variant alleles in tumor necrosis factor, TNF-308G>A (OR, 1.29; 95% CI, 1.14-1.46), and in interleukin-10, IL10-3575T>A genes (OR, 1.15; 95% CI, 1.04-1.26), and the risk of DLBCL.
and similar to other risk factors mentioned above and below in this article. However, when both \( TNF^{-308G} > A \) and \( IL10^{-3575T} > A \) occurred, the OR for developing DLBCL was 2.13. These and other data support a genetic basis for etiologic commonality and heterogeneity for lymphoma subtypes\(^\text{23-25} \) and suggest to some investigators that immune dysfunction is of greater etiologic importance for DLBCL than some other lymphoma subtypes.\(^\text{26} \)

**Environmental Factors That May Influence DLBCL Risk**

Since the development of DLBCL likely involves both genetic and environmental risk factors and their interactions, case-control studies have explored environmental exposures potentially associated with DLBCL. One study of NHL in 4 Surveillance, Epidemiology, and End Results (SEER) regions of the United States examined the residential locations of 864 cases and 684 controls during the 10-year period before recruitment to characterize the impact that proximity to industrial facilities had on developing NHL. This study found that living within 2 miles of a lumber facility was associated with increased DLBCL risk (OR, 1.7; 95% CI, 1.0-3.0) but did not provide strong evidence that living near manufacturing industries increases NHL risk overall.\(^\text{27} \) In another InterLymph study, data from 10 case-control studies covering 8243 cases and 9697 controls in the United States, Europe, and Australia examined the relations between self-reported sun exposures and developing NHL. Increased recreational sun exposure was associated with decreased risk of NHL and DLBCL (pooled ORs, 0.76; 95% CI, 0.63-0.91 and 0.69; 95% CI, 0.55-0.87, respectively) for the highest exposure category,\(^\text{28} \) but this level of sun exposure may be associated with other health risks. In other studies, ultraviolet radiation, herbicides, insecticides, and oxidative dye products have also been associated with increased lymphoma risk, but debate remains concerning their relative influence on DLBCL development specifically.\(^\text{12,13,29-31} \)

**Clinical Conditions That May Influence DLBCL Risk**

Increased risk of lymphoma and DLBCL have been observed in association with viral infections and treatments and diseases that suppress the immune system, including autoimmune diseases, organ transplants, and primary or acquired immunodeficiencies.\(^\text{31} \) Several infectious organisms have been linked to the risk of lymphoma, including Epstein-Barr virus (EBV), Kaposi sarcoma-associated human herpes virus 8 (HHV 8), *Helicobacter pylori*, *Chlamydia psittaci*, and hepatitis C virus (HCV). For example, certain subsets of DLBCL, namely immunoblastic and primary central nervous system DLBCLs, have been highly associated with the EBV.\(^\text{32} \) Primary effusion lymphoma represents an unusual DLBCL subtype occurring predominantly in immunodeficient patients in association with infection by HHV8.\(^\text{33,34} \) \( H. \text{ pylori} \) and \( C. \text{ psittaci} \) are more commonly associated with indolent lymphomas that can later transform into DLBCL.\(^\text{35} \) In an InterLymph study of 4784 patients with NHL diagnosed between 1988 and 2004 and 6269 controls matched by age, sex, and study center, evidence of HCV infection was detected in 172 (3.60%) NHL cases and in 169 (2.70%) controls (OR, 1.78; 95% CI, 1.40-2.25). When analyzed by NHL subtype, HCV prevalence was associated with lymphoplasmacytic lymphoma (OR, 2.57), marginal zone lymphoma (OR, 2.47), and DLBCL (OR, 2.24; 95% CI, 1.68-2.99).\(^\text{30} \) Other data suggest that neither total antibiotic use nor antibiotic use by site is associated with risk of developing NHL, or any NHL subtype,\(^\text{36} \) suggesting that infectious agents rather than their treatments are involved. Whether infectious pathogens are responsible for specific host mutations that initiate lymphomagenesis, antigenic stimulation leading to B-cell proliferation, and increased potential of random cell-replication errors, immunosuppression that thereby promotes tumor growth, or some combination of these effects has not been clearly delineated.\(^\text{31} \)

Data from the SEER-Medicare Assessment of Hematopoietic Malignancy Risk Traits (SMAHRT) Study have been used to investigate the associations between autoimmune conditions and lymphoma subtypes in 44,350 cases with lymphoid malignancies and in 122,531 controls. An increased risk of DLBCL in the Medicare population was associated with a diagnosis of rheumatoid arthritis (OR, 1.4), Sjogren syndrome (OR, 2.0), and autoimmune hemolytic anemia (OR, 3.3).\(^\text{37} \) In another study, systemic lupus erythematosus and hemolytic anemia also were associated with increased risk of...
DLBCL. A history of blood transfusion has been inconsistently associated with risk of lymphoma, with the majority of studies finding no association. Transformation from indolent B-cell NHL can occur at a rate of 2% to 5% per year, making this an additional risk factor for the development of DLBCL.

To date and for a number of reasons, epidemiological studies have provided limited insight into the impact of genetic, nutritional, and environmental exposures on DLBCL risk. Older studies attempted to identify risk factors for NHL and grouped DLBCL with indolent B-cell and T-cell lymphomas, but they have distinct biological origins. Even within DLBCL, there are biological variants (discussed below) that may have different etiologies. Studies that focus on pathologically defined DLBCL have been limited by statistical power, exposure measurement that addresses temporal and dose-response relations, and controls for potential confounding variables. Moreover, risk factors for DLBCL are likely to be multifactorial, have temporal or other dependent relations, and/or have minimal independent impact on disease development, all of which have frustrated their discovery and, thus, limited research efforts directed toward DLBCL prevention.

Comprehensive analyses using genome-wide association studies combined with assessments of environmental and nutritional exposures in specific biological subsets of DLBCL through large international collaborations, such as InterLymph, are needed to recruit sufficient numbers of patients for defining risk factors for DLBCL development and for initiating prevention efforts. Although the advances in treatment discussed below have established our ability to cure the majority of patients who have DLBCL, these treatments still carry substantial morbidity and cost. Renewed efforts to identify risk factors for DLBCL and other lymphomas may help define public health efforts that can reduce the occurrence of these cancers.

Clinical Prognostic Factors

Originally proposed in 1993, the international prognostic index (IPI) remains the primary clinical tool used to predict outcome for patients with DLBCL. Stage III/IV disease, elevated LDH, age >60 years, Eastern Cooperative Oncology Group (ECOG) performance status ≥2, and involvement of >1 extranodal site form the IPI score, with one point given for each factor. The IPI scoring system stratifies patients into 4 discrete groups with a 5-year overall survival (OS) ranging from 26% to 73% (Table 1). However, the IPI was developed in the era before rituximab was routinely included in treatment regimens (discussed below). To address this issue, Sehn and colleagues performed a population-based, retrospective, cohort analysis of 365 patients with newly diagnosed DLBCL treated with rituximab.
plus standard chemotherapy. Although the IPI remained prognostic in this study, it no longer distinguished 4 outcome groups. With redistribution of the IPI factors into a Revised IPI (R-IPI) grouping, 3 separate categories were defined that provided more accurate prediction of outcome. Patients with zero risk factors had a >90% chance of 4-year progression-free survival (PFS); those with 1-2 risk factors had 80% expected PFS; and those with ≥3 risk factors had 50% PFS (Table 1). Other studies have refuted this reclassification of the IPI and demonstrated improved outcomes for all IPI categories with modern treatment regimens, but the benefits were not evenly distributed across IPI groups. Currently, the original IPI remains as a prospectively designed and validated measure for assessing DLBCL risk.

### PET Scanning as a Prognostic Indicator in DLBCL

A novel approach for predicting outcomes in DLBCL is evaluating interval response to therapy with functional imaging such as fluorine-18 2-fluoro-2-deoxy-D-glucose-positron emission tomography (FDG-PET) scanning. In 2007, the revised International Working Group response criteria for malignant lymphoma strongly recommended the use of PET for patients with routinely FDG-avid, potentially curable lymphomas such DLBCL, 1) before treatment to better delineate the extent of disease, 2) 6 to 8 weeks after completion of therapy for assessment of complete response (CR) because CR is required for cure in DLBCL, and 3) in the context of a clinical trial midtreatment to evaluate the prognostic ability of interval PET to predict the ultimate response to therapy and long-term outcomes. However, at present, there is no role for interim PET scans during treatment or after completion of therapy for patients in CR.

Modern technology creates fused PET and CT images with their data shown together to provide information on structure, tumor size, and activity. However, the CT scan performed for anatomic localization of FDG activity in a fused PET-CT scan is often not at the same degree of resolution as an individual diagnostic CT. Early restaging PET scans performed after 1 to 4 cycles of therapy have been shown to be predictive of outcome in some, but not all, studies. Spaepen and colleagues prospectively assessed the usefulness of PET scans performed after 3-4 cycles of doxorubicin-based chemotherapy in 70 patients with newly diagnosed aggressive NHL and found that none of the 33 patients who had a positive PET scan achieved a durable CR, whereas 31 of 37 patients with a negative early PET scan achieved a durable CR. In this study, midtreatment PET was a stronger predictor for progression-free survival (PFS) and overall survival (OS) than the IPI.

Dupuis and colleagues investigated the use of PET in patients with DLBCL after 2 and 4 cycles of first-line treatment with CHOP or CHOP-like chemotherapy with or without rituximab (49% of

### Table 1. Expected Outcomes for Patients by Risk Group with the International Prognostic Index and Revised International Prognostic Index for Patients Treated with R-CHOP

| RISK GROUP | NO. OF RISK FACTORS | 4-YEAR PFS PERCENTAGE | 4-YEAR OS PERCENTAGE |
|------------|---------------------|-----------------------|----------------------|
| Very Good  | 0                   | 94                    | 94                   |
| Good       | 1, 2                | 80                    | 79                   |
| Poor       | 3-5                 | 53                    | 55                   |

CR indicates complete remission; OS, overall survival; PFS, progression free survival.
patients with and 51% without). The 5-year event-free survival (EFS) was 36% for patients with a positive PET versus 80% for those with a negative PET; similar findings were observed in patients treated with and without rituximab.\textsuperscript{50} Dupuis et al also found that although semiquantitative means of evaluating PET scans using standard uptake values (SUV) help to reduce the occurrence of false-positive interim PET interpretations at 2 cycles, these methods were equivalent to simple visual analysis at 4 cycles.\textsuperscript{49}

However, a study of 98 patients treated at the Memorial Sloan Kettering Cancer Center with DLBCL who received initial treatment with CHOP plus rituximab and risk-adapted therapy on the basis of PET scan results after 4 treatment cycles raised concerns about the value of interim PET. In this study, 51 of 59 patients who had a negative PET scan were progression free at a median follow up of 44 months. However, among 38 patients who had a positive PET after 4 cycles and who underwent repeat biopsy, 33 were negative for DLBCL, and 26 remained progression free after consolidation therapy.\textsuperscript{51} Together, these data suggest that a negative interim PET scan after 4 cycles of CHOP-based chemotherapy can have prognostic significance for patients with DLBCL, but positive interim PET scans need to be followed by biopsy before additional treatment is implemented. The timing of PET with respect to chemotherapy and radiation administration must also be carefully considered because treatment-related effects may lead to falsely positive results. Given its potential predictive power and its apparent independence from the treatment selected,\textsuperscript{52} interim fused PET/CT imaging or other functional imaging modalities may become important clinical tools for predicting outcomes for DLBCL patients in the future.

**Pathology**

**Histological Features and Pathological Classification**

As its name implies, DLBCL is a cancer of large B-cells that most commonly grows in a diffuse pattern completely effacing the normal lymph node architecture.\textsuperscript{53} In 2008, the International Agency for Research on Cancer published the fourth edition of the World Health Organization (WHO) Classification of Tumors of Hematopoietic and Lymphoid Tissues, an effort involving 62 clinical specialists with expertise in lymphoid and myeloid disorders.\textsuperscript{54} Given that DLBCL represents a clinically, biologically, and pathologically heterogeneous entity, the WHO system modified DLBCL classification to recognize multiple morphologic variants based on an improved understanding of the variety of molecular abnormalities associated with DLBCL (Table 2).\textsuperscript{55} However, complete discussion of many of these clinical subtypes is beyond the scope of the current article. The distinct genetic, molecular, pathological, and clinical features of new and previously recognized DLBCL variants have been addressed in more detail in recent reviews.\textsuperscript{54,56} This classification system demonstrates the importance of site or clinical factors in defining variants of DLBCL by including subtypes such as EBV\textsuperscript{+} DLBCL of the elderly and DLBCL associated with chronic inflammation. This WHO system provides additional clarity by removing the categories of follicular lymphoma grade 3A and 3B, so that cases of grade 3 follicular lymphoma with diffuse

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**TABLE 2. World Health Organization Classification of the Mature Large B-cell Neoplasms**

| CLASSIFICATION | DIFFERENTIATION |
|---------------|-----------------|
| Diffuse large B-cell lymphoma (DLBCL), NOS | - |
| T-cell/histiocyte rich large B-cell lymphoma | - |
| Primary DLBCL of the CNS | - |
| Primary cutaneous DLBCL, leg type | - |
| EBV\textsuperscript{+} DLBCL of the elderly\textsuperscript{a} | - |
| DLBCL associated with chronic inflammation | - |
| Lymphomatoid granulomatosis | - |
| Primary mediastinal (thymic) large B-cell lymphoma | - |
| Intravascular large B-cell lymphoma | - |
| ALK\textsuperscript{+} large B-cell lymphoma | - |
| Plasmablastic lymphoma | - |
| Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease | - |
| Primary effusion lymphoma | - |
| B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma | - |
| B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma | - |

NOS indicates not otherwise specified; CNS, central nervous system.\textsuperscript{a} Provisional entities for which the WHO Working Group felt there was insufficient evidence to recognize as distinct diseases at this time.
growth patterns (common in the former grade 3B) might be properly classified as DLBCL. The 2008 WHO classification system also appreciates the ambiguity that arises due to morphologic and immunophenotypic overlap between classic Hodgkin lymphoma and some DLBCL variants (usually primary mediastinal large B-cell lymphoma [PMBL]) and recognizes a provisional category of B-cell neoplasms with features somewhere between DLBCL and classic Hodgkin lymphoma.54,55 A similar provisional category termed unclassifiable B-cell lymphoma, with features intermediate between DLBCL and Burkitt lymphoma was established for a comparable clinical conundrum. Although these efforts may appear at first glance to be missteps that lack the specificity we seek with modern pathological tools, they do provide a common definition so that consistent treatment algorithms can be applied to these diagnostically challenging cases of DLBCL.

**Biological Prognostic Factors**

Despite our recognition and classification of the morphologic and clinical heterogeneity within DLBCL, we have been challenged to define therapies that uniquely benefit each subgroup. To segregate DLBCL into biologically meaningful subgroups that might identify rational therapeutic targets, the Leukemia and Lymphoma Molecular Profiling Project began gene expression analyses of DLBCL biopsy samples by using DNA microarrays and identified biologically distinct and prognostically meaningful molecular subgroups of DLBCL.4 The first group had a gene expression profile pattern clustered with normal germinal center B cells and was labeled as the GCB variant. The second group had a contrasting set of signature genes similar to activated B-cells, and was thus, termed the ABC variant.

Patients in the GCB subgroup had a higher 5-year survival rate (60% vs 35%; P < .001). Molecular subtype was shown to predict survival independent of IPI risk.4 A third uncommon subtype, PMBL (mentioned above), tends to occur in younger patients in the anterior mediastinum with a GEP signature that significantly overlaps with Hodgkin lymphoma cell lines suggesting that the 2 diseases share biological features.5,57 Subsequent gene expression studies have upheld these findings and expanded on them.3,5,58 Other biological markers including the antiapoptotic protein, Bcl-2, Bcl-6 (a marker of germinal center derivation), and Myc (a proto-oncogene transcription factor) carry prognostic significance in DLBCL, and these are now being explored for interacting effects with ABC and GCB subtypes, PET imaging, and modern therapies.50,59-65

As noted above, GCB, ABC, and PMBL subtypes of DLBCL are not merely prognostic factors but biologically distinct entities because they are associated with unique driver mutations and pathways. For example, translocation of \( BCL2 \) is more commonly present in GCB-like DLBCL,65 whereas \( BCL6 \) translocation more commonly occurs in ABC-like DLBCL.63 GEP studies have demonstrated that the activation of nuclear factor-kappaB (NF-\( \kappa \)B) pathway is much more frequently observed in the ABC and PMBL subtype of DLBCL compared with the GCB-subtype, but the implications of NF-\( \kappa \)B in ABC and PMBL are radically different.57 In PMBL, gains in chromosome region 2p14–16, which contains the gene \( cREL \), occur commonly. Because the \( cREL \) gene is known to be important in human B-cell survival and differentiation, \( cREL \) gain or amplification may be an important regulator of NF-\( \kappa \)B activity for PMBL, but its precise role in lymphomagenesis remains unclear.57

For ABC-like DLBCL, recent data suggest that the signaling adaptor, \( CARD11 \), leads to constitutive activation of the NF-\( \kappa \)B signaling pathway and contributes to tumorigenesis.23-25 These biological differences in DLBCL subtypes have been described in greater detail in a recent review.57 Because oncogenic pathways appear to be differentially activated in these subtypes of DLBCL, future advances in therapy should target these differences.

Despite its usefulness, gene expression profiling technology has not moved easily into community practices. As a result, immunohistochemical algorithms have been proposed and validated for classification of DLBCL into GCB and ABC types. An initial algorithm proposed by Hans et al66 used CD20, CD10, Bcl-6, and MUM1 to distinguish GCB and non-GCB subtypes (mostly ABC). More recently, a consortium of hematopathologists improved on the Hans method by employing different immunostains, GCET1, CD10, BCL6, MUM1, and FOXP1, and derived a new algorithm with 93% concordance with gene expression profiling.67
The new algorithm predicted survival in a validation set and was an independent predictor of survival in multivariate analyses.

Independent of IPI, GCB and ABC subtypes defined by IHC demonstrated significant differences in event-free survival (EFS) and OS among patients treated with and without rituximab-based regimens. The GCB subtype had significantly better outcome (3-year OS, 87% GCB vs 44% ABC; \( P < .001 \)).

However, the GBC and ABC subtypes were not formally incorporated into the 2008 WHO classification system because of 1) the lack of availability of GEP as a routine diagnostic test, 2) the imperfect correlation of immunohistochemical surrogate markers with GEP (based on the Hans algorithm with a positive predictive value of 87% for the GCB group and 73% for the non-GCB group), and 3) a lack of distinct management strategies for DLBCL subtypes. However, it remains to be seen how the next revision of the WHO classification system will view immunohistochemical algorithms for segregating DLBCL with the increased accuracy of the Choi method and the use of these algorithms to direct therapy in current clinical trials.

**DLBCL Treatment and Outcomes**

**First-Line Treatment**

Although DLBCL is associated with a median survival of less than 1 year in untreated patients, this disease is commonly curable with conventional anthracycline-based chemotherapy. Advances in the management of DLBCL during the last decade, including the advent of monoclonal antibodies, have led to excellent outcomes for many patients. Common treatment algorithms for the management of DLBCL are divided into strategies for patients with localized disease (Ann Arbor stage I and II) and advanced-stage disease (stages III and IV).

Until recently, the CHOP regimen, developed in the 1970s, remained the standard therapy for DLBCL. In the 1980s, several pilot studies that used aggressive combination-chemotherapy regimens for patients with advanced stage aggressive NHL reported increased CR and survival rates, but these new treatment programs were costly, difficult to administer, and appeared to be more toxic than CHOP.

The Southwest Oncology Group (SWOG) and the Eastern Cooperative Oncology Group (ECOG) initiated a prospective, randomized, phase 3 trial that compared CHOP to 3 aggressive multiagent regimens. When compared with these intensive chemotherapy regimens in patients with advanced NHL, the standard CHOP regimen produced similar survival outcomes. Fatal toxic reactions were less common in patients treated with CHOP, thus establishing CHOP as the standard of care for patients with DLBCL. This finding has been confirmed by other trials comparing more aggressive chemotherapy regimens to standard CHOP therapy.

Together, these studies suggest that there is a small margin beyond the chemotherapy dosing in the CHOP regimen to intensify chemotherapy without incurring substantial toxicity.

In 1997, rituximab became the first monoclonal antibody approved for use by the Food and Drug Administration (FDA) for follicular lymphoma, and this immunotherapy was soon applied to DLBCL and other B-cell NHLs. Although not completely understood, rituximab is thought to induce lymphoma cell lysis through complement-mediated cytolysis, antibody-dependent cell cytotoxicity, and direct induction of apoptosis. In addition, rituximab acts synergistically with chemotherapy. On the basis of phase 2 studies in which rituximab in combination with CHOP had a good safety profile and induced response rates in more than 90% of patients with indolent and aggressive lymphoma, the Groupe d’Étude des Lymphomes de l’Adulte (GELA) published a study in 2002 that compared CHOP plus rituximab (R-CHOP) with CHOP alone in patients older than age 60 years. The complete response rates were significantly higher in patients who received R-CHOP than in the group who received CHOP alone (76% vs 63%; \( P = .0005 \)), and 2-year OS improved from 57% to 70% (\( P = .007 \)). Updates of this trial have demonstrated that EFS, PFS, and OS remained statistically significant in favor of R-CHOP and actually continued to improve. R-CHOP did not produce substantially more toxicity than CHOP alone, leading R-CHOP to become the treatment regimen of choice in elderly patients with DLBCL.

Results from the GELA trial were confirmed in a US Intergroup trial in older patients. The US trial randomized patients to induction with R-CHOP...
(n = 318) or CHOP (n = 314) and a second randomization of responders (n = 415) to maintenance rituximab (n = 207) or observation (n = 208). The 3-year failure-free survival rate was 53% for R-CHOP patients and 46% for CHOP patients (P = .04). Only those patients who received CHOP as induction therapy appeared to benefit from maintenance rituximab. The administration of maintenance rituximab was of no benefit to patients who received R-CHOP; thus, maintenance rituximab has no role in the initial management of the vast majority of patients with DLBCL.

The question of whether rituximab could benefit younger patients was addressed by the MabThera International Trial (MInT), in which 824 patients from 18 countries were randomly assigned to 6 cycles of CHOP or CHOP-like chemotherapy and rituximab or 6 cycles of the same chemotherapy regimen alone. After a median follow up of 34 months, patients assigned R-CHOP had increased 3-year EFS and OS compared with those assigned chemotherapy alone. On the basis of these observations, it is clear that rituximab-containing regimens improve survival for DLBCL patients regardless of age.

Several trials have explored the possible benefit of administering more dose-intense chemotherapy regimens with rituximab, based in part on the findings of the German Lymphoma Study Group (GLSG) in the prerituximab era that CHOP given every 14 days instead of every 21 days benefited older (but not younger) patients with DLBCL. In the RICOVER-60 trial, the GLSG addressed the question of the number of cycles to administer on a dose-dense schedule. Patients older than the age of 60 years were randomized to 6 or 8 cycles of CHOP14 with or without rituximab. Six cycles of R-CHOP14 significantly improved EFS, PFS, and OS when compared with 6 cycles of CHOP14 treatment, and these endpoints were not improved further by giving 8 cycles of R-CHOP14. These results call into question the widely practiced standard approach of response-adapted therapy (giving 2 cycles beyond best response) for DLBCL patients who receive 6 cycles of chemotherapy.

**TABLE 3. Outcomes Following CHOP and R-CHOP by Age Group and Number of Cycles**

| REGIMEN                  | CYCLES                        | OUTCOMES                  | 2-YEAR PERCENTAGE | 3-YEAR PERCENTAGE | 5-YEAR PERCENTAGE |
|--------------------------|-------------------------------|---------------------------|-------------------|-------------------|-------------------|
|                          |                               | NO. CR/CUR PERCENTAGE     | EFS               | OS                | EFS               | OS                |
| CHOP-14                  |                               | X 6 (younger: NHL-B1)     | 172               | 79                | 61               | 85                |
|                          |                               | X 6 (older: NHL-B2)       | 172               | 76                | 54               | 69                |
|                          |                               | X 6 (older: RICOVER-60)   | 307               | 68                | 47               | 68                |
|                          |                               | X 8 (older: RICOVER-60)   | 305               | 72                | 53               | 66                |
| CHOP-21                  |                               | X 6 (younger: NHL-B1)     | 176               | 80                | 55               | 75                |
|                          |                               | X 6 (older: NHL-B2)       | 178               | 60                | 41               | 49                |
|                          |                               | X 6-8 (older: E4494)      | 314               | NR                | 46               | 58                |
|                          |                               | X 8 (older: LNH 98.5)     | 197               | 63                | 38               | 57                |
| R-CHOP-14                |                               | X 6 (older: RICOVER-60)   | 306               | 78                | 67               | 78                |
|                          |                               | X 8 (older: RICOVER-60)   | 304               | 76                | 63               | 73                |
|                          |                               | X 8 (older: LNH 03-68)    | 103               | 67                | 49 PFS           | 67                |
| R-CHOP-21                |                               | X 6-8 (older: E4494)      | 318               | NR                | 53               | 67                |
|                          |                               | X 8 (older: LNH 98.5)     | 202               | 76                | 57               | 70                |
|                          |                               | X 8 (older: LNH 03-68)    | 99                | 75                | 63 PFS           | 70                |
cycles of dose-intensive therapy. Based on their preceding standard of CHOP14 for patients older than 60 years, 6 cycles of R-CHOP14 became the preferred treatment of the GLSG for elderly patients with DLBCL. The outcomes observed with CHOP and R-CHOP in these trials are shown in Table 3.

Because competing standards currently exist for DLBCL initial therapy, Cunningham and colleagues in the United Kingdom and the GELA designed randomized trials to compare outcomes associated with R-CHOP14 and R-CHOP21 in newly diagnosed patients with DLBCL. The UK trial completed accrual with 1080 patients randomized, and early results show no differences in CR or OR rates. The GELA performed a similar trial, LNH03-6B, randomizing patients between the ages of 60 and 80 years to R-CHOP given every 14 days compared with R-CHOP given every 21 days, administering both regimens for 8 cycles. Patients’ characteristics were similar in both groups with a slightly higher proportion of patients with age-adjusted IPI 2-3 in the R-CHOP14 arm (67% vs 59%). CR rates were 67% in the R-CHOP14 arm and 75% in the R-CHOP21 arm, but these differences were not statistically significant. The 2-year EFS, PFS, and OS were 48%, 49%, and 67% for the R-CHOP14 arm compared with 61%, 63%, and 72% for R-CHOP21. Grade 3-4 hematological toxicity was more frequent in the R-CHOP14 group, resulting in a higher proportion of patients hospitalized for adverse events. However, white blood-cell growth factors were not routinely given to patients who received R-CHOP14 in this trial as is typically performed for patients who receive dose-dense regimens in the United States. Additional follow-up is needed to determine whether a single preferred standard has been established. Until then, it appears that 6 cycles of CHOP14 (for older patients based on GLSG data) and CHOP21 (given for at least 6 cycles) are standard-of-care therapies as long as rituximab is given with chemotherapy.

The role for first-line high-dose therapy with autologous stem cell transplantation (ASCT) in DLBCL has been debated for several years. Conflicting results of high-dose therapy as part of first-line therapy have been reported in randomized controlled trials. A 2008 meta-analysis that included data from 15 trials with a total of 3079 patients showed that overall treatment-related mortality was the same in patients treated with chemotherapy alone versus those treated with high-dose therapy. Thirteen studies including 2018 patients showed significantly higher response rates in the group receiving high-dose therapy; however, this did not result in improved EFS or OS. However, it is possible that patients with high risk features could benefit from more intensive treatment regimens.

The US Intergroup S9704 trial comparing 8 cycles of R-CHOP21 versus 5 cycles of R-CHOP21 + high-dose therapy and the Groupe Ouest Est Leucemies Aigues Myeloblastiques (GOELAM; Western Group for Acute Myeloblastic Leukemias) trial comparing 8 cycles of R-CHOP14 versus 2 cycles of R-CEEP (cyclophosphamide, epirubicin, vindesine, prednisone) followed by high-dose therapy should provide additional data to address this question.

Approximately 25% of patients present with localized DLBCL, most commonly defined as stage I and nonbulky stage II disease (with tumor size up to 10 cm in greatest diameter). For patients with limited-stage DLBCL, chemotherapy given with rituximab for 3 cycles followed by radiation has produced impressive early results, leading this to become a recommended standard of care. However, there has not been a randomized clinical trial in the rituximab era that conclusively addresses the need for radiation therapy in localized DLBCL, and controversy over the optimal approach for these patients remains.

The Southwest Oncology Group (SWOG) Study 8736 initially demonstrated that, for patients with localized DLBCL, 3 cycles of CHOP chemotherapy followed by involved-field radiation therapy was superior to 8 cycles of CHOP alone in terms of 5-year PFS (77% and 64%, respectively; \( P = .03 \)) and 5-year OS (82% and 72%, respectively; \( P = .02 \)). Patients who achieved CR in the study were randomized to receive 3000 cGy or were monitored. Patients with partial remission were not randomized, and all received 4000 cGy. However, with additional follow-up, the PFS and OS curves for the arms of this overlapped because of higher relapse rates for the group treated with a shorter course of CHOP and radiation. Furthermore, in a French study of the dose-intensive chemotherapy regimen—doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone (ACVBP)—given at 2-week intervals followed by sequential consolidation was superior to 3 cycles of CHOP plus 4000 cGy of involved-field radiation.
radiotherapy in patients with localized disease (5-year EFS, 82% vs 74%, respectively; \( P < .0001 \) and 5-year OS, 90% versus 81%, respectively; \( P = .001 \)).

Another French trial of DLBCL found that 5-year EFS and OS were similar for patients aged more than 60 years who received 4 cycles of CHOP with or without 4000 cGy of involved-field radiation administered one month after their last cycle of CHOP.

Although the SWOG phase 2 trial of 3 cycles of CHOP with rituximab and 4000-4600 cGy of locoregional radiation demonstrated excellent early PFS (93% at 2 years and 88% at 4 years) and OS (95% at 2 years and 92% at 4 years), concern exists that this shorter course of chemoimmunotherapy with radiation may be associated with late relapse similar to a prior SWOG trial. Among patients who present with bulky disease, R-CHOP followed by radiation has been considered standard therapy, but controversy exists here as well.

### Salvage Treatment for Patients With Relapsed DLBCL

Although the adoption of R-CHOP as the new standard of care has improved outcomes for DLBCL, patients still relapse. The standard approach for fit patients with DLBCL has been to proceed toward salvage therapy and consolidation with autologous stem cell transplantation (ASCT). The question of how ASCT compares with conventional salvage therapy for relapsed disease was addressed by a multicenter trial known as the PARMA trial (because the group formed in 1986 during a meeting in Parma, Italy). In this trial, 215 patients in first or second relapse received 2 cycles of intensive combination chemotherapy. The 109 patients who responded were randomly assigned to receive 4 more cycles of chemotherapy or ASCT. With a 5-year median follow-up, EFS and OS were significantly improved with transplantation (46% vs 12% and 53% vs 32%, respectively). On the basis of these observations, high-dose therapy followed by ASCT became the treatment of choice for relapsed or refractory patients who respond to salvage therapy.

A recent evidence-based review on the role of ASCT in the management of DLBCL continues to recommend ASCT as salvage therapy for patients with chemosensitive relapsed DLBCL. However, the success of R-CHOP in the upfront setting also appears to select for patients with a poorer prognosis in the relapsed setting (discussed below). Several standard regimens exist for salvage lymphoma therapy including ICE (ifosphamide, carboplatin, etoposide), ESHAP (etoposide, methyl prednisolone, high-dose cytarabine, cisplatin), DHAP (dexamethasone, cisplatin, cytarabine), and GDP (dexamethasone, cisplatin, gemcitabine) with various response rates. The choice of salvage therapy is still debated, but the addition of rituximab to the salvage regimen appears to benefit relapsed patients as well.

For example, the Hemato-Oncologie voor Volwassenen Nederland (HOVON; Hemato-Oncology Foundation for Adults in the Netherlands) group randomized relapsed patients to DHAP with or without rituximab. Following 2 cycles, 75% of the patients in the R-DHAP arm had responsive disease versus 54% in the DHAP arm. With a median follow-up of 24 months, there was a significant difference in PFS (52% vs 31%; \( P < .002 \)) and OS in favor of the R-DHAP arm. Moreover, rituximab does not appear to impair stem cell engraftment or adversely affect transplantation toxicity and is associated with improved PFS when administered before ASCT for DLBCL.

In another study validating the use of rituximab at relapse, Kewalramani and colleagues conducted a retrospective review of patients treated with (rituximab with ICE) R-ICE and compared them to historical controls treated with ICE alone. R-ICE given for 3 cycles produced CR in 53%, and no patient had R-ICE–related toxicity that precluded ASCT. It is important to note that patients in both studies had received prior induction therapy without the addition of rituximab.

Among patients with relapsed or refractory DLBCL who received R-ESHAP as salvage therapy with curative intent, those previously exposed to rituximab had very low CR and OR rates. For example CR and OR rates for patients with primary refractory disease were 8% and 33%, respectively, compared with those in first partial remission (41% and 86%) or who had relapsed disease (50% and 75%). A comparison of patients with and without prior exposure to rituximab revealed that 60% underwent ASCT after salvage therapy with a PFS of 17% versus 57% and an OS of 38% versus 67% at 3 years.
The choice of salvage chemotherapy after R-CHOP failure was addressed by a prospective multicenter phase 3 study, the Collaborative Trial in Relapsed Aggressive Lymphoma (CORAL). DLBCL patients were randomized to receive salvage R-ICE \((n = 202)\) or R-DHAP \((n = 194)\). After 3 courses, responders underwent high-dose therapy and ASCT. The overall response rates \((63.5\% \text{ vs } 62.8\%)\), 3-year PFSs \((31\% \text{ vs } 42\%)\), and 3-year OSs \((47\% \text{ vs } 51\%)\) for R-ICE and R-DHAP were not statistically different, suggesting that either regimen can be used for salvage therapy. Factors that affect 3-year OS include 1) second-line age-adjusted IPI of \(\geq 2\) \((32\% \text{ vs } 62\%)\), 2) relapse <12 months after completion of first-line therapy \((39\% \text{ vs } 64\%)\), and 3) prior rituximab exposure in the front-line setting \((40\% \text{ vs } 66\%)\). Given that nearly all patients with DLBCL currently receive front-line rituximab, these data call into question our current strategies for salvage therapy, particularly for patients who relapse within one year of initial therapy.

**Novel Therapies for DLBCL**

Although rituximab and R-chemotherapy regimens have greatly improved response rates and survival for patients with DLBCL, relapse remains a consistent clinical problem. Of particular concern are preliminary data from the CORAL trial indicating that although DLBCL is commonly cured with first-line R-CHOP, and many patients have been salvaged at relapse with ASCT in the past, current DLBCL patients are at higher risk when they relapse early following upfront R-CHOP chemotherapy and have a poor response to second-line rituximab-containing regimens even when these regimens are consolidated with high-dose therapy and ASCT. Novel approaches clearly are needed for DLBCL patients who relapse early after R-CHOP chemotherapy. The improved understanding of DLBCL subtypes and molecular profiles has led to subsequent development of targeted drugs and regimens for DLBCL, which may address this clinical problem. Several drugs are undergoing evaluation in DLBCL, both as single agents in the relapsed setting and in combination with R-CHOP. These include other antibody therapies, lenalidomide, SGN-40, bevacizumab, Syk inhibitors, enzastaurin, histone deacetylase inhibitors, bortezomib, antisurvivin agents, and mTOR inhibitors.

For example, protein kinase C beta (PKC\(\beta\)) was identified by gene expression profiling, preclinical evaluation, and independent immunohistochemical analysis as a rational therapeutic target in DLBCL. A multicenter phase 2 study of a potent inhibitor of PKC\(\beta\), enzastaurin, was performed administering the drug orally once daily until disease progression or unacceptable toxicity occurred in 55 patients with relapsed or refractory DLBCL. This trial demonstrated that enzastaurin was well tolerated. Four patients, including 3 who achieved CR, experienced freedom from progression for at least 20 months after study entry. A randomized placebo-controlled study of enzastaurin maintenance therapy after R-CHOP administration in DLBCL patients at high risk for relapse has completed enrollment.

Another target identified through gene expression profiling, B-cell receptor (BCR)-mediated survival signals, can be blocked by an inhibitor of spleen tyrosine kinase (Syk), fostamatinib disodium, which induces apoptosis in B-cell lymphoma cell lines and primary tumors. These data prompted a phase 1/2 clinical trial in patients with relapsed B-cell NHL. Five of 22 patients with relapsed DLBCL who received 200 mg of the drug orally twice daily responded, and the drug was well tolerated. On the basis of this trial, the authors proposed that disrupting BCR signaling by inhibiting Syk represents a novel and active therapeutic approach for DLBCL and other B-cell NHLs.

Although there is evidence that tonic low-grade signaling through the B-cell receptor contributes to the survival of DLBCL, the BCR signaling pathway appears to be of most importance for the ABC subtype and may limit the activity of enzastaurin and Syk inhibitors to subsets of ABC-like DLBCL. Conversely, chronic activation of BCR signaling may be an important pathogenetic event in some cases of ABC DLBCL that emerge without mutations that directly activate the NF-\(\kappa\)B pathway; thus, Syk inhibition may be particularly effective for this group of patients.

A proteasome inhibitor, bortezomib, demonstrated single-agent activity, which led to its approval for use in relapsed mantle cell lymphoma, and is another agent hypothesized to have distinct activity in DLBCL on the basis of its molecular profile.
ABC-like DLBCL and PMBL subtypes are known to have high levels of activity in the NF-κB pathway, which may be targeted by bortezomib. In a trial examining whether addition of bortezomib to dose-adjusted infusion of etoposide, vincristine, and doxorubicin, with cyclophosphamide and prednisone (DA-EPOCH-B) would preferentially improve the survival of patients with ABC-like DLBCL, the overall response rate was 13% in GCB DLBCL compared with 83% in ABC DLBCL (P < .001). In a phase 1/2 trial, patients with non-GCB DLBCL had PFSs and OSs similar to GCB patients when bortezomib was added to R-CHOP, suggesting that bortezomib may mitigate adverse outcomes associated with the ABC subtype. Supporting this approach are preclinical data that suggest use of bortezomib can improve therapeutic response in rituximab and chemotherapy-resistant lymphoma cell lines. A multicenter clinical trial that uses the Hans method to subtype DLBCL patients and then randomizes non-GCB patients to bortezomib plus R-CHOP alone is now underway.

Lenalidomide, another approved agent that is used in myelodysplastic syndrome and myeloma, has been studied in patients with relapsed aggressive lymphomas and has produced responses in 5 of 26 DLBCL patients. Lenalidomide is currently being investigated in combination with R-CHOP and as maintenance therapy for patients with DLBCL.

Although these new agents offer promise for improving outcomes for DLBCL patients, considerable challenges remain in identifying the appropriate DLBCL patients for investigating particular agents: in selecting the ideal therapeutic setting (eg, combination with R-CHOP, maintenance therapy, at relapse as a single agent or in combination), in demonstrating statistically and clinically that the new agents add value to existing therapy, and in integrating and sequencing these new therapies with current treatment regimens.

Whereas rituximab was the first monoclonal antibody approved for B-cell NHL and clearly has revolutionized therapy for DLBCL, other antibodies targeting B-cell lymphomas are now available on an investigational basis, including AME-133, GA101, epratuzumab (CD22), dace-tuzumab (CD40), galiximab (CD80), lexatumumab (TRAIL), as are other approaches to improve antibody therapy such as conjugation with radioisotopes or toxins. Systematically developing each of these agents and integrating them into treatment strategies for DLBCL will require an improved understanding of their specific activity and mechanisms of action in each DLBCL subtype, alone and in combination with the existing regimens described above. Moreover, the degree to which the benefits of adding rituximab to chemotherapy in DLBCL is a class effect that may be observed with other anti-CD20 antibodies (or other B-cell targeted antibodies) is unknown, and it will remain so unless randomized trials comparing rituximab plus chemotherapy to another antibody plus chemotherapy are performed.

Given the excellent outcome in PFS of the first-line studies described, R-CHOP may have set the bar sufficiently high that such studies will not be performed as pathways to drug approval. Notably, the ongoing bortezomib trial for patients with non-GCB DLBCL and a proposed ECOG trial with epratuzumab suggest that randomized clinical trials of additions to R-CHOP may still be feasible. Ultimately, understanding mechanisms by which malignant B-cells become resistant to rituximab and chemotherapy and determining means to address these mechanisms may provide pathways for approval of novel agents. Moreover, defining the biology of resistance and activity for various agents across DLBCL subtypes will become increasingly important in the future as we attempt to select among regimens for newly diagnosed and relapsed patients.

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