Advances in the biology and therapy of diffuse large B-cell lymphoma: moving toward a molecularly targeted approach

Jeremy S. Abramson and Margaret A. Shipp

Diffuse large B-cell lymphoma (DLBCL) displays striking heterogeneity at the clinical, genetic, and molecular levels. Clinical prognostic models can define a population at high risk for relapse following empiric chemotherapy, although such models do not account for underlying biologic differences among tumors. Commonly observed genetic abnormalities that likely contribute to pathogenesis include translocations of BCL6, BCL2, cMYC, and FAS(CD95) mutations, and aberrant somatic hypermutation. Despite recent advances in empiric chemotherapy, including interval reduction of CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) and the incorporation of anti-CD20 monoclonal antibodies, a significant proportion of patients still die of their disease. Gene expression profiling has shed light on the molecular heterogeneity within DLBCL by highlighting similarities between subsets of tumors and normal B cells, identifying features associated with unfavorable responses to empiric combination chemotherapy, and defining robust subtypes with comprehensive transcriptional signatures. Such strategies have suggested distinct routes to lymphomagenesis and have identified promising rational therapeutic targets. Additional novel therapies under investigation include those targeting BCL6 and BCL2, as well as development of novel monoclonal antibody-based therapies. Our increasing molecular understanding of the heterogeneous subsets within DLBCL will likely improve the current empiric therapy of DLBCL by identifying rational therapeutic targets in specific disease subtypes. (Blood. 2005;106:1164-1174)

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Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoid malignancy in adults, accounting for approximately 30 000 new cases each year and nearly 40% of all non-Hodgkin lymphomas (NHLs). Although the cause of most DLBCLs remains unknown, predisposing factors include congenital and acquired immunodeficiency states that are often associated with dysregulated apoptosis or defective DNA repair. Although DLBCL has proven one of the most chemotherapy-responsive human malignancies, many newly diagnosed patients will not be cured with conventional anthracycline-based chemotherapy. The variability in response to therapy suggests underlying heterogeneity in a disease that is largely treated with a homogeneous approach. Recent developments in biology and therapy are now changing the face of DLBCL.

Pathologic classifications

The evolution of lymphoma classification is largely of historical interest, although understanding these systems is essential in interpreting the literature on DLBCL therapy. Early systems by Rappaport and others were based exclusively on architecture and morphology. Emerging knowledge of lymphoid biology led to the Kiel and Lukes-Collins systems in 1974 and to the Working Formulation for Clinical Usage (WF) in 1982. The WF classified tumors on the basis of cell size, nodal pattern, and morphology and assigned grade based on untreated natural history with a low, intermediate, and high grade corresponding to survival measured in years, months, and weeks, respectively. Although the WF was broadly applicable and prognostically useful, it lacked some of the biologic insights of Kiel and Lukes-Collins.

The Revised European-American lymphoma classification system (REAL), published in 1994, incorporated genetics, immunophenotyping, lymphoid lineage, and further insights on lymphocyte development. REAL created a practical approach to identifying discrete entities based on biologic features and dispensed with the artificial grouping of entities based on untreated natural history. Prior to being named DLBCL in the REAL classification, this disease was included under several different descriptors in prior classifications (Table 1). In the REAL classification, the diagnosis of DLBCL was expanded to include B-cell tumors previously designated as immunoblastic lymphomas. DLBCLs were also clearly distinguished from other aggressive lymphomas including peripheral T-cell, mantle cell, anaplastic large (T) cell, follicular large cell, and Burkitt-like, all of which had been included with DLBCL in previous randomized trials.

Although the REAL classification was subsequently updated as the World Health Organization (WHO) classification in 2001, the diagnosis of DLBCL remained largely unchanged. DLBCL is so named because the malignant B lymphocytes diffusely efface the normal architecture of the lymph node or extranodal site. The cells are large transformed lymphocytes, which have been further divided into morphologic variants: centroblastic, immunoblastic, T-cell/histiocyte-rich, and anaplastic (B). DLBCLs typically express pan-B-cell markers CD19, CD20, CD22, CD79a, and often have surface immunoglobulin (50%-75% of cases). CD30 expression may occur, most commonly with the anaplastic variant. A small number of tumors may express CD10 or CD5, although they...
are cyclin D1 negative, distinguishing them from blastic mantle cell lymphoma.6

By contrast, the morphologic variant T-cell/histiocyte-rich B-cell lymphoma (T/HRBCL), is characterized histologically by increased numbers of polyclonal T cells infiltrating the tumor and fewer than 10% neoplastic B cells.8 These malignant B cells, however, share the immunohistochemical characteristics of DLBCL.

Several clinical variants of DLBCL are further defined based on their major site of clinical presentation, including primary mediastinal, intravascular, and primary effusion lymphomas.6 Primary mediastinal (thymic) large B-cell lymphoma (MLBCL) typically presents as a localized, sclerotic mass in young female patients, unlike DLBCL, which commonly arises in elderly patients of both sexes.7,8 MLBCL may be difficult to differentiate histologically from DLBCL, although MLBCL is often characterized by interdispersed dense fibrosis and a polyclonal infiltrate of host inflammatory cells, reminiscent of classical Hodgkin lymphoma (cHL).8 MLBCLs express pan-B-cell markers and have rearranged immunoglobulin genes, although they do not express surface immunoglobulin like DLBCL. These tumors often demonstrate weak CD30 staining, a feature more commonly associated with cHL than DLBCL.8

Several genetic abnormalities have been identified in subsets of DLBCLs (Table 2). Recurring chromosomal translocations occur in approximately 50% of cases10 and DNA imbalances in as many as 67%.11 The 3 most frequently deregulated genes, BCL6, BCL2, and cMYC, share a common mechanism whereby chromosomal translocation brings the target gene under inappropriate control of an immunoglobulin regulatory element.

### BCL6

Chromosomal translocations involving the BCL6 gene on band 3q27 are the most common genetic abnormalities in DLBCL, occurring in 35% to 40% of cases.12 Although several chromosomes may partner with 3q27, the most common translocations involve the immunoglobulin heavy-chain promoter, resulting in constitutive expression of this normally developmentally regulated gene.13,14 BCL6 is a zinc-finger transcription repressor normally expressed exclusively within GC B cells, suggesting a critical role in the GC reaction. Indeed, BCL6 null animals fail to generate GCs in response to antigen.15 The down-regulation of BCL6 may be necessary for normal GC B cells to further differentiate into memory B cells or plasma cells. In DLBCL, dysregulated constitutive expression of BCL6 may lead to maturation arrest and confer a proliferative advantage.

Recent studies identify a mechanism whereby BCL6 may regulate GC formation and lymphomagenesis via down-regulation of p53.16 Investigators postulate that BCL6 functions normally to suppress p53-mediated apoptosis of GC B cells in response to DNA damage during the GC reaction. Constitutive expression of BCL6 might decrease the p53-mediated apoptotic response to DNA damage, promoting persistence of malignant clones. A recently developed BCL6 transgenic mouse provides further insight into the precise role of this gene in lymphomagenesis.17

Clinically, BCL6 rearrangements occur primarily in de novo DLBCL.14 No uniform effect on prognosis has been observed, likely due to multiple other contributing factors, including differential biology of the partner chromosome, concomitant genetic defects, SHM, and unidentified molecular substructure.

### BCL2

BCL2 is a proto-oncogene located at 18q21 that promotes B-cell survival via inhibition of apoptosis and confers chemotherapy resistance.18 The BCL2 family includes both antiapoptotic and proapoptotic members that form heterodimers and homodimers. Following death signals, proapoptotic homodimers alter mitochondrial membrane potential, trigger cytochrome c release, and caspase-mediated apoptosis. Increased abundance of antiapoptotic BCL2 proteins favors the formation of antiapoptotic/proapoptotic heterodimers rather than proapoptotic/proapoptotic homodimers, limiting the effects of death signals at the mitochondrial membrane.

### Genetic and biologic heterogeneity

DLBCL is thought to arise from normal antigen-exposed B cells that have migrated to or through germinal centers (GCs) of lymph nodes or secondary lymphoid organs. Somatic hypermutation (SHM) of immunoglobulin variable region (IgV) genes, a process requiring double-strand DNA breakage, occurs during GC B-lymphocyte development and serves as a marker for this stage of maturation.8 SHM generates antibody diversity and increases antigen affinity, but also creates a setting for chromosomal translocations and mutagenesis to occur.8

### Table 1. DLBCL in NHL pathologic classification systems

<table>
<thead>
<tr>
<th>Classification System</th>
<th>Type of Lymphoma</th>
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<tbody>
<tr>
<td>Rappaport (1966)</td>
<td>Diffuse histiocytic lymphoma</td>
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<tr>
<td>Kiel (1974)</td>
<td>Centroblastic lymphoma</td>
</tr>
<tr>
<td>Lukes-Collins (1974)</td>
<td>Large cleaved follicular center cell lymphoma</td>
</tr>
<tr>
<td>Working Formulation</td>
<td>Diffuse mixed small and large cell lymphoma (group F)</td>
</tr>
<tr>
<td>REAL (1994) and</td>
<td>Diffuse large B-cell lymphoma (group H)</td>
</tr>
<tr>
<td>WHO (2001)</td>
<td>Large noncleaved follicular center cell lymphoma</td>
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</table>

### Table 2. Major recurring genetic events in DLBCL

<table>
<thead>
<tr>
<th>Genetic defect</th>
<th>Frequency</th>
<th>Location</th>
<th>Mechanism of deregulation</th>
</tr>
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<tbody>
<tr>
<td>BCL6</td>
<td>35%-40%12</td>
<td>3q27</td>
<td>t(3;...), SHM</td>
</tr>
<tr>
<td>BCL2</td>
<td>t(14;18)</td>
<td>18q21</td>
<td>t(14;18) and gene amplification</td>
</tr>
<tr>
<td>cMYC</td>
<td>15%20</td>
<td>8q24</td>
<td>t(8;...), SHM</td>
</tr>
<tr>
<td>FAS(CD95)</td>
<td>20%20</td>
<td>1q24</td>
<td>Death domain mutations, SHM</td>
</tr>
<tr>
<td>SHM</td>
<td>45%21</td>
<td>Physiologic: IgV, FAS, BCL6, Aberrant: BCL6, PIM1, cMYC, PAX5, Rhod/TF, FAS</td>
<td>SHM</td>
</tr>
<tr>
<td>p53</td>
<td>16%11</td>
<td>17p</td>
<td>Mutation, deletion</td>
</tr>
</tbody>
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Either the relative excess of antiapoptotic BCL2 family members or the deficiency of proapoptotic isoforms may confer a survival advantage and contribute to lymphomagenesis. Proof of principle is provided by models in which mice overexpressing BCL2 protein due to the t(14;18) developed follicular hyperplasia and extended survival of B lymphocytes; conversely, mice deficient for a proapoptotic BCL2 family member, BAD, developed DLBCL of GC origin.

BCL2 expression is normally down-regulated in the GC where apoptosis plays a critical role in negative B-cell selection. BCL2 deregulation is most commonly associated with the t(14;18), present in approximately 15% of DLBCLs. BCL2 protein expression, however, can be detected in approximately 50% of DLBCLs, independent of the t(14;18). Interestingly, increased expression of the BCL2 protein is associated with an inferior outcome in DLBCL, though the t(14;18) alone has no predictive value.

Because the t(14;18) is the hallmark abnormality in follicular lymphoma and transformed follicular lymphomas resemble de novo DLBCL, the reported frequencies of t(14;18) in DLBCL series may depend on how accurately included tumors and patients were prescreened.

**cMYC**

cMYC, a transcription factor associated with Burkitt lymphoma, is deregulated in approximately 15% of DLBCLs, although some of these cases may actually represent Burkitt-like lymphoma, because the distinction can be difficult. Deregulation occurs most commonly in the setting of t(8;14), which brings the cMYC gene on 8q24 under the control of an immunoglobulin promoter. cMYC rearrangements have no clear effect on survival.

**FAS(CD95)**

FAS(CD95) is a proapoptotic protein expressed within GCs where it plays an important role in negative selection of B cells. FAS ligand cross-links the transmembrane FAS death receptor, leading to the assembly of a death-inducing signaling complex and initiating caspase-mediated apoptosis. FAS mutations have been reported in up to about 20% of DLBCLs, most commonly within the last exon, which encodes the death domain. Such mutations likely act in a dominant-negative manner, destabilizing trimeric FAS receptors. Loss of FAS leads to defective affinity maturation and failure to negatively select autoreactive B cells, which may lead to autoimmune disease and persistence of malignant clones. Indeed, mice with FAS and FAS ligand mutations are prone to development of B-cell lymphomas, as are families with germline FAS mutations and associated autoimmune lymphoproliferative syndrome.

**Aberrant SHM**

Normal GC B cells undergo immunoglobulin gene editing via SHM of their rearranged IgV region genes. This process specifically targets regulatory sequences downstream of the IgV gene promoter. DLBCLs typically have evidence of SHM, indicating that these tumors likely originate from GC or post-GC B cells. The 5′ regulatory regions of other GC genes, BCL6 and CD95 (FAS), are also known targets of physiologic SHM (Figure 1). SHM may also occur aberrantly and is postulated to be an additional pathogenetic mechanism in DLBCL. Genes aberrantly targeted by SHM include BCL6, PIM1, cMYC, PAX5, and RhoH/TTF (Figure 1). Like BCL6, FAS(CD95) has been postulated to be targeted by both physiologic and aberrant SHM. Because the SHM machinery targets broad regions of multiple genes, the specific consequences of aberrant SHM may be quite different in individual tumors.

**p53**

Although occurring rarely as an isolated event in DLBCL, mutation of the p53 tumor suppressor gene has been associated with poor outcome. p53 is located on chromosome 17p, deletion of which has also correlated with an inferior prognosis. Mutations of p53 in animal models have been associated with B-cell malignancies. In a murine model of impaired DNA repair, mice deficient for both histone 2AX (H2AX) and p53 had markedly increased numbers of B-cell lymphomas and solid tumors.

**Chromosomal imbalances**

Numerous chromosomal imbalances have been observed in DLBCL, some of which may influence prognosis. Poor outcome has been associated with abnormalities of chromosomes 1q, 5, 7q, 12p, and 6q. Whereas gain of 3p has been associated with improved prognosis. Defects with no clear influence on prognosis include abnormalities of Xq, 7q, 12p, and 6q. The role of these abnormalities in DLBCL pathogenesis is unclear.

**REL**

The reported amplification of chromosome 2p13 in up to 14% of DLBCL has focused attention on genes located at this locus, such as cREL. Because cREL encodes a component of the nuclear factor kB (NF-kB) heterodimer, previous studies have suggested that increased cREL abundance might be associated with increased NF-kB activity. Recent data, however, indicate that 2p13 amplifications are more common in subsets of DLBCL without evidence of NF-kB activation, suggesting a different functional target at this locus. Interest, the BCL11A proto-oncogene has been found to be simultaneously amplified with cREL in tumors with gains of chromosome 2p13.

**Clinical heterogeneity: stratification according to risk**

Although a subset of patients with DLBCL can be cured with standard chemotherapy, many will still die of their disease. Clinical risk stratification models such as the International Prognostic Index (IPI) were developed to identify patients at high risk for relapse.
after anthracycline-based chemotherapy and to allow interpretation of clinical trials based on the clinical heterogeneity of included patients.

In patients with aggressive lymphoma who received anthracycline-based chemotherapy, pretreatment variables that independently predicted outcome were advanced age, poor performance status, elevated lactic dehydrogenase (LDH) levels, advanced Ann Arbor stage, and multiple extranodal sites. Four risk groups—low, low-intermediate, high-intermediate, and high—were generated by summing the indicated risk factors present at diagnosis. More recent studies of lymphomas classified according to REAL/WHO criteria indicate that the IPI is more predictive of outcome in DLBCL than other aggressive lymphomas.

### Evolution of empiric therapy

Successful cure of advanced stage DLBCL was first reported in 1972, at which time the disease was known as reticulum cell sarcoma. The CHOP regimen (cyclophosphamide, doxorubicin, vincristine, and prednisone) was subsequently considered to be standard therapy based on phase 2 studies in which approximately 35% of patients were cured of their disease. Thereafter, second- and third-generation regimens were developed that appeared to improve on the success of CHOP.

Improving on the gold standard

Several recently reported randomized trials have focused on increasing dose frequency and intensity of CHOP. For example, the 21-day CHOP regimen was compared to CHOP given every 2 weeks (CHOP-14), with or without etoposide (CHOEP), in young and elderly patients with aggressive lymphoma, respectively. Using a 2 × 2 factorial design, patients were randomized to receive CHOP-21, CHOP-14, CHOEP-21, or CHOEP-14.

### Table 3. Randomized trials of CHOP versus other regimens in newly diagnosed previously untreated aggressive lymphomas

<table>
<thead>
<tr>
<th>Author/Year</th>
<th>Regimens</th>
<th>Patient characteristics</th>
<th>Diseases</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td>Early trials</td>
<td></td>
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<tr>
<td>Gordon et al (1992)</td>
<td>CHOP vs m-BACOD IV</td>
<td>325 patients, all ages, stages III, IV</td>
<td>WF* groups F, G</td>
<td>No difference in TTF, OS</td>
</tr>
<tr>
<td>Cooper et al (1994)</td>
<td>CHOP vs MACOP-B</td>
<td>236 patients, aged &gt;16 y, stage I (bulky), II-IV</td>
<td>WF groups D, E, F, G, H</td>
<td>No difference in FFS, OS</td>
</tr>
<tr>
<td>Fisher et al (1994)</td>
<td>CHOP vs m-BACOD vs ProMACE-CytarBOM vs MACOP-B</td>
<td>899 patients, all ages, stage II (bulky), III, IV</td>
<td>WF groups D, E, F, G, H, J (80% were F, G, H)</td>
<td>No difference in TTF, OS</td>
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<tr>
<td>Recent trials</td>
<td></td>
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<tr>
<td>Tilly et al (2003)</td>
<td>CHOP vs ACVBP</td>
<td>635 patients, aged 61-69 y, stage I-IV, at least one adverse prognostic factor by aaIPI</td>
<td>WF groups F, G, H, I, J (79% were DLBCL)</td>
<td>5 y EFS: ACVBP 39% vs CHOP 29% (P = .005); 5 y OS: ACVBP 46% vs CHOP 38% (P = .036)</td>
</tr>
<tr>
<td>Pfreundschuh et al (2004)</td>
<td>CHOP-14 vs CHOEP-21 vs CHOP-14/21 vs CHOEP-14</td>
<td>710 patients, aged 18-60, stage I-IV, normal LDH level</td>
<td>REAL/WHO: DLBCL (60%), MLBL (3.0%), Follicular grade III, Burkitt, aggressive marginal zone, anaplastic large-cell lymphoblastic, PTCL, angioimmunoblastic, extranodal NK/T, nasal type, aggressive NOS</td>
<td>5 y EFS: CHO(E)P-14 65% vs CHO(E)P-21 58% (P = .004); 5 y OS: CHO(E)P-14 85% vs CHO(E)P-21 58% (P = .004), CHOPE-14/21 53% vs CHOPE-14/21 58% (P = NS)</td>
</tr>
<tr>
<td>Pfreundschuh et al (2004)</td>
<td>CHOP-14 vs CHOEP-14 vs CHOEP-21</td>
<td>689 patients, aged 61-75 y, stage I-IV</td>
<td>REAL/WHO: DLBCL (71%), MLBL (0.6%), Follicular grade III, Burkitt, marginal zone, anaplastic large cell, lymphoblastic, PTCL, angioimmunoblastic, extranodal NK/T, nasal type, aggressive NOS</td>
<td>5 y EFS: CHOEP-14 44% vs CHOEP-21 33% (P = .003), CHOEP-21 41% vs CHOEP-21 33% (P = NS); 5 y OS: CHOEP-14 53% vs CHOEP-21 41% (P &lt; .001), CHOEP-21 46% vs CHOEP-21 41% (P = NS)</td>
</tr>
</tbody>
</table>

**Table Legend:**
- CHOP indicates cyclophosphamide, doxorubicin, vincristine, prednisone; m-BACOD, methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone; TTF, time to treatment failure; OS, overall survival; MACOP-B, methotrexate, doxorubicin, vincristine, cyclophosphamide, prednisone, bleomycin; FFS, failure-free survival; ProMACE-CytarBOM, prednisone, procarbazine, doxorubicin, cyclophosphamide, etoposide/cytarabine, bleomycin, vincristine, methotrexate; ACVBP, induction: doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone, intrathecal methotrexate; consolidation: intravenous methotrexate, etoposide, ifosfamide, cytotoxic-arabinoside; aaIPI, age-adjusted International Prognostic Index; EFS, event-free survival; CHOP, cyclophosphamide, doxorubicin, vincristine, etoposide, prednisone; LDH, lactic dehydrogenase; MLBL, mediastinal large B-cell lymphoma; PTCL, peripheral T-cell lymphoma; NOS, not otherwise specified; CHO(E)P, cyclophosphamide, doxorubicin, vincristine, prednisone with or without etoposide; NS, not significant.
- Working Formulation: group D, follicular large cleaved; E, diffuse small cleaved; F, diffuse mixed; G, diffuse large cell; H, large cell immunoblastic; I, lymphoblastic; J, small noncleaved cell (Burkitt).
- †Treatment-related mortality: ACVBP 13% versus CHOP 7% (P = .014).

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In the trial of elderly patients, CHOP-14 showed a significant improvement in both event-free survival (EFS) and overall survival (OS) versus traditional CHOP-21.55 The addition of etoposide did not further improve outcome, but did enhance toxicity.

In the trial of patients younger than age 60 years with a normal LDH level, EFS was significantly improved with the addition of etoposide, although OS was not.54 Patients who received the 14-day regimen had improved OS. The lack of survival benefit for etoposide despite the favorable EFS suggests that many of the patients in the control arm could be salvaged following relapse, a reasonable assumption given their favorable prognostic status.

Dose intensification was further evaluated in a randomized trial comparing CHOP to ACVB69 (doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone) in elderly patients with aggressive lymphomas (Table 3). At 5 years of follow-up, ACVB was associated with improved EFS and OS, although the rate of treatment-related death was nearly double. Of note, the ACVB regimen included intensive central nervous system (CNS) prophylaxis, which was associated with a significantly lower risk of CNS recurrence.56

**Table 4. Randomized trials of induction therapy followed by HDT/ASCT consolidation in newly diagnosed previously untreated aggressive lymphomas**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Protocol</th>
<th>Patient characteristics</th>
<th>Included diseases</th>
<th>Significant results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gianni et al68 (1997)</td>
<td>MACOP-B vs sequential HDT/ASCT</td>
<td>98 patients, aged 17-60 y, stage I (bulky), II (bulky), III, IV</td>
<td>WF* groups G, H</td>
<td>7-y EFS: HDT 76% vs MACOP-B 49% (P = .004); 7-y OS: HDT 81% vs MACOP-B 55% (P = NS)</td>
</tr>
<tr>
<td>Santini et al69 (1998)</td>
<td>VACOP-B plus salvage DHAP vs VACOP-B plus HDT/ASCT</td>
<td>124 patients, aged 15-60 y, stage II (bulky), III, IV</td>
<td>WF groups D, E, F, G, H, I, J</td>
<td>No difference in EFS and OS</td>
</tr>
<tr>
<td>Halouin et al70 (2000)</td>
<td>Induction with either ACVBP or NCBV; patients in CR randomized to: consolidation with MTX, ifosfamide, L-asparaginase and Ara-C vs MTX and HDT/ASCT</td>
<td>236 patients, aged &lt;55 y, stage I-IV, at least one poor prognostic variable (poor PS, multiple extranodal sites, bulky disease, BM, CNS, Burkitt or lymphoblastic)</td>
<td>WF groups D, E, F, G, H, I, J (73% were F, G, H)</td>
<td>Propective analysis: no difference in DFS and OS; Retrospective analysis on IPI H/risk patients: 8-y DFS: ASCT 55% vs chemotherapy 39% (P = .02); 8-y OS: ASCT 64% vs chemotherapy 49% (P = .04)</td>
</tr>
<tr>
<td>Klein-Nelemans et al71 (2001)</td>
<td>Induction with 3 cycles CHVmP/BV; patients in CR/PR with negative BM randomized to: 3 cycles CHVmP/BV HDT/ASCT vs 5 cycles CHVmP/BV</td>
<td>311 patients, aged 15-65 y, stage I (bulky), II-IV</td>
<td>WF groups D, E, F, G (51% were DLBCL)</td>
<td>No difference in FFP and OS</td>
</tr>
<tr>
<td>Gisselbrecht et al72 (2002)</td>
<td>4 cycles ACVB, 2 cycles HD MTX, 4 cycles EI, 2 cycles Ara-C vs 1 cycle CEEP, 2 cycles ECVPB, HDT/ASCT</td>
<td>370 patients, aged 15-60 y, stage I-IV, at least 2 IPI risk factors</td>
<td>REAL/WHO: DLBCL (61%), PTCL, lymphoblastic, Burkitt, diffuse aggressive NOS</td>
<td>5-y EFS: ASCT 52% vs ACVB 39% (P = .01); 5-y OS: ASCT 46% vs ACVB 60% (P = .007)</td>
</tr>
<tr>
<td>Milpied et al73 (2004)</td>
<td>4 cycles CHOP; patients in CR/PR receive 4 additional cycles vs 2 cycles CEEP; patients in CR/PR receive 1 cycle MC; HDT/ASCT</td>
<td>197 patients, aged 15-60 y, stage II (bulky), III, IV; aaIPI L, LI, or HI</td>
<td>WF groups D, E, F, G, H (75% were DLBCL)</td>
<td>Prospective analysis by ITT: 5-y EFS: ASCT 55% vs CHOP 37% (P = 0.037); 5-y OS: HDT 71% vs CHOP 56% (P = NS); Retrospective analysis on IPI H/risk patients: 5-y EFS: ASCT 56% vs CHOP 28% (P = .003); 5-y OS: HDT 74% vs CHOP 44% (P = .001)</td>
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</tbody>
</table>

MACOP-B indicates melphalan, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin; HDT, high-dose therapy; ASCT, autologous stem cell transplantation; EFS, event-free survival; OS, overall survival; NS, not significant; VACOP-B, etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin; DHAP, dexamethasone, cytarabine, cisplatin; DFS, disease-free survival; ACVB, doxorubicin, cyclophosphamide, vindesine, bleomycin; NCBV, mitoxantrone, cyclophosphamide, vinblastine, bleomycin; CR, complete response; MTX, methotrexate; Ara C, cytarabine; PS, performance status; BM, bone marrow; CNS, central nervous system; IPI, International Prognostic Index; H/H, high/high intermediate; CHVmP/BV, cyclophosphamide, doxorubicin, teniposide, prednisone, bleomycin, vincristine; PR, partial response; FFP, free from progression; ACVB, doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone; HD MTX, high-dose methotrexate; EI, etoposide, ifosfamide; CEEP, cyclophosphamide, epirubicin, vincristine, prednisone; ECVPB, epirubicin, cyclophosphamide, vindesine, bleomycin, prednisone; PTCL, peripheral T-cell lymphoma; NOS, not otherwise specified; CEEP, cyclophosphamide, epirubicin, vindesine, prednisone; MC, methotrexate, cytarabine; aaIPI, age-adjusted International Prognostic Index; L, low; LI, low intermediate; HI, high intermediate; ITT, intention to treat.

*Working Formulation groups are as described in Table 3.
†Crossover allowed at relapse, which may account for lack of survival benefit.

**Autologous stem-cell transplantation**

A more intensive strategy involves high-dose chemotherapy (HDT) followed by autologous stem-cell transplantation (ASCT). This approach, which cures nearly half of patients with chemotherapy-sensitive relapsed/refractory DLBCL,73 has been associated with more variable results in the upfront setting (Table 4).56-60 One of the earliest randomized trials of full-dose induction therapy followed by HDT/ASCT or low-dose consolidation in complete responders was LNH-87.61 Among all eligible patients evaluated prospectively, HDT/ASCT did not affect OS; however, in retrospective analyses of IPI high-intermediate/high (HI/H) risk groups, HDT/ASCT improved both disease-free survival (DFS) and OS. In a follow-up study, LNH-93, HI/H-risk patients were randomized to receive full induction therapy followed by sequential consolidative chemotherapy or an abbreviated induction phase followed by HDT/ASCT for those achieving complete response (CR).62 This study showed an inferior survival in the transplantation arm,
prompted early closure of the study. Several additional studies of abbreviated induction followed by HDT/ASCT likewise suggested lack of benefit for this approach.\textsuperscript{63-65} A recent prospective trial of CHOP or abbreviated induction therapy followed by HDT/ASCT, however, suggested that the HDT improved EFS in all patients and OS in IPI HI-risk patients.\textsuperscript{66} Given the variable results of upfront HDT/ASCT and additional promising treatment options, upfront HDT/ASCT can only be recommended in the context of a clinical trial.

Addition of rituximab

Rituximab is a recombinant humanized monoclonal antibody against the pan-B-cell marker CD20.\textsuperscript{67,68} Although the precise mechanism of action of rituximab is unknown, it likely includes antibody-dependent cell-mediated cytotoxicity, complement-mediated lysis, and induction of apoptosis.\textsuperscript{65} The benefit of adding rituximab to CHOP (R-CHOP) was demonstrated in a randomized trial of R-CHOP versus CHOP in elderly patients with aggressive B-cell lymphomas (Table 5).\textsuperscript{69} At a median of 2 years of follow-up, there was a superior OS in the rituximab-containing arm, with only minimal additional toxicity. These data were recently updated at 5 years and showed persistent benefit for R-CHOP.\textsuperscript{70} Of interest, in this study the rituximab benefit was primarily limited to patients who overexpressed BCL2 protein, suggesting that rituximab may act in part by overcoming BCL2-induced chemotherapy resistance.\textsuperscript{71}

An additional randomized trial of CHOP versus R-CHOP in elderly patients with aggressive lymphomas, with a second randomization to maintenance rituximab (MR) or no further therapy, was recently completed.\textsuperscript{72} In preliminary analysis, there was an improved time-to-treatment failure (TTF) favoring R-CHOP, but no survival difference; likewise, TTF was improved in the MR group, but again without survival benefit. These data must be interpreted in the context of the second randomization, which resulted in approximately 40% of the CHOP group receiving MR. In subgroup analysis, the benefit of MR was limited to patients who received CHOP alone for induction, whereas MR conferred no additional benefit to those who had received R-CHOP. These data, in concert with the previous randomized trial, suggest that rituximab is beneficial for elderly patients with DLBCL as part of a CHOP induction regimen, or as maintenance therapy, but that both are not required. An ongoing study (RICOVER-60) compares the efficacy of CHOP-14 to that of CHOP-14 with rituximab in elderly patients with DLBCL.

The benefit of rituximab in young, low-risk patients (defined as IPI \(\leq 1\)) has recently been reported in the first interim analysis of the MabThera International Trial (MInT trial).\textsuperscript{73} Patients aged 18 to 60 years with aggressive lymphoma were randomized to CHOP-like therapy, with or without rituximab. At 2 years of follow-up, there was a significantly improved TTF and OS in the rituximab-containing arm. Recently, a retrospective observational study further supported the benefit of rituximab in younger patients.\textsuperscript{74} These data suggest that the merits of rituximab are broadly applicable and not limited to elderly patients who simply could not tolerate aggressive chemotherapy.

Table 5. Randomized trials of rituximab added to CHOP in newly diagnosed previously untreated aggressive lymphomas

<table>
<thead>
<tr>
<th>Trial</th>
<th>Design/regimen</th>
<th>Patients/diseases</th>
<th>Diseases</th>
<th>Significant results</th>
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<tbody>
<tr>
<td>Coiffier et al\textsuperscript{69} (2002)</td>
<td>R-CHOP vs CHOP</td>
<td>399 patients, aged 60-80 y, stage II-IV</td>
<td>REALWHO: DLBCL (84%), Burkitt, mantle-cell, marginal-zone, follicular lymphoma, SLL, Hodgkin lymphoma, B-cell lymphoma NOS, T-cell lymphoma</td>
<td>2-y EFS*: R-CHOP 43% vs CHOP 61% (P = .002); 2-y OS: R-CHOP 70% vs CHOP 57% (P = .007)</td>
</tr>
<tr>
<td>Habermann et al\textsuperscript{72} (2003)</td>
<td>First randomization: R-CHOP vs CHOP; second randomization: MR vs no maintenance</td>
<td>632 patients, aged &gt;60 y</td>
<td>REALWHO: DLBCL</td>
<td>2.7-y TTF favors R-CHOP (P = .025) and MR (P = .01); no difference in OS</td>
</tr>
<tr>
<td>Pfreundschuh et al\textsuperscript{73} (2004)</td>
<td>R-CHOP-like vs CHOP-like</td>
<td>326 patients, aged 18-60 y, stage I (bulky), II-IV, low risk (IPI of 0-1)</td>
<td>REALWHO: DLBCL</td>
<td>2-y TTF: R-CHOP-like 76% vs CHOP-like 60% (P &lt; .001); 2-y OS: R-CHOP-like 94% vs CHOP-like 87% (P = .001)</td>
</tr>
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</table>

R-CHOP indicates rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; SLL, small lymphocytic lymphoma; NOS, not otherwise specified; EFS, event-free survival; OS, overall survival; TTF, time to treatment failure; MR, maintenance rituximab; IPI, International Prognostic Index.

*Updated results at 5 years\textsuperscript{75} show 5-y EFS: R-CHOP 47% vs CHOP 29% (P < .001); 5-y OS R-CHOP 58% vs CHOP 45% (P = .007).
Initially, 2 groups of DLBCLs were identified—GC-like and activated B cell-like (ABC)—based on similarities in expression of approximately 375 genes in the tumors and normal GC B cells or in vitro activated peripheral B cells (Figure 2A).76 Subsequently, this cell-of-origin (COO) signature was revised to include only 100 genes and to identify GC- and ABC-like DLBCLs, and a third group of tumors without either signature (“type 3”; Figure 2A).78 Using the same large data set, the COO signature was further refined to include only 27 genes, again identifying GC- and ABC-like tumors and a third unspecified category, termed “other” (Figure 2A).81 Although the sequential 100- and 27-gene models largely identified the same tumors as GC- and ABC-like, there was poor agreement on the third category, indicating additional heterogeneity beyond the COO distinction.77,81 Of importance because the unspecified (“other”) group includes 17% to 40% of tumors in recent series.79,81 Of interest, DLBCLs with features common to normal GC B cells responded more favorably to standard empiric chemotherapy.76,78,81 In contrast to the original approximately 375-gene COO signature, the more robust 27-gene COO signature also identified GC-like tumors with more favorable outcome in an independent series of DLBCLs.77,81

Distinct DLBCL subtypes

MLBCL versus DLBCL. One of the best characterized subtypes of LBCL is primary MLBCL, which is defined by a combination of clinical and pathologic features. To elucidate unique molecular features of MLBCL, investigators recently compared the gene expression profiles of newly diagnosed MLBCL and DLBCL.7,80

Primary MLBCLs expressed low levels of B-cell receptor (BCR) signaling pathway components and a distinctive cytokine signature that was strikingly similar to that of a clinically related disorder, cHL. Given the known role of NF-κB activation in cHL and the increased expression of certain NF-κB targets in primary MLBCLs, one group also assessed NF-κB activation in MLBCLs using immunohistochemical methods.7 They found near-uniform nuclear localization of the cREL NF-κB subunit in primary MLBCLs, implicating the NF-κB survival pathway in these tumors.7,41

Similarities in transcription profiles and survival pathways in MLBCL and cHL were of particular interest because these diseases have similar clinical presentations and share specific genetic lesions.8 In addition, both diseases exhibit increased sclerosis and have a prominent inflammatory infiltrate highlighting the likely important role of tumor microenvironment and host inflammatory response.

DLBCL comprehensive clusters. Given the striking genetic heterogeneity and recognized histologic variants of DLBCL, likely additional subtypes of this disease remain to be defined. To obtain comprehensive transcriptional profiles of robust DLBCL subtypes, investigators recently analyzed a large series of newly diagnosed DLBCLs with 3 different clustering algorithms and the top 5% of genes with the highest reproducibility across duplicate samples and largest variation across tumors.79 Using an approach that selected the most stable numbers of clusters with each algorithm, 3 biologically robust clusters were defined that were independent of prior distinctions, such as COO (Figure 3).79 The 3 clustering algorithms (hierarchical clustering, probabilistic clustering, and self-organizing maps) demonstrated excellent agreement, with more than 84% of DLBCLs being assigned to concordant clusters by any 2 clustering algorithms (Figure 3).79 These findings were further validated in an independent data set.79

The 3 discrete subsets of DLBCL, “oxidative phosphorylation (Ox Phos),” “B-cell receptor/proliferation (BCR),” and “host response (HR),” were further characterized using gene set enrichment analyses.79 Within each cluster, genes and pathways were identified that proffer clues to lymphomagenesis. The Ox Phos cluster showed increased expression of genes involved in mitochondrial function, electron transport, regulation of apoptosis, and

Outcome

In additional profiling studies, the molecular signatures of DLBCLs with different responses to standard chemotherapy were directly examined.77 Signatures predictive of outcome (cured versus fatal/refractory disease) were identified, which included genes involved in B-cell receptor signaling, regulation of apoptosis, and serine/threonine phosphorylation, among others.77 Of the genes and pathways associated with poor responses to current regimens, 2 have already been credentialed and targeted for possible therapeutic intervention (protein kinase C β [PKCβ]82 and the cyclic adenosine monophosphate [cAMP]–specific phosphodiesterase PDE4B83).

Additional analyses of DLBCLs sorted according to COO identified other features predictive of survival, including signatures of proliferation and expression of major histocompatibility complex (MHC) class II molecules and reactive stromal cells and host immune cells.78 In another recent study, DLBCL array data sets were used to describe signatures of outcome with as few as 6 genes.84 As new information regarding specific subtypes of DLBCL emerges,7,79,80 it will be important to consider molecular prognostic features in light of this additional biologic heterogeneity.
proteosomal degradation. Genetically, these tumors were more likely than others to harbor the t(14;18). The BCR cluster demonstrated increased expression of cell cycle regulatory genes, DNA repair genes, components of the BCR signaling cascade, and numerous B-cell–specific transcription factors, such as BCL6, MYC, and signal transducer and activator of transcription 6 (STAT6), and more commonly had the t(3;...). Unlike the Ox Phos and BCR clusters, the HR signature was largely determined by the host inflammatory response, rather than tumor cells themselves. In HR tumors, overexpressed genes included those involved in T-cell receptor signaling, CD2, T-cell and natural killer (NK) cell activation, monocyte/macrophage activators, complement pathway proteins, cytokine receptors, tumor necrosis factor (TNF)–related proteins, and adhesion molecules. Consistent with these observations, HR tumors included increased numbers of tumor-infiltrating lymphocytes and immunohistochemically defined CD2+ and CD3+ T cells and gamma-interferon–responsive lysosomal thiol reductase–positive (GILT+) interdigitating dendritic cells. Despite their brisk host immune response, HR tumors did not have a more favorable outcome, suggesting that the response itself was ineffective, or perhaps inhibited by counterregulatory measures. Alternatively, the host immune/inflammatory response might facilitate growth, an attractive hypothesis if the infiltrating host T cells and the lymphoma precursor B cells are directed against the same antigen. Of interest, HR DLBCLs lacked the common genetic lesions seen in Ox Phos and BCR tumors, prompting speculation regarding a unique mechanism of transformation in the HR lymphomas.

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The T-cell/dendritic cell infiltrates in HR tumors resemble those of the provisional (WHO) DLBCL subtype, T/HRBCL. Notable similarities exist between these entities, including their predilection for younger patients, involvement of liver, spleen, and bone marrow, and few recurring genetic abnormalities. As observed in the HR cluster, T/HRBCLs do not have an improved prognosis, despite the increased inflammatory response.

**Figure 3. Identification of DLBCL consensus clusters.** (A) The left panel shows consensus matrices (clusters) produced by hierarchical clustering (HC), self-organizing maps (SOMs), and probabilistic clustering (PC). Clusters were generated using the top 5% of genes with the highest reproducibility across duplicate samples and largest variation across tumors and an approach that selected the most stable numbers of clusters with each algorithm (consensus clustering). The right panel shows comparisons of the cluster assignments of different algorithms (PC versus HC, HC versus SOM, and PC versus SOM, respectively). Clusters are denoted as C[1..], C[2..], and C[3..]. The 3 clustering algorithms demonstrated excellent agreement with more than 84% of DLBCLs assigned to the same clusters by any 2 algorithms. (B) Expression profiles of the 3 DLBCL comprehensive clusters. The top 50 genes associated with each cluster are shown. Each column is a sample and each row is a gene. Color scale at bottom indicates relative expression and SDs from the mean. Red indicates high-level expression; blue, low-level expression. Adapted from Monti et al with permission.

**Figure 4. DLBCL, gray zone lymphomas, and Hodgkin lymphomas.** MBCL and T-cell/histiocyte–rich large B-cell lymphoma (T/HRBCL) are considered “gray zone lymphomas,” which share characteristics of both non-Hodgkin and Hodgkin lymphomas, including increased host inflammatory response (Figure 4). The intriguing similarities among these entities points to a group of tumors defined, and
possibly driven, by their interaction with the host microenvironment (Figure 4).

**GEP in clinical practice**

GEP has proven useful for identifying features and disease subsets with biologic and clinical significance. Given that these distinctions will likely lead to novel therapeutic approaches, an additional challenge has been translating these findings into a readily available platform, such as immunohistochemistry (IHC). In a recent study of newly diagnosed DLBCLs, the combination of 3 markers (CD10, BCL6, and MUM1) was used to assign COO and discriminate between GC-like DLBCLs and other tumors. Similarly, IHC markers derived from the MLBCL transcriptional profile have been used to distinguish MLBCL from DLBCL, an important consideration for the development of MLBCL-specific clinical trials.

**Rational therapeutic targets**

Novel therapeutic approaches have been identified by a combination of GEP, insights into molecular pathogenesis, and improvements on existing therapies. To date, targets identified via GEP include PKCβ, PDE4B, and NF-κB. PKCβ is a serine/threonine kinase that modulates BCR downstream signaling and downstream activation of the NF-κB survival pathway in B cells and vascular endothelial growth factor signaling in the tumor microvasculature. Recent studies have validated this target in vitro, and clinical trials of a PKCβ inhibitor are ongoing in patients with relapsed/refractory DLBCLs.

Investigation is also under way in manipulating PDE4B, a regulator of apoptosis, PDE4B inactivates cAMP, and prevents cAMP-induced apoptosis, which appears to be mediated by the phosphatidylinositol 3-kinase (PI3K)/AKT pathway. Inhibition of this process at either PDE4B or PI3K/AKT may restore cAMP-induced apoptosis of PDE4B-overexpressing DLBCLs.

GEP has identified a prominent role for NF-κB in certain subsets of DLBCL and in MLBCL. Studies of DLBCL cell lines found higher levels of NF-κB and constitutive activation of IκB kinase in the ABC-like DLBCLs. In these studies, NF-κB inhibition resulted in preferential growth arrest and apoptosis of ABC-like cell lines. MLBCL tumors have consistent nuclear localization of cREL and up-regulation of NF-κB target genes, suggesting that the NF-κB survival pathway may be important in this disease. Consistent with this hypothesis, NFκB inhibition markedly increases apoptosis and decreases proliferation of MLBCL cells in vitro. Novel treatments for MLBCL are of critical importance given that standard therapy including mediastinal radiation in young patients may have long-term sequelae. Of interest, the GEP of HR DLBCLs is also enriched for NFκB target genes, potentially implicating the NFκB survival pathway may be important in this disease.

**References**


Advances in the biology and therapy of diffuse large B-cell lymphoma: moving toward a molecularly targeted approach

Jeremy S. Abramson and Margaret A. Shipp