Advances in the Biology and Therapy of Diffuse Large B-Cell Lymphoma—
Moving Towards a Molecularly Targeted Approach

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ABSTRACT
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, though such models do not account for underlying biologic differences among tumors. Commonly observed genetic abnormalities that likely contribute to pathogenesis include translocations of \textit{BCL6}, \textit{BCL2} and \textit{cMYC}, \textit{FAS(CD95)} mutations, and aberrant somatic hypermutation. Despite recent advances in empiric chemotherapy, including interval reduction of CHOP 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.
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’s lymphomas. Although the cause of most DLBCLs remains unknown, predisposing factors include congenital and acquired immunodeficiency states that are often associated with dysregulated apoptosis and/or defective DNA repair. Though 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, though 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 based on cell size, nodal pattern, and morphology, and assigned grade based on untreated natural history with low, intermediate and high-grade corresponding to survival measured in years, months, and weeks, respectively. While successful in being broadly applicable and providing prognostic information, the WF sacrificed some of the biologic advances 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’s-like, all of which have been included with DLBCL in previous randomized trials.

Although the REAL classification was subsequently updated as the 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 Ig (50-75% of cases). CD30 expression may occur, most commonly with the anaplastic variant. A small number of tumors may express CD10 or CD5, though they will be cyclin D1 negative, distinguishing them from blastic mantle cell lymphoma.
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. 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. Primary mediastinal (thymic) large B-cell lymphoma (MLBCL) typically presents as localized, sclerotic masses in young female patients, unlike DLBCL which commonly arises in elderly patients of both sexes. MLBCL may be difficult to differentiate histologically from DLBCL, though MLBCL is often characterized by interspersed dense fibrosis and a polyclonal infiltrate of host inflammatory cells, reminiscent of classical Hodgkin’s lymphoma (cHL). MLBCLs express pan-B-cell markers and have rearranged immunoglobulin genes, though they do not express surface Ig like classical DLBCL. These tumors often demonstrate weak CD30 staining—a feature more commonly associated with cHL than DLBCL.

GENETIC AND BIOLOGIC HETEROGENEITY

DLBCL is thought to arise from normal antigen-exposed B-cells that have migrated to or through germinal centers (GC) 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. SHM generates antibody diversity and increases antigen affinity, but also creates a setting for chromosomal translocations and mutagenesis to occur.

Several genetic abnormalities have been identified in subsets of DLBCLs (Table 2). Recurring chromosomal translocations occur in approximately 50% of cases, and DNA imbalances in as many as 67%. The three 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 35-40% of cases. Although several chromosomes may partner with 3q27, the most common translocations involve the Ig heavy chain promoter, resulting in constitutive expression of this normally developmentally regulated gene. 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. 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. 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 \textit{BCL6} transgenic mouse promises to provide further insight into this gene’s precise role in lymphomagenesis.\textsuperscript{17}

Clinically, \textit{BCL6} rearrangements occur primarily in \textit{de novo} DLBCL.\textsuperscript{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.

**\textit{BCL2}**

\textit{BCL2} is a proto-oncogene located at 18q21 that promotes B-cell survival via inhibition of apoptosis, and confers chemotherapy resistance.\textsuperscript{18} The \textit{BCL2} family includes both anti-apoptotic and pro-apoptotic members that form hetero- and homodimers. Following death signals, pro-apoptotic homodimers alter mitochondrial membrane potential, trigger cytochrome C release and caspase-mediated apoptosis. Increased abundance of anti-apoptotic \textit{BCL2} proteins favors the formation of anti-/pro-apoptotic heterodimers rather than pro-/pro-apoptotic homodimers, limiting the effects of death signals at the mitochondrial membrane. Either the relative excess of anti-apoptotic \textit{BCL2} family members or the deficiency of pro-apoptotic isoforms may confer a survival advantage and contribute to lymphomagenesis. Proof of principle is provided by models where mice overexpressing \textit{BCL2} protein due to the t(14;18) developed follicular hyperplasia and extended survival of B-lymphocytes;\textsuperscript{19} conversely, mice deficient for a pro-apoptotic \textit{BCL2} family member, \textit{BAD}, developed DLBCL of GC origin.\textsuperscript{20}

\textit{BCL2} expression is normally down regulated in the GC where apoptosis plays a critical role in negative B-cell selection. \textit{BCL2} deregulation is most commonly associated with the t(14;18), present in approximately 15\% of DLBCL.\textsuperscript{21,22} \textit{BCL2} protein expression, however, can be detected in approximately 50\% of DLBCLs, independent of the t(14;18).\textsuperscript{21} Interestingly, increased expression of the \textit{BCL2} protein is associated with an inferior outcome in DLBCL, though the t(14;18) alone has no predictive value.\textsuperscript{21,22} Since the t(14;18) is the hallmark abnormality in follicular lymphoma and transformed follicular lymphomas resemble \textit{de novo} DLBCL, the reported frequencies of t(14;18) in DLBCL series may depend on how accurately included tumors and patients were pre-screened.

**\textit{cMYC}**

\textit{cMYC}, a transcription factor associated with Burkitt’s lymphoma, is deregulated in approximately 15\% of DLBCL,\textsuperscript{22} though some of these cases may actually represent Burkitt’s-like lymphoma, as the distinction can be difficult. Deregulation occurs most commonly in the setting of t(8;14), which brings the \textit{cMYC} gene on 8q24 under the control of an immunoglobulin promoter.\textsuperscript{23} \textit{cMYC} rearrangements have no clear effect on survival.\textsuperscript{22,23}

**\textit{FAS(CD95)}**

\textit{FAS(CD95)} is a pro-apoptotic protein expressed within GCs where it plays an important role in negative selection of B-cells.\textsuperscript{24} \textit{FAS} ligand crosslinks the transmembrane \textit{FAS} death receptor, leading to the assembly of a death-inducing signaling complex and initiating caspase-mediated apoptosis.\textsuperscript{24} \textit{FAS} mutations have been reported in up to $\approx 20\%$ of DLBCLs, most commonly within the last exon which encodes the death domain.\textsuperscript{24} Such mutations likely act in a dominant-
negative manner, destabilizing trimeric FAS receptors.\textsuperscript{24} 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.\textsuperscript{24-26}

**Aberrant SHM**

Normal GC B-cells undergo immunoglobulin gene editing via SHM of their rearranged IgV-region genes.\textsuperscript{9,27} This process specifically targets regulatory sequences downstream of the IgV gene promoter.\textsuperscript{9} DLBCLs typically have evidence of SHM, indicating that these tumors likely originate from GC or post-GC B-cells.\textsuperscript{27,28} The 5’ regulatory regions of other GC genes, BCL6 and CD95 (FAS), are also known targets of physiologic SHM (Fig. 1).\textsuperscript{24,29,30} SHM may also occur aberrantly, and is postulated to be an additional pathogenetic mechanism in DLBCL.\textsuperscript{31} Genes aberrantly targeted by SHM include BCL6, PIM1, cMYC, PAX5, and RhoH/TTF (Fig. 1).\textsuperscript{31} Like BCL6, FAS(CD95) has been postulated to be targeted by both physiologic and aberrant SHM.\textsuperscript{24} Since the SHM machinery targets broad regions of multiple genes, the specific consequences of aberrant SHM may be quite different in individual tumors.

**Fig. 1**

![Diagram showing somatic hypermutation](image)

**Fig. 1 Somatic hypermutation.** Physiologic SHM targets regulatory sequences of the IgV gene and the additional GC genes, BCL6 and (CD95)Fas. Aberrant SHM targets additional GC genes including PIM1, cMYC, PAX5, RhoH/TTF as well as BCL6, and, possibly, FAS. Figure modified from R. Dalla-Favera, Institute of Genetics, Columbia University, New York, NY.

**p53**

Though occurring rarely as an isolated event in DLBCL, mutation of the p53 tumor suppressor gene has been associated with poor outcome.\textsuperscript{32-34} p53 is located on chromosome 17p, deletion of
which has also correlated with an inferior prognosis.\textsuperscript{11,35} Mutations of $p53$ in animal models have also been associated with B-cell malignancies. In a murine model of impaired DNA repair, mice deficient for both \textit{histone 2AX (H2AX)} and $p53$ had markedly increased numbers of B-cell lymphomas and solid tumors.\textsuperscript{36}

**Chromosomal Imbalances**
Numerous chromosomal imbalances have been observed in DLBCL, some of which may influence prognosis.\textsuperscript{11} Poor outcome has been associated with abnormalities of chromosomes 1q,\textsuperscript{37} 5,\textsuperscript{35} 6,\textsuperscript{35} 7q,\textsuperscript{38} and 14,\textsuperscript{35,39} while gain of 3p has been associated with improved prognosis.\textsuperscript{39} Defects with no clear influence on prognosis include abnormalities of Xq, 7q, 12p, and 6q.\textsuperscript{39} 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 \textit{cREL}.\textsuperscript{11,40,41} Since \textit{cREL} encodes a component of the NFkB heterodimer, previous studies have suggested that increased \textit{cREL} abundance might be associated with increased NFkB activity.\textsuperscript{42,43} Recent data, however, indicate that 2p13 amplifications are more common in subsets of DLBCL without evidence of NFkB activation, suggesting a different functional target at this locus.\textsuperscript{40,41} Of interest, the \textit{BCL11A} proto-oncogene has been found to be simultaneously amplified with \textit{cREL} in tumors with gains of chromosome 2p13.\textsuperscript{11,44,45}

**CLINICAL HETEROGENEITY: STRATIFICATION ACCORDING TO RISK**
Although a subset of DLBCL patients 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.\textsuperscript{46} In aggressive lymphoma patients who received anthracycline-based chemotherapy, pre-treatment variables that independently predicted outcome were advanced age, poor performance status, elevated LDH, advanced Ann Arbor stage, and multiple extranodal sites.\textsuperscript{46} Four risk groups – low, low-intermediate, high-intermediate, and high -- were generated by summing the above-mentioned risk factors present at diagnosis. More recent studies of lymphomas classified according to REAL/WHO criteria indicate that the IPI is a better classifier of DLBCL than of other aggressive lymphomas.\textsuperscript{47}

**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.\textsuperscript{48} The CHOP regimen (cyclophosphamide, hydroxydaunomycin, vincristine, and prednisone) was subsequently considered to be standard therapy based on phase II studies in which approximately 35% of patients were cured of their disease.\textsuperscript{49} Thereafter, second and third generation regimens were developed which appeared to cure 55-65% of patients; however, despite these encouraging results, randomized trials of these regimens versus CHOP showed no survival differences (Table 3a).\textsuperscript{50-52} Results of these studies secured CHOP’s role as the standard of care in DLBCL, so much so that it was nearly a decade until additional trials sought to improve on CHOP’s success.\textsuperscript{53}
The striking differences between the encouraging phase II trials and subsequent phase III studies may reflect the inclusion of better risk patients in early trials and more representative patients in the later randomized studies. For this reason, only randomized studies of additional treatment options will be reviewed here. Although the majority of patients in these studies had DLBCL or its equivalent in prior classifications (Table 1), these randomized trials also included other aggressive lymphomas (Tables 3-5).

**IMPROVING ON THE GOLD STANDARD:**
Several recently reported randomized trials have focused on increasing dose frequency and intensity of CHOP (Table 3b). For example, the 21-day CHOP regimen was compared to CHOP given every two weeks (CHOP-14), with or without etoposide (CHOEP), in young and elderly patients with aggressive lymphoma, respectively. Using a 2x2 factorial design, patients were randomized to receive CHOP-21, CHOP-14, CHOEP-21, or CHOEP-14.

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. The addition of etoposide did not further improve outcome, but did enhance toxicity.

In the trial of patients younger than age 60 with a normal LDH, EFS was significantly improved with the addition of etoposide, though OS was not. 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 upon relapse, a reasonable assumption given their favorable prognostic status.

Dose intensification was further evaluated in a randomized trial comparing CHOP to ACVBP in elderly patients with aggressive lymphomas (Table 3b). At five years follow-up, ACVBP was associated with improved EFS and OS, though the rate of treatment-related death was nearly double. Of note, the ACVBP regimen included intensive CNS prophylaxis, which was associated with a significantly lower risk of CNS recurrence.

**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, has been associated with more variable results in the upfront setting (Table 4). 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. Among all eligible patients evaluated prospectively, HDT/ASCT did not impact OS; however, in retrospective analyses of IPI high-intermediate/high (HI/H) risk groups, HDT/ASCT improved both 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 CR. This study showed an inferior survival in the transplant arm, prompting early closure of the study. Several additional studies of abbreviated induction followed by HDT/ASCT likewise suggested lack of benefit for this approach. 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. Given the variable results of
upfront HDT/ASCT and additional promising treatment options (discussed below), 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. 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. 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). At a median of two years follow-up, there was a superior OS in the rituximab-containing arm, with only minimal additional toxicity. These data were recently updated at five years, and showed persistent benefit for R-CHOP. 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.

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. 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 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, while 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.

The benefit of rituximab in younger patients has recently been reported in the first interim analysis of the MabThera International Trial (MInT trial). Patients aged 18-60 with aggressive lymphoma were randomized to CHOP-like therapy, with or without rituximab. At two years follow-up, there was a significantly improved TTF and OS in the rituximab-containing arm. Recently, a retrospective observational study further supported the survival benefit for rituximab in younger patients. By demonstrating the benefit of rituximab in younger patients, these data suggest that the merits of rituximab are broadly applicable and not limited to elderly patients who simply could not tolerate aggressive chemotherapy.

**GENE EXPRESSION PROFILING**

Striking clinical and genetic heterogeneity suggest additional substructure within DLBCL. Until recently, analyses of biological heterogeneity have focused on individual genes, emphasizing those associated with treatment outcome, known function in other malignancies, and in normal lymphocyte development. To understand the bases of clinical and molecular heterogeneity in DLBCL, it would be useful to have comprehensive molecular signatures of tumors that share similar features. In addition to highlighting potential pathogenetic mechanisms, such signatures might identify promising subtype-specific targets for therapeutic intervention. With the advent of gene expression profiling (GEP), it is now possible to obtain such signatures of DLBCL.
subtypes. To date, transcriptional profiling of DLBCLs has been used to: 1) highlight similarities between subsets of tumors and normal B-cells;76 2) identify features associated with unfavorable responses to empiric combination chemotherapy;77,78 and 3) define robust and highly reproducible DLBCL subtypes with comprehensive transcriptional signatures.7,79,80

**Cell-of-origin**

A series of molecular models have been described that relate subsets of DLBCL to stages of normal B-cell development. 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 blood B-cells (Fig. 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”) (Fig. 2a).78 Using the same large dataset, 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” (Fig. 2a).81 Although the sequential 100- and 27-gene models largely identified the same tumors of GC- and ABC-like, there was poor agreement on the third category, indicating additional heterogeneity beyond the COO distinction (Fig. 2b). This is of particular importance because the unspecified (“Other”) group includes 17% - 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 ≈ 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
Fig. 2  Cell of origin: concordance across studies. (A) Sequential models relating subsets of DLBCL to stages of normal B-cell development on the basis of shared transcriptional profiles. Two groups of DLBCLs were initially identified – GC-like and activated B-cell-like (ABC) – based on similarities in expression of ≈375 genes in the tumors and normal GC B-cells or in vitro activated peripheral blood B-cells (Study 1). The cell-of-origin (COO) signature was subsequently 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”) (Study 2). Using the same large dataset, 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.” (Study 3). (B) Concordance between the sequential COO models. 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.

Outcome

In additional profiling studies, the molecular signatures of DLBCLs with different responses to standard chemotherapy were directly examined. Signatures predictive of outcome (cured vs. fatal/refractory disease) were identified which included genes involved in B-cell receptor signaling, regulation of apoptosis, and serine/threonine phosphorylation, among others. Of the genes and pathways associated with poor responses to current regimens, two have already been credentialed and targeted for possible therapeutic intervention (PKCβ and the cyclic AMP-specific phosphodiesterase PDE4B).

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. In another recent study, DLBCL array data sets were used to describe signatures of outcome with as few as
six genes. As new information regarding specific subtypes of DLBCL emerges, it will be important to consider molecular prognostic features in light of this additional biological heterogeneity.

Distinct DLBCL subtypes
MLBCL vs. 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. 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 NFκB activation in cHL and the increased expression of certain NFκB targets in primary MLBCLs, one group also assessed NFκB activation in MLBCL using immunohistochemical methods. They found near-uniform nuclear localization of the cREL NFκB subunit in primary MLBCLs, implicating the NFκB survival pathway in these tumors. 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. 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, there are likely to be additional subtypes of this disease that remain to be defined. To obtain comprehensive transcriptional profiles of robust DLBCL subtypes, investigators recently analyzed a large series of newly diagnosed DLBCL with three different clustering algorithms and the top 5% of genes with the highest reproducibility across duplicate samples and largest variation across tumors. Using an approach which selected the most stable numbers of clusters with each algorithm, three biologically robust clusters were defined that were independent of prior distinctions, such as COO (Fig. 3). The three clustering algorithms (hierarchical clustering, probabilistic clustering and self-organizing maps) demonstrated excellent agreement, with greater than 84% of DLBCLs being assigned to concordant clusters by any two clustering algorithms (Fig. 3). These findings were further validated in an independent dataset.
The three 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. 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 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 B-cell receptor signaling cascade, and numerous B-cell specific transcription factors, such as BCL6, MYC, and 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 NK-cell activation, monocyte/macrophage activators, complement pathway proteins, cytokine receptors, 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 positive T-cells and GILT-positive 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 counter-regulatory measures. If such a mechanism exists, the host inflammatory response may provide stimulatory/proliferative cues to the tumor, or alternatively, the host T-lymphocytes and malignant B-lymphocytes may be directed against a common antigen. Of interest, HR DLBCLs lacked the common genetic lesions seen in OxPhos and BCR tumors, prompting speculation regarding a unique mechanism of transformation in the HR lymphomas.

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. Of further interest is the clinical and histologic similarity between T/HRBCL and another Hodgkin’s lymphoma subtype, nodular lymphocyte-predominant Hodgkin’s lymphoma (NLPHL). These diseases and MLBCL are considered “gray zone lymphomas” which share characteristics of both non-Hodgkin’s and Hodgkin’s lymphomas, including increased host inflammatory response (Fig. 4). The intriguing similarities among these entities points to a group of tumors defined, and possibly driven, by their interaction with the host microenvironment (Fig. 4).

**Fig. 4**

**DLBCL, gray-zone lymphomas, and Hodgkin’s lymphomas.** MLBCL and T/HRBCL are considered “gray-zone lymphomas” which share characteristics of large B-cell lymphomas and Hodgkin’s lymphomas (cHL and NLPHL, respectively), including increased host inflammatory response. The similarities between these entities points to a group of tumors defined, and possibly driven, by their interaction with the host microenvironment. Images provided by J. Kutok, Department of Pathology, Brigham and Women’s Hospital, Boston, MA.
GEP in Clinical Practice
GEP has proven useful for identifying features and disease subsets with biological 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, like immunohistochemistry (IHC). In a recent study of newly diagnosed DLBCLs, the combination of three 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 could be 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, PDE4β, and NFκB. PKCβ is a serine/threonine kinase that modulates BCR- signaling and downstream activation of the NFκB 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 relapsed/refractory DLBCL patients.

Investigation is also underway in manipulating PDE4B, a regulator of apoptosis. PDE4B inactivates cAMP, and prevents cAMP-induced apoptosis, which appears to be mediated by the phosphatidylinositol3-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 NFκB in certain subsets of DLBCL and in MLBCL. Studies of DLBCL cell lines found higher levels of NFκB and constitutive activation of Ikappa kinase in the ABC-like DLBCLs. In these studies, NFκB inhibition resulted in preferential growth arrest and apoptosis of ABC-like cell lines. MLBCL tumors have consistent nuclear localization of cREL and upregulation of NFκB target genes, suggesting that the NFκB survival pathway may be important in this disease. Novel treatments for MLBCL are of critical importance given that standard therapy including mediastinal radiation in young patients may have devastating long-term sequelae. Possible strategies for targeting NFκB in MLBCL include inhibiting proteosomal degradation of IκB (inhibitor of κB) or reducing Iκ kinase-mediated activation of NFκB.

Given the likely etiologic role of BCL6 in a subset of DLBCL, regulation of this pathway has garnered significant attention. Acetylation plays a key role in down-regulating BCL6, with histone deacetylase (HDAC) being required to lift this repression. Pharmacologic inhibition of HDAC in tumors expressing BCL6 may lead to tonic acetylation and inhibition of this pathway. A second deacetylation pathway, silent information regulator 2 (SIR2), also regulates BCL6. Both of these deacetylation pathways activate the p53 pathway, providing further insight into the interplay between BCL6 and p53, and further rationale for inhibiting deacetylation in DLBCL. Indeed, multiple NHL cell lines undergo apoptosis when treated with trichostatin A (TSA) and nicotinamide, which inhibit HDAC and SIR2, respectively. Several other inhibitors...
of HDAC, including depsipeptide, SAHA (suberoylanilide hydroxamic acid), and valproic acid are also under investigation in DLBCL.

An inhibitory peptide to the lateral groove of the BTB/POZ domain of BCL6 was recently described.95 This peptide blocked repression of \textit{BCL6} target genes in a dose-dependent manner, with resultant reactivation of these pathways. In addition, when injected into mice, the peptide blocked GC formation, simulating a \textit{BCL6} null model.95 \textit{BCL6} positive DLBCL cell lines treated with the peptide also underwent cell cycle arrest and apoptosis.

\textit{BCL2} is a critical regulator of apoptosis that confers chemotherapy resistance.18 Numerous therapeutic approaches directed at \textit{BCL2} are being developed, including antisense \textit{BCL2} oligonucleotides and small molecule inhibitors.96 One group has recently used hydrocarbon stapling to generate stable native BH3 peptides called “stabilized alpha-helix of BCL2 domains,” or SAHBs.97 These SAHBs were capable of entering leukemic cells, binding to the death domain of \textit{BCL2}, and inducing apoptosis.

Recent years have seen notable advances in empiric chemotherapy for DLBCL. Anti-CD20 antibodies may be the greatest recent advance given their broad applicability, tolerability, and efficacy. More potent anti-CD20 antibodies have been developed that show promise in rituximab-resistant CD20 positive cell lines and may ultimately become useful in humans.98 Anti-CD20 targeted radioimmunoconjugates are approved for use in low-grade B-cell lymphomas, and are currently being investigated in aggressive lymphomas. Additional targets for monoclonal antibody therapy are being investigated as well, including CD22, HLA-DR, and CD80, among others.99,100

Though recent years have seen encouraging advances in DLBCL therapy, our increased understanding of the molecular heterogeneity within this disease will likely change the treatment paradigm from empiric therapy to a rationally targeted approach directed at discrete biologic subsets. As we continue to learn more from GEP and other techniques, we will ultimately look upon DLBCL as a panoply of distinct entities, treated with consideration of their unique molecular features.
REFERENCES

## TABLES

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

<table>
<thead>
<tr>
<th>Classification System</th>
<th>Categorization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rappaport (1966)</td>
<td>Diffuse Histiocytic Lymphoma</td>
</tr>
<tr>
<td>Kiel (1974)</td>
<td>Centroblastic Lymphoma&lt;br&gt;B-immunoblastic Lymphoma&lt;br&gt;B-large cell anaplastic Lymphoma</td>
</tr>
<tr>
<td>Lukes-Collins (1974)</td>
<td>Large Cleaved Follicular Center Cell Lymphoma&lt;br&gt;Large Noncleaved Follicular Center Cell Lymphoma&lt;br&gt;B-immunoblastic Lymphoma</td>
</tr>
<tr>
<td>Working Formulation (1982)</td>
<td>Diffuse Mixed Small and Large Cell Lymphoma (Group F)&lt;br&gt;Diffuse Large Cell Lymphoma (Group G)&lt;br&gt;Large Cell Immunoblastic Lymphoma (Group H)</td>
</tr>
<tr>
<td>REAL (1994) and WHO (2001)</td>
<td>Diffuse Large B-cell Lymphoma</td>
</tr>
</tbody>
</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>
</thead>
<tbody>
<tr>
<td>BCL6</td>
<td>35-40%17</td>
<td>3q27</td>
<td>t(3;…) and SHM</td>
</tr>
<tr>
<td>BCL2</td>
<td>t(14;18)</td>
<td>13%19</td>
<td>18q21</td>
</tr>
<tr>
<td></td>
<td>amplification</td>
<td>24%21</td>
<td>t(14;18) and gene amplification</td>
</tr>
<tr>
<td>cMYC</td>
<td>15%22</td>
<td>8q24</td>
<td>(8;…) and SHM</td>
</tr>
<tr>
<td>FAS(CD95)</td>
<td>20%24</td>
<td>10q24</td>
<td>Death domain mutations, ?SHM</td>
</tr>
<tr>
<td>SHM</td>
<td>45%79</td>
<td></td>
<td>Physiologic: Ig(v), FAS, BCL6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Aberrant: BCL6, PIM1, cMYC,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PAX5, RhoH/ITF, ?FAS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SHM</td>
</tr>
<tr>
<td>p53</td>
<td>16%11</td>
<td>17p</td>
<td>Mutation, deletion</td>
</tr>
</tbody>
</table>

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

### a. Early trials

<table>
<thead>
<tr>
<th>First Author (year)</th>
<th>Regimens</th>
<th>Patients Characteristics</th>
<th>Diseases</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gordon et al. (1992)</td>
<td>CHOP vs. m-BACOD</td>
<td>325 patients</td>
<td>All ages, Stage III, IV</td>
<td>WF groups F,G</td>
</tr>
<tr>
<td>Cooper, et al. (1994)</td>
<td>CHOP vs. MACOP-B</td>
<td>236 patients</td>
<td>Age &gt;16, Stage I (bulky), II-IV</td>
<td>WF groups D,E,F,G,H</td>
</tr>
<tr>
<td>Fisher et al. (1994)</td>
<td>CHOP vs. m-BACOD vs. ProMACE-CytaBOM vs. MACOP-B</td>
<td>899 patients</td>
<td>All ages, Stage II (bulky), III, IV</td>
<td>WF groups D,E,F,G,H,J</td>
</tr>
</tbody>
</table>

### b. Recent trials

<table>
<thead>
<tr>
<th>First Author (year)</th>
<th>Regimens</th>
<th>Patients Characteristics</th>
<th>Diseases</th>
<th>Results</th>
</tr>
</thead>
</table>
| Tilly et al. (2003) | CHOP vs. ACVBP | 635 patients | Age 61-69, Stage I-IV, At least one adverse prognostic factor by aaIPI | WF groups F,G,H,I,J (79% were DLBCL) | 5 yr EFS: ACVBP 39% vs. CHOP 29% (p=0.005)  
5 yr OS†: ACVBP 46% vs. CHOP 38% (p=0.036) |
| Pfreundschuh et al. (2004) | CHOP-14 vs. CHOP-21 vs. CHOEP-21 vs. CHOEP-14 | 710 patients | Age 18-60, Stage I-IV, Normal LDH | REAL/WHO: DLBCL (60%), MLBCL (3.0%), Follicular grade III Burkitt’s, Aggressive Marginal zone, Anaplastic large cell Lymphoblastic PTCL, Angioimmunoblastic Extranodal NK/T, Nasal type, Aggressive NOS | 5 yr EFS: CHOP-E/P-14 65% vs. CHOEP-E/P-21 62% (p=NS)  
CHOEP-14/21 69% vs. CHOP-14/21 58% (p=0.004)  
5 yr OS: CHOP-E/P-14 85% vs. CHOEP-E/P-21 79% (p=0.004)  
CHOEP-14/21 84% vs. CHOP-14/21 80% (p=NS) |
| Pfreundschuh et al. (2004) | CHOP-14 vs. CHOP-21 vs. CHOEP-14 vs. CHOEP-21 | 689 patients | Age 61-75, Stage I-IV | REAL/WHO: DLBCL (71%), MLBCL (0.6%), Follicular grade III Burkitt’s, Marginal zone, Anaplastic large cell Lymphoblastic PTCL, Angioimmunoblastic Extranodal NK/T, Nasal type, Aggressive NOS | 5 yr EFS: CHOP-14 44% vs. CHOP-21 33% (p=0.003)  
CHOEP-21 41% vs. CHOEP-21 33% (p=NS)  
5 yr OS: CHOP-14 53% vs. CHOP-21 41% (p<0.001)  
CHOEP-21 46% vs. CHOEP-21 41% (p=NS) |

aaIPI indicates age-adjusted International Prognostic Index; ACVBP, induction: doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone, and intrathecal methotrexate; consolidation: intravenous methotrexate, etoposide, ifosfamide, and cytotoxic ara-bisamide; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CHOE, cyclophosphamide, doxorubicin, vincristine, etoposide, and prednisone; CHO(E)P, cyclophosphamide, doxorubicin, vincristine, prednisone +/- etoposide; EFS, event-free survival; FFS, failure-free survival; m-BACOD, methotrexate, bleomycin, doxorubicin, vincristine, and dexamethasone; MACOP-B, methotrexate, doxorubicin, cyclophosphamide, vincristine, and bleomycin; MLBCL, mediastinal large B-cell lymphoma; NOS, not otherwise specified; NS, not significant; OS, overall survival; ProMACE-CytaBOM, prednisone, procarbazine, doxorubicin, cyclophosphamide, etoposide/cytarabine, bleomycin, vincristine, and methotrexate; PTCL, peripheral T-cell lymphoma; and TTF, time to treatment failure.

* 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’s)

† Treatment-related mortality: ACVBP 13% vs. CHOP 7% (p=0.014).
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>Patients Characteristics</th>
<th>Included Diseases</th>
<th>Significant Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gianni et al. (1997)&lt;sup&gt;58&lt;/sup&gt;</td>
<td>MACOP-B vs. Sequential HDT/ASCT</td>
<td>98 patients</td>
<td>Age 17-60, Stage I (bulky), II(bulky), III,IV</td>
<td>WF&lt;sup&gt;+&lt;/sup&gt; groups G,H 7 year EFS HDT 76% vs. MACOP-B 49%, (p=0.004) 7 year OS&lt;sup&gt;+&lt;/sup&gt; ASCT 81% vs. MACOP-B 55%, (p=NS)</td>
</tr>
<tr>
<td>Santini et al. (1998)&lt;sup&gt;59&lt;/sup&gt;</td>
<td>VACOP-B plus salvage DHAP vs. VACOP-B plus HDT/ASCT</td>
<td>124 patients</td>
<td>Age 15-60, Stage II (bulky), III,IV</td>
<td>WF groups D,E,F,G,H,I,J No difference in DFS and OS</td>
</tr>
<tr>
<td>Haioun et al. (2000)&lt;sup&gt;61&lt;/sup&gt;</td>
<td>Induction with either ACVB or NCVB—Patients in CR randomized to: Consolidation with MTX, Ifosfamide, L-asparaginase and Ara-C vs. Methotrexate and HDT/ASCT</td>
<td>236 patients</td>
<td>Age &lt;55, Stage I-IV At least one poor prognostic variable (poor PS, multiple extranodal sites, bulky disease, BM, CNS, Burkitts or lymphoblastic)</td>
<td>WF groups D,E,F,G,H,I,J Propective analysis: No difference in DFS and OS Retrospective analysis on IPI HI/H risk pts: 8yr DFS ASCT 55% vs. chemo 39%, (p=0.02) 8yr OS ASCT 64% vs. chemo 49%, (p=0.04)</td>
</tr>
<tr>
<td>Kluin-Nelemans et al. (2001)&lt;sup&gt;60&lt;/sup&gt;</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</td>
<td>Age 15-65, Stage I(bulky), II-IV</td>
<td>WF groups D,E,F,G (51% were DLBCL) No difference in FFP and OS</td>
</tr>
<tr>
<td>Gisselbrecht, et al. (2002)&lt;sup&gt;62&lt;/sup&gt;</td>
<td>4 cycles ACVB vs. 4 cycles E1 4 cycles Ara-C vs. 1 cycle CEOP 2 cycles ECVBP HDT/ASCT</td>
<td>370 patients</td>
<td>Age 15-60, Stage I-IV At least 2 IPI risk factors</td>
<td>REAL/WHO: DLBCL (61%) PTCL Lymphoblastic Burkitt’s Diffuse aggressive NOS 5 yr EFS: ASCT 39% vs. ACVB 51% (p=0.01) 5 yr OS: ASCT 46% vs. ACVB 60% (p=0.007)</td>
</tr>
<tr>
<td>Milpied et al. (2004)&lt;sup&gt;66&lt;/sup&gt;</td>
<td>4 cycles CHOP—Patients in CR/PR receive 4 additional cycles vs. 2 cycles CEEP Pts in CR/PR receive 1 cycle MC HDT/ASCT</td>
<td>197 patients</td>
<td>Age 15-60, Stage II(bulky), III,IV aIPI L, LI, or HI</td>
<td>WF groups D,E,F,G,H (75% were DLBCL) Prospective analysis by ITT: 5 yr EFS: ASCT 55% vs. CHOP 37%, (p=0.037) 5yr OS: HDT 71% vs. CHOP 56%, (p=NS) Retrospective analysis on IPI HI risk pts: 5 yr EFS: ASCT 56% vs. CHOP 28%, (p=0.003) 5yr OS: HDT 74% vs. CHOP 44%, (p=0.001)</td>
</tr>
</tbody>
</table>

ASCT indicates autologous stem cell transplantation; CEEP, cyclophosphamide, epirubicin, vincristine, and prednisone; CEOP, cyclophosphamide, epirubicin, vincristine, and prednisone; CHVmP/BV, cyclophosphamide, doxorubicin, teniposide, prednisone, bleomycin, and
vincristine; DFS, disease free survival; DHAP, dexamethasone, cytarabine, and cisplatin; ECVBP, epirubicin, cyclophosphamide, vindesine, bleomycin, prednisone; EI, etoposide and ifosfamide; HD MTX, high dose methotrexate; HDT, high dose therapy; H/HI, high/high intermediate; HI, high intermediate; IPI, International Prognostic Index; ITT, intention to treat; L, low; LI, low intermediate; MACOP-B, methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin; MC, methotrexate and cytarabine; NCVB, mitoxantrone, cyclophosphamide, vinblastine, and bleomycin; PFS, progression free survival; PR, partial response; PS, performance status; and VACOP-B, etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin.

* Crossover allowed at relapse, which may account for lack of survival benefit.
<table>
<thead>
<tr>
<th>Trial</th>
<th>Design/Regimen</th>
<th>Patients/Diseases</th>
<th>Diseases</th>
<th>Significant Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coiffier et al. (2002)69</td>
<td>R-CHOP vs. CHOP</td>
<td>399 patients</td>
<td>REAL/WHO: DLBCL (84%)</td>
<td>2yr EFS: R-CHOP 57% vs. CHOP 38%, (p&lt;0.001).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Age 60-80</td>
<td>Burkitt’s, Follicular lymphoma, SLL, Hodgkin’s lymphoma, B-cell lymphoma NOS, T-cell lymphoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stage II-IV</td>
<td></td>
<td>2yr OS R-CHOP 70% vs. CHOP 57%, (p=0.007).</td>
</tr>
<tr>
<td>Habermann et al. (2003)72</td>
<td>First randomization: R-CHOP</td>
<td>632 patients</td>
<td>REAL/WHO: DLBCL</td>
<td>2.7 yr TTF Favors R-CHOP (p=0.025) and MR (p=0.01)</td>
</tr>
<tr>
<td></td>
<td>vs. CHOP</td>
<td>Age greater than 60</td>
<td></td>
<td>No difference in OS</td>
</tr>
<tr>
<td></td>
<td>Second randomization: MR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>vs. No maintenance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pfreundschuh et al. (2004)37</td>
<td>R-CHOP-like vs. CHOP-like</td>
<td>326 patients</td>
<td>REAL/WHO: DLBCL</td>
<td>2 yr TTF R-CHOP-like 76% vs. CHOP-like 60%, (p&lt;0.00001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Age 18-60</td>
<td></td>
<td>2 yr OS R-CHOP-like 94% vs. CHOP-like 87%, (p=0.001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stage I (bulk),II-IV</td>
<td>Low risk (IPI of 0-1)</td>
<td></td>
</tr>
</tbody>
</table>

MR indicates maintenance rituximab; SLL, small lymphocytic lymphoma; TTF, time to treatment failure; and R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone.

* Updated results at 5 years\textsuperscript{10} show 5yr EFS: R-CHOP 47% vs. CHOP 29%; 5yr OS R-CHOP 58% vs. CHOP 45%.
Advances in the biology and therapy of diffuse large B-cell lymphoma-moving towards a molecularly targeted approach

Jeremy S Abramson and Margaret A Shipp