The classification of diffuse large B-cell lymphoma (DLBCL) has been significantly refined as a result of novel insights into the biology of lymphoid tumors. Although it has been known for some time that DLBCL is a clinically and biologically diverse disease, new diagnostic technologies, such as gene expression profiling (GEP), have defined a new molecular taxonomy for DLBCL and led to the identification of driver mutations and druggable targets. DLBCL can now be divided into at least three molecular subtypes that correspond to distinct stages of B-cell differentiation. It is critical to understand and consider these pathobiologic distinctions in the context of novel targets and strategies in DLBCL.

The most recent World Health Organization classification of tumors of hematopoietic and lymphoid tissues divides DLBCL into four major groupings—and these are further subdivided according to clinicopathologic and molecular characteristics.[1] DLBCL not otherwise specified is the most common group; on the basis of GEP results, it can be further subdivided into the germinal center B-cell–like (GCB) and activated B-cell–like (ABC) subgroups (Figure 1).[2,3] Genes that are associated with the GCB subtype include markers of germinal center differentiation, such as CD10 and the BCL6 gene.[4] In the ABC type, the nuclear factor kappa B (NF-κB) pathway is constitutively active, with high expression of NF-κB target genes. Although both GCB and ABC subtypes express B-cell lymphoma 2 (BCL2)—which is induced > 30-fold during peripheral B-cell activation—its expression is > 4-fold higher in most ABC DLBCLs compared with GCB DLBCLs.[5,6] The distinct genetic characteristics of the GCB and ABC subtypes suggest their derivation from different stages of B-cell differentiation, with the GCB subtype arising from germinal center B cells and the ABC subtype from post–germinal center B cells that are blocked during plasmacytic differentiation (Figure 2). Note that in patients who undergo standard immunochemotherapy with R-CHOP (rituximab + cyclophosphamide, doxorubicin, vincristine, and prednisone), overall survival is significantly worse for those with the ABC subtype than for those with the GCB subtype (Figure 3).[7]

The third molecular subtype of DLBCL, primary mediastinal B-cell lymphoma (PMBL), arises from a thymic B cell. This disease entity occurs predominantly in girls and young women and shares many clinical features with classic Hodgkin lymphoma of the nodular sclerosis type (CHL-NS).[1,8] GEP studies have demonstrated that PMBL is a distinct biologic entity that shares many molecular similarities with CHL-NS.[9] Although B-cell transcription factors such as OCT-2 and BOB-1 are expressed in PMBL, immunoglobulin production is defective, in contrast to other subtypes of DLBCL.

**Standard Chemotherapy Platforms in DLBCL**

In the early 1970s, the addition of doxorubicin to cyclophosphamide, vincristine, and prednisone (CVP)—CHOP—resulted in the first curative regimen for DLBCL and underlined the importance of anthracyclines in DLBCL therapeutics. Subsequently, the empiric addition of drugs to CHOP did not improve outcomes in patients with DLBCL, as shown in the landmark randomized study that compared CHOP with second- and third-generation regimens.[10] Further attempts were made to improve the curative ability of CHOP. In the Deutsche Studiengruppe für Hochmaligne Non-Hodgkin Lymphome (DSHNHL) four-arm studies, CHOP was administered every 14 or 21 days, without or with etoposide (CHOEP), to patients aged > 60 years and to low-risk...
patients aged ≤ 60 years. The results demonstrated the benefits of CHOEP-21 in younger patients and CHOP-14 in older patients.[11,12] However, similar trials showed that these survival benefits were lost when rituximab was added (results of the Groupe d’Étude des Lymphomes de l’Adul
te [GELA] study, which demonstrated superior survival with R-CHOP compared with CHOP in patients aged ≥ 60 years).[13-15] The DSHNHL also performed a randomized study (RICOVER-60) of 6 vs 8 cycles of CHOP-14, with or without rituximab, in elderly patients with DLBCL.[14] They found no difference in the outcomes of patients who received 6 vs 8 cycles of treatment, but based on a historical comparison, the researchers suggested that R-CHOP-14 should be the new standard.[14] However, when R-CHOP-14 was compared with R-CHOP-21 in two randomized trials, no benefit of R-CHOP-14 was confirmed; hence, R-CHOP-21 continues to be the standard chemotherapy platform.[16-18]

A randomized study compared dose-intense R-ACVBP (rituximab + doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone) with R-CHOP-21 in patients aged < 60 years with a low-risk International Prognostic Index score.[19] At 3-year follow-up, the progression-free survival (PFS) of patients who received R-ACVBP (87%) was significantly superior to the PFS of those who received R-CHOP (73%); however, significant hematologic toxicity limited the use of R-ACVBP in younger patients. Although this study demonstrates that the R-CHOP platform can be improved, the clinical limitations of R-ACVBP and the absence of information on its activity within DLBCL molecular subgroups restrict its use as a universal platform replacing R-CHOP. Other dose-intensity approaches, including autologous stem cell transplant, have been studied as initial therapy for DLBCL but have not shown a clear benefit over R-CHOP alone.[20]

The current National Comprehensive Cancer Network guidelines include both R-CHOP and DA-EPOCH-R (dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin, with rituximab) as suggested regimens for the front-line treatment of DLBCL.[17,18] Although R-CHOP-14 is included along with R-CHOP-21, recent randomized studies support the use of the latter.

The DA-EPOCH-R regimen was developed from in vitro modeling of drug resistance and drug pharmacodynamics and employs infusional drug scheduling, topoisomerase II targeting, and pharmacodynamic dosing.[21] A multicenter Cancer and Leukemia Group B (CALGB) cooperative group study of 69 patients who received the regimen reported a 5-year time to progression of 81% and overall survival of 88%; the toxicity profile was similar to that of R-CHOP.[22] Other phase II trials have reported similarly promising results with DA-EPOCH-R, and a randomized comparison of DA-EPOCH-R and R-CHOP, with analysis of outcome according to molecular subtype, has recently completed accrual.[23-25]

**Treating GCB DLBCL**

By far the most common molecular subtype of DLBCL, GCB DLBCL typically occurs in children and young adults.[26] While it has a much better prognosis than ABC DLBCL, approximately 30% of patients with GCB DLBCL are not cured with R-CHOP chemotherapy.[7] One of the most interesting targets in GCB DLBCL is BCL6; while it is typically highly expressed in the GCB subtype, it is rarely expressed in ABC DLBCL. BCL6 is a key transcription factor that represses many target genes involved in such processes as lymphocyte activation, apoptosis, and the DNA damage response.[27] Chromosomal translocations can lead to the deregulation of BCL6, or it may be altered by multiple somatic mutations. These translocations/mutations enhance the inhibitory effect of BCL6 on the apoptotic stress response, leading to tumor proliferation and treatment failure.[28] It is challenging to target BCL6 directly, but specific inhibitors of BCL6 are in development. One of these, the 79-6 complex, is a small molecule inhibitor of BCL6 that binds to the corepressor binding groove of the BCL6 BTB domain and kills BCL6-positive cell lines.[29] It is possible to target other BCL6 domains, and strategies such as inhibition of histone deacetylation to overcome the effects of BCL6 repression on p53 and cell cycle inhibitory proteins may also be useful.[30] In the context of targeting GCB DLBCL, the effect of topoisomerase II inhibition on BCL6 expression is potentially interesting. Inhibiting topoisomerase II with agents such as etoposide—through ubiquitin-mediated protein degradation and possibly transcriptional inhibition—results in downregulation of BCL6 expression.[31] This may help explain the improvement in event-free survival in younger patients who received etoposide in addition to CHOP (CHOEP vs CHOP alone) in the DSHNHL study. Because the incidence of GCB DLBCL is higher in younger persons than in older ones, younger patients may benefit more from the addition of etoposide.[11,12] Although the benefit of adding etoposide was lost when rituximab was added to CHOEP (R-CHOEP), the results
nonetheless suggest that topoisomerase inhibition may be an important strategy in GCB DLBCL.[13] This relationship between topoisomerase II inhibition and BCL6 suggests that regimens that can more effectively inhibit topoisomerase II may be more effective in patients with GCB DLBCL, even when considering regimens that include rituximab. The DA-EPOCH-R regimen incorporates two topoisomerase II inhibitors, etoposide and doxorubicin. Inhibition of topoisomerase II is optimized by both continuous delivery of the drugs over 96 hours and pharmacodynamic dose adjustment on successive cycles, which ensures adequate steady-state concentrations.[21] In two studies of the regimen in patients with untreated DLBCL, the outcome for those with the GCB subtype was particularly good: event-free survivals ranged from 95% to 100% after 5 years of follow-up.[22,32] Another promising therapeutic target in GCB DLBCL is EZH2. Gain-of-function mutations in EZH2 result in increased H3K27 methylation and are present in 25% of patients with GCB DLBCL, and inhibitors of EZH2 are toxic to GCB cell lines.[33]

**Treating ABC DLBCL**

ABC DLBCL, which is associated with poorer outcomes compared with GCB DLBCL, is characterized by the constitutive activation of NF-κB genes, and this leads to tumor survival and proliferation. Staudt et al conducted early studies to validate NF-κB as a therapeutic target. They tested an inhibitor of the IkB kinase, which is necessary for NF-κB activation, in both ABC DLBCL and GCB DLBCL cell lines and demonstrated that ABC lines were differentially sensitive to it. These findings led to a subsequent “proof of principle” clinical study that set out to test whether inhibition of NF-κB might sensitize ABC DLBCL but not GCB DLBCL to chemotherapy.[34] Bortezomib is a proteasome inhibitor that prevents the degradation of phosphorylated IkBα in the proteasome, and this leads to inhibition of NF-κB activity in ABC DLBCL cell lines. On the basis of these in vitro findings, bortezomib was tested in combination with DA-EPOCH in patients with relapsed and refractory DLBCL; molecular subtype was determined on study entry (Figure 4). Compared with patients who had GCB DLBCL, those with ABC DLBCL had a much higher overall response rate (83% vs 13%; P = .0004) as well as an improved median overall survival (10.8 vs 3.4 months; P = .0026). These results support the development of rational therapeutic strategies based on distinct molecular subtypes of DLBCL. Currently, several randomized studies are testing R-CHOP ± bortezomib in patients with newly diagnosed DLBCL.

Another agent that has shown activity in the ABC subtype is the immunomodulatory drug lenalidomide. In vitro studies have demonstrated that the drug selectively kills ABC DLBCL cells by augmenting interferon-β production through its effects on interferon regulatory factor 4.[35] In a phase II study of patients with relapsed and refractory DLBCL, the overall response rate was 55% in those with the ABC subtype vs 9% in those with the GCB subtype; these results suggest differential activity.[36] Although downstream pathways involved in NF-κB activation have been understood for some time, many upstream pathways that lead to NF-κB activation have only recently been elucidated (Figure 5). It is now known that chronic B-cell receptor (BCR) signaling, as well as activation of mutations in caspase recruitment domain-containing protein 11 (CARD11) and myeloid differentiation primary response gene 88 (MYD88), is an important driver of NF-κB activation, and this discovery has led to the identification of several novel targets.[37-39] One of the most exciting of these targets is Bruton tyrosine kinase (BTK), and specific inhibitors of BTK are in clinical development. Ibrutinib is an orally administered, selective, and covalent inhibitor of BTK that is selectively toxic to cell lines with chronic active BCR signaling.[37] Because of this activity, the drug was tested in 70 patients with relapsed and refractory DLBCL in a phase II multicenter study. Twenty-nine patients had ABC DLBCL, and 20 had GCB DLBCL; in 21 patients, the molecular subtype was unclassified.[40] The overall response rate was 23%; the rate was 41% in those with ABC DLBCL and 5% in those with GCB DLBCL (P = .007). In addition, there was a trend toward better overall survival in patients with the ABC subtype compared with those who had the GCB subtype (9.76 vs 3.35 months; P = .099). This differential response supports the role of BCR signaling in ABC DLBCL but not in GCB DLBCL.[37] Another objective of the study was to test the mutational status of individual tumors and to correlate this status with outcome. Although patients with mutations of BCR and MYD88 had a high rate of response, those with CARD11 mutations did not respond, indicating the dominance of downstream signaling.[40]

Another exciting kinase in development is protein kinase C beta (PKCβ). This serine/threonine kinase is amplified through the BCR signaling pathway and also appears to play a critical role in NF-κB activation (see Figure 5). Enzastaurin is a potent inhibitor of PKCβ that is administered orally and has
been studied as a single agent in relapsed/refractory DLBCL and in combination with R-CHOP in DLBCL.[41] Spleen tyrosine kinase (Syk) is activated by BCR signaling, and fostamatinib is a Syk inhibitor—it has not specifically been studied in ABC lymphoma but has activity across a wide range of lymphomas; in one study it had a response rate of up to 22% in patients with DLBCL.[42]

Other studies have used mammalian target of rapamycin (mTOR) inhibitors to target the phosphoinositide 3 kinase (PI3K)/AKT/mTOR signaling pathway. Both temsirolimus and everolimus have induced complete remissions across lymphoma subtypes.[43,44] This suggests that different types of lymphomas, including DLBCL, are dependent on an activated PI3K/AKT/mTOR pathway. Although the ideal target for this pathway is unknown, studies underway are targeting upstream molecules such as AKT and PI3K. GS 1101—a first-in-class, potent specific inhibitor of PI3K p110δ that blocks constitutive PI3K signaling in vitro—has been tested in DLBCL but has not demonstrated any activity thus far.[45]

The NF-κB signaling pathway may also be activated by stimulation of MYD88, which is mutated in 30% of patients with ABC DLBCL (see Figure 5).[39] MYD88 activates NF-κB by a signaling cascade that involves interleukin-1 receptor–associated kinase (IRAK) 1 and IRAK4; activity of the latter is required for the oncogenic effect of MYD88. Small molecule inhibitors of IRAK4 have selective toxicity for ABC DLBCL cell lines and are another rational therapeutic strategy for this subtype.

**Targeting MYC and BCL2 in Both ABC and GCB DLBCL**

Another potentially important target that is expressed in both GCB DLBCL and ABC DLBCL is c-MYC.[2,6] Although a c-MYC translocation is characteristic of Burkitt lymphoma, approximately 10% of DLBCLs harbor it and most of these cases are of GCB origin. Harboring a c-MYC rearrangement is important clinically because it has been associated with high tumor proliferation and worse outcomes (compared with MYC-negative cases) following standard R-CHOP therapy.[46,47] Recently, there has been strong interest in investigating the prognostic significance of immunohistochemistry for MYC in concert with BCL2 irrespective of translocation status.[48,49] Novel strategies that target MYC include epigenetic manipulation of BET bromodomain protein 4 (BRD4) by the compound JQ1, which has shown promise in inhibiting c-MYC in preclinical models of multiple myeloma and primary effusion lymphoma.[50,51] Other epigenetic strategies that target c-MYC are also under investigation.

BCL2 is a potentially important druggable target that is expressed in both GCB and ABC DLBCL, albeit through different mechanisms.[52] In GCB DLBCL, BCL2 expression is associated with t(14:18) in most cases, although some patients who overexpress BCL2 have no translocations. In the ABC subtype, BCL2 overexpression is associated with the constitutive activation of NF-κB.[2,6] Although some older studies identified an association between BCL2 expression and poor outcomes in DLBCL, later studies have demonstrated a more complex association.[53,54] It is likely that BCL2 is an important therapeutic target, and several inhibitors of BCL2, such as navitoclax and ABT-199, are in development.[55,56]

**Treating PMBL**

PMBL is a subtype of DLBCL that arises in the mediastinum and is derived from a thymic B cell.[1] It makes up 10% of cases of DLBCL and predominantly affects girls and young women; its clinical presentation is distinct from the other subtypes of DLBCL and closely resembles that of CHL-NS.[1] Its molecular profile is also unique and is much closer to that of CHL-NS than to the profile of either GCB DLBCL or ABC DLBCL.[9]

Among other reasons, the rarity of this entity and the paucity of prospective studies have resulted in a lack of consensus on what the optimal therapeutic approach for PMBL should be.[8] Early studies suggested that radiotherapy to the mediastinum was a necessary component of curative therapy, and most strategies today continue to include it. One of the key early studies that led to this practice evaluated the MACOP-B regimen (methotrexate, leucovorin, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin) followed by consolidative radiation in patients with PMBL. Only 19% of patients had a positive gallium scan at the completion of radiotherapy, compared with 66% who had a positive scan after chemotherapy alone. These results supported a combined modality approach, and 80% of patients who received this treatment were event-free at 39 months median follow-up.[57]

Because dose intensity is important therapeutically in Hodgkin lymphoma, it would not be unexpected for this to be true as well in PMBL, given the shared clinical and biologic characteristics of the two entities.[58] Several retrospective studies have assessed the role of dose intensity in
PMBL.[59-61] In one such study, MACOP-B and VACOP-B (etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin) were compared with CHOP; patients who received CHOP had worse outcomes, which suggests a role for dose intensity.[59] Other studies also found poorer outcomes with CHOP compared with more dose-intensive approaches.[60,61] However, there have not been prospective comparisons of dose-intensive vs conventional approaches in PMBL. In the Southwest Oncology Group (SWOG) study that prospectively compared CHOP with second- and third-generation regimens in DLBCL, PMBL was not recognized as a distinct entity and its outcome was not assessed.[10]

The additive role of rituximab in PMBL has not been well studied. In a British Columbia retrospective study that compared PMBL outcomes in the pre- and post-rituximab eras, the addition of rituximab to CHOP did not confer a survival advantage (the number of patients in the R-CHOP arm was small, however, and the follow-up time was short).[62] Other studies, meanwhile, have suggested a benefit to adding rituximab. In the randomized, phase III MabThera International Trial (MiNT), a subgroup analysis in patients with PMBL evaluated the additive role of rituximab in combination with CHOP-like regimens.[63] The rituximab arm was superior in terms of 3-year event-free survival (78% vs 52% in the chemotherapy-alone arm), but no statistically significant difference in overall survival was detected because of the small number of patients. It is important to note, however, that in the MiNT and more recent analyses, the majority of patients received preplanned mediastinal radiation following R-CHOP, and the addition of radiation improved remission rates.[64,65]

Although high cure rates can be achieved in patients with PMBL following combined modality treatment, it is clear, particularly from the collective experience in Hodgkin lymphoma, that the use of mediastinal radiation causes significant and devastating late-term sequelae, such as secondary tumors and cardiac disease.[66,67] The excess risk of breast cancer is particularly problematic, given that the majority of patients with PMBL are young women. Lower doses of radiation may reduce the risk of these complications, but this has not been proven. A survivorship study in pediatric Hodgkin lymphoma demonstrated that secondary tumors occurred with similar latency and frequency in patients who received low-dose or high-dose radiation.[68]

DA-EPOCH-R was tested in PMBL, based on its efficacy in DLBCL and the evidence from historical studies that dose intensity is important in PMBL.[69,70] One important goal of the regimen in PMBL is to obviate the need for mediastinal radiation and eliminate the risk of long-term sequelae. In a recent study of 51 patients with untreated PMBL, event-free survival was 93% and overall survival was 97% at a median follow-up time of 5 years.[71] Only two patients required radiotherapy, and no patients died of PMBL. These results suggest that DA-EPOCH-R is a highly curative strategy in PMBL that obviates the need for radiation in almost all cases. On the basis of these results and to provide confirmatory evidence of this strategy in PMBL, an international trial of DA-EPOCH-R is ongoing in children with PMBL.[72] Although patients with PMBL who are treated with regimens such as DA-EPOCH-R have excellent outcomes, future strategies should continue to focus on reducing the toxicity and the duration of therapy while maintaining high cure rates. In that regard, it will be important to test targeted agents such as inhibitors of Janus kinase pathways.[73]

Conclusions

Although the treatment of most patients with DLBCL is similar, we now appreciate that this disease is heterogeneous at the molecular level and can be divided into subtypes by GEP. Each of these subtypes has distinct mechanisms of oncogenic activation, and therapies that target individual subtypes and driver mutations are in development and showing promise. It is hoped that continued progress in developing new strategies based on an improved understanding of the molecular biology of DLBCL will pave the way for more effective treatment of this disease.

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Figure 1: Differentiating Diffuse Large B-Cell Lymphoma (DLBCL) Into M...

Figure 2: Oncogenic Pathways for Three Subtypes of Diffuse Large B-Cell...

Figure 3: Outcome of GCB DLBCL and ABC DLBCL With R-CHOP

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Figure 5: B-Cell Receptor (BCR) and MYD88 Signaling Pathways and Poten...

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