Treatment strategies for aggressive lymphomas: what works?

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Introduction

We are now in the era of targeted therapy for diffuse large B-cell lymphoma (DLBCL) that is moved forward by a rapidly increasing knowledge of tumor biology, driver pathways, and clinical successes. The first targeted treatment, rituximab, has been an unqualified albeit empirical success. Rational drug discovery now leverages our understanding of tumor pathogenesis and tumor-host interactions. The discovery of new signaling pathways through gene expression profiling (GEP), transcriptome sequencing, RNA interference screens, and DNA sequencing has identified an array of new targets for DLBCL. The division of DLBCL into at least 3 distinct molecular diseases, germinal center B-cell (GCB), activated B-cell (ABC), and primary mediastinal B-cell (PMBL) DLBCL, is essential for advancing treatment.

The application of empiricism over scientific rigor, inadequate translational end points, and the entrenchment of R-CHOP (rituximab plus cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone/prednisolone) has hampered the development and acceptance of new standards for DLBCL. Fortunately, insights into the molecular taxonomy of DLBCL has led to the identification of “driver” pathways, druggable targets, and more effective immunotherapy regimens, which highlights the importance of conducting studies within the molecular DLBCL subtypes.

Pathobiology

Conceptual therapeutic advances usually emerge from biological foundations. The major genetic and biological insights have been codified into the diagnostic criteria of the World Health Organization (WHO) classification of tumors of the lymphoid tissues. The classification of DLBCL has been among the greatest beneficiaries of recent biological discoveries in lymphoid tumors. Although it has long been recognized that DLBCL is clinically and biologically diverse, it was not possible to subdivide it into distinct disease entities because of overlapping morphology and pathogenetic features. As a result, treatment strategies have depended and still depend on clinical features such as stage and age as validated by the International Prognostic Index (IPI) score. However, with the application of large-scale GEP, DLBCL is now divided into at least 3 molecular subtypes. Although still retaining the histological description of a neoplasm of large B-lymphoid cells with a diffuse growth pattern, these subtypes derive from B cells at different stages of differentiation with distinctive molecular and clinical characteristics. When considering treatment, either in the research or clinical setting, it is essential to understand these pathobiological distinctions.

Presently, DLBCL is divided into 4 major groups within the WHO, which are further divided along molecular, pathological, and/or clinical grounds. Of these divisions, the most common group is DLBCL not otherwise specified (NOS), which can be further subdivided into the GCB and ABC molecular subgroups by GEP (Figure 1A).1,2 Genes associated with GCB DLBCL include known markers of germinal center differentiation such as CD10 and the bcl-6 gene, which may be translocated or mutated in DLBCL. In contrast, most genes that define ABC DLBCL are not expressed by normal GCB cells, but instead are induced during in vitro activation of peripheral B cells. The ABC DLBCL signature also includes the IRF4 (MUM1) gene that is transiently induced during normal lymphocyte activation and is necessary for antigen receptor–driven B-cell proliferation. A noteworthy feature of ABC DLBCL is the expression of bcl-2 that is induced >30-fold during peripheral B-cell activation.3 These results suggested that the GCB and ABC DLBCL subtypes are derived from B cells at different stages of differentiation. GCB DLBCL appears to arise from GCB cells, whereas ABC DLBCL likely arises from post-GCB cells that are blocked during plasmacytic differentiation. Clinically, GCB DLBCL has a higher overall survival compared with ABC DLBCL with R-CHOP–based treatment (Figure 1B).4

PMBL is the third molecular subtype of DLBCL, which occurs mostly in young patients (Figure 1A).5,6 PMBL is pathologically defined by a combination of clinical and histological features and some cases may have features reminiscent of Hodgkin’s lymphoma, all of which can confound an accurate diagnosis. Two studies using GEP have confirmed the unique biological identity of PMBL and have shown a strong relationship between PMBL and nodular sclerosis Hodgkin lymphoma. Unlike other types of DLBCL, PMBL tumors...
have defective immunoglobulin production despite the expression of the B-cell transcription factors OCT-2, BOB.1, and PU.1.

Chemotherapy platforms
The addition of doxorubicin to CVP (CHOP) in the early 1970s ushered in the first curative regimen for DLBCL. Since then, anthracyclines have been an essential drug class for DLBCL. However, the empiric addition of drugs to CHOP did not improve the outcome of DLBCL, as shown by the landmark randomized study comparing CHOP with second- and third-generation regimens in 1993.7 Later attempts had only qualified success. The Deutsche Studiengruppe für Hochmaligne Non-Hodgkin Lymphome (DSHNL) 4-arm studies of CHOP every 14 or 21 days with or without etoposide (CHOEP) in patients >60 years and low-risk patients ≤60 years of age showed a benefit of CHOEP-21 in younger patients and CHOP-14 in older patients.8,9 These benefits, however, were lost when similar trials were done with rituximab.10,11 They also performed a randomized study of 6 versus 8 cycles of CHOP-14 with or without rituximab in elderly patients with DLBCL.11 In that study, termed RICOVER-60, there was no difference between 6 and 8 cycles of treatment, but the investigators suggested that R-CHOP-14 should be the new standard.11 This conclusion was based on a historical comparison and when assessed in 2 randomized trials (Groupe d’Etude des Lymphomes de l’Adulte and a United Kingdom study), it was not confirmed.12 Therefore, R-CHOP-21 continues to be the standard.

The GELA group recently reported a randomized study of dose-intense R-ACVBP (rituximab plus doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone) versus R-CHOP-21 in patients under 60 years of age with low-risk IPI.13 At 3-years, the progression-free survival of R-ACVBP was 87% compared with 73% for R-CHOP, a significant difference, although hematologic toxicity limits its use to younger patients. Although this study confirms that the R-CHOP platform can be improved, the clinical limitations of R-ACVBP and absence of information on its performance within the molecular subtypes of DLBCL restrict its use as a universal platform to replace R-CHOP. Other dose-intensity approaches have
also been studied as initial therapy in DLBCL. A dose-intensified R-CHOP showed a failure-free survival of 65% at 3 years in high-risk DLBCL, but was associated with several toxic deaths, suggesting that it is not an optimal approach.\textsuperscript{14} Autologous transplantation showed some benefit in the pre-rituximab era, but the lack of substantive evidence for benefit in the rituximab era and its considerable toxicities make it an unacceptable standard of care.

The DA-EPOCH-R (dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, rituximab) regimen was developed from in vitro modeling of drug resistance and drug pharmacodynamics and employs infusional drug scheduling, topoisomerase II targeting, and pharmacodynamic dosing. A multicenter Cancer and Leukemia Group B (CALGB) cooperative group study of 69 patients reported a 5-year time to progression and overall survival of 81% and 88%, respectively.\textsuperscript{15} DA-EPOCH-R showed superior outcome within all IPI and age groups compared with published reports of R-CHOP. DA-EPOCH-R was also assessed within the GCB and post-GCB molecular subtypes; time to progression was 100% in GCB and 67% in non-GCB DLBCL at 62 months.\textsuperscript{15} Other phase 2 trials have reported similarly promising results with DA-EPOCH-R.\textsuperscript{16-18} A randomized comparison of DA-EPOCH-R and R-CHOP with analysis of outcome within the molecular subtypes of DLBCL is nearing completion. Although the DA-EPOCH-R regimen presents logistical administration issues compared with R-CHOP, its toxicity profile is similar.

**Activated B-cell DLBCL**

The constitutive activation of NF-κB activates genes that are characteristic of ABC DLBCL promotes survival and proliferation. To help assess the clinical utility of this target, Staudt et al showed that ABC DLBCL cell lines were differentially sensitive to an IkB kinase inhibitor, which is necessary for NF-κB activation. Dunleavy et al undertook a “proof of principle” clinical study to test whether inhibition of NF-κB might sensitize ABC but not GCB DLBCL to chemotherapy.\textsuperscript{19} Based on in vitro evidence that bortezomib, a proteasome inhibitor, blocked degradation of phosphorylated IkBa and consequently inhibited NF-κB activity in ABC DLBCL cell lines, bortezomib was combined with DA-EPOCH in patients with relapsed/refractory DLBCL. Tumor tissue was analyzed to identify molecular DLBCL subtypes. Patients with ABC DLBCL had a significantly higher response (83% vs 13%; \( P = .0004 \)) and median overall survival (10.8 vs 3.4 months; \( P = .0026 \)) compared with GCB DLBCL (Figure 2). These results provide a rational therapeutic approach based on genetically distinct DLBCL subtypes. Several randomized studies of R-CHOP with or without bortezomib in untreated DLBCL patients have been initiated.

Lenalidomide, an immunomodulatory agent, may also have activity in ABC DLBCL. As a single agent, lenalidomide demonstrated a response rate of 55% in patients with ABC DLBCL compared with only 9% in patients with GCB DLBCL, suggesting differential activity.\textsuperscript{20} In vitro, lenalidomide selectively kills ABC DLBCL cells by augmenting IFN-β production through its effects on IRF4.\textsuperscript{21} In ABC DLBCL cell lines, lenalidomide leads to the reduction of IRF4, which requires the expression of the E3 ubiquitin ligase complex coreceptor protein cereblon.

It is also important to understand and target upstream targets involved in NF-κB activation (Figure 3). Chronic BCR signaling and activating mutations of CARD11 and MYD88 promote NF-κB activation, suggesting several targets. One potential target is Bruton tyrosine kinase (Btk), in which the selective inhibitor ibrutinib is selectively toxic to cell lines with chronic active BCR signaling (Figure 2).\textsuperscript{22} The position of molecular lesions in the BCR and MYD88 signaling pathways could help guide therapy of ABC DLBCL.

Based on these studies, a phase 2 multicenter study of ibrutinib was performed in patients with relapsed/refractory DLBCL. The objectives were to assess whether ibrutinib had differential activity in ABC versus GCB DLBCL and the role of MYD88, CARD11, and CD79 mutations on overall response rate. Seventy patients were enrolled with a median age of 64 years and 3 (range 1-7) prior regimens. Overall, there were 29 ABC, 20 GCB, and 21 unclassified/unknown patients. Twenty-three percent of patients responded; 41% ABC and 5% GCB DLBCL (\( P = .0007 \)), supporting the role of BCR signaling in ABC but not GCB DLBCL.\textsuperscript{22} Furthermore, there was a trend toward improved overall survival in patients with ABC compared with GCB DLBCL (9.76 vs 3.35 months, \( P = .009 \)). The investigators also assessed the relationship between mutations and overall response rate (Figure 4). Responses were documented in 71% (5/7) of patients with mutant CD79B and 34% (10/29) of patients with wild-type CD79B, suggesting the presence of chronic BCR signaling. Interestingly, 80% (4/5) of patients with both mutant CD79B and MYD88 responded, whereas patients with wild-type CD79B and mutant MYD88 did not respond, suggesting a MYD88-independent pathway for NF-κB activation. Patients with CARD11 mutations did not respond, indicating dominance of downstream signaling.\textsuperscript{23}

PKCβ is a serine/threonine kinase amplified through the BCR signaling pathway that may also play an essential role in the activation of the NF-κB pathway in B cells (Figure 4). GEP identified PKCβ as an unfavorable prognostic marker in DLBCL and in vitro evidence supported PKCβ as a rational therapeutic target. Enzastaurin is a potent oral inhibitor of PKCβ that has been studied in relapsed/refractory DLBCL and in combination with R-CHOP in patients with intermediate- and high-risk DLBCL.\textsuperscript{24} Unfortunately, it has shown little activity.

Studies have also targeted the PI3K/AKT/mTOR signaling pathway using mTOR inhibitors. Although the patients have been heterogeneous, mTOR inhibitors (temsirolimus and everolimus) have induced complete remissions across lymphoma subtypes.\textsuperscript{25,26} These
results suggested that different types of lymphomas are dependent on an activated PI3K/AKT/mTOR pathway, including DLBCL. Although the ideal target for the PI3K/AKT/mTOR pathway is unknown, investigators are targeting upstream molecules such as AKT and PI3K. GS 1101 is a potent small-molecule inhibitor of PI3K p110α/H9254 that blocks constitutive PI3K signaling in vitro.27 GS 1101 was studied in 9 patients with DLBCL and was well tolerated, but did not result in clinical responses.

Alternative activation of the classical NF-κB signaling pathway occurs through stimulation of MYD88 (Figure 3). MYD88 mutations are present in 30% of ABC DLBCL cases and promote NF-κB activation through this pathway via the kinase activity of IRAK1 and IRAK4. In ABC DLBCL cell lines, it is the activity of IRAK4 but not IRAK1 that is required for the oncogenic effect of MYD88. Small-molecule inhibitors of IRAK4 have demonstrated selective toxicity for ABC DLBCL cell lines and represent another potential therapeutic target in ABC DLBCL.

GCB DLBCL

Although GCB DLBCL has a better prognosis than ABC DLBCL, upwards of 30% of patients are not cured with R-CHOP–based treatment (Figure 1A). Bcl-6 is a key transcription factor expressed by GCBs, including GCB DLBCL, that regulates cell growth and apoptosis. Bcl-6 suppresses genes that are involved in lymphocyte activation, differentiation, and cell cycle arrest and the DNA damage response genes p53 and ATR. In GCB DLBCL, chromosomal translocations affecting the Bcl-6 locus juxtapose heterologous promoters from the partner chromosome with intact Bcl-6 coding sequences, leading to deregulated expression of Bcl-6; in addition, Bcl-6 can be altered by multiple somatic mutations. These mutations/translocations in Bcl-6 enhance its inhibitory effect on the apoptotic stress response and promote proliferation, both of which are associated with treatment failure. These results suggest that BCL6 is an important target for GCB DLBCL. BCL6 is difficult to target directly. Recently, a small molecule known as the 79-6 complex that specifically disrupts the activity of BCL6 by blocking its corepressors was identified.28 Targeting other Bcl-6 domains or using histone deacetylase inhibitors to overcome Bcl-6 repression of p53 and cell cycle inhibitory proteins may also be useful.

A potentially important observation is the effect of topoisomerase II inhibition on Bcl-6 expression. Inhibition of topoisomerase II by etoposide leads to the down-regulation of Bcl-6 expression through ubiquitin-mediated protein degradation and possibly transcriptional inhibition.29 This could account for the in vitro finding that sustained exposure of tumor cells to etoposide and low-dose doxorubicin promotes the p53-p21 pathway and activates the check-point kinase (Chk2), effects that are inhibited in cells engineered to overexpress Bcl-6. This raises the possibility that inhibition of topoisomerase II may be important in GCB DLBCL and may partially explain the finding by the German cooperative group (DSHNHL) that the addition of etoposide to CHOP (CHOEP) improved the event-free survival (EFS) of younger patients, who have a higher incidence of GCB DLBCL compared with older patients.1,9,30 Although the benefit of etoposide in CHOEP was lost

Figure 3. BCR and MYD88 signaling pathways and potential targets. (A) Signaling through BCR leads to downstream activation of the NF-κB transcription factor, which is a driver pathway in ABC DLBCL. Signaling also activates the AKT/MTOR and MAP kinase pathways. Constitutive MYD88 signaling is an alternative pathway leading to NF-κB activation. (B) Inhibition of Btk by ibrutinib is toxic in ABC, but not in GCB DLBCL cell lines, providing evidence for the clinical relevance of the BCR signaling pathway.

Figure 4. Blockade of BCR signaling in ABC DLBCL with ibrutinib, an irreversible inhibitor of BTK. Shown is the pilot analysis of ABC DLBCL gene mutations and response to ibrutinib.
The association between topoisomerase II inhibition and inhibition of Bcl-6 raises the hypothesis of whether regimens that more effectively inhibit topoisomerase II would be more effective in GCB DLBCL, even in the setting of rituximab. In this regard, the DA-EPOCH-R regimen inhibits topoisomerase II through several strategies: (1) it incorporates 2 topoisomerase II inhibitors, etoposide and doxorubicin; (2) it optimizes topoisomerase II inhibition through a prolonged 96-hour infusion; and (3) it maximizes steady-state concentrations through pharmacodynamic dose adjustment. An analysis of outcome of GCB DLBCL in 2 DA-EPOCH-R trials showed a 95% EFS at 5 years in HIV+ GCB DLBCL, and a 100% EFS at 5-years in GCB DLBCL (CALGB study). The polycomb-group oncopgene EZH2 has now been reported as a gain-of-function mutation in > 21% of GCB DLBCL and is essentially absent from ABC DLBCL. EZH2 is an epigenetic regulator gene and mutant EZH2 protein results in decreased histone-lysine methyltransferase activity. GCB DLBCL cell lines and mouse xenograft models with EZH2 mutations have demonstrated selective sensitivity to inhibition of EZH2, with GSK126 confirming its potential role as a target in lymphomas of germinal center origin.

MYC is another potentially important target that is expressed in both GCB and ABC DLBCL, and its expression level is associated with tumor proliferation. Recent studies have shown that up to 10% of DLBCL cases harbor myc translocations, mostly in GCB DLBCL, which lead to high protein expression and is associated with a poor outcome with standard R-CHOP treatment. The Myc oncoproteins (c-Myc, N-Myc, and L-Myc) have generally been considered “undruggable” targets because the protein structures are not amenable to small-molecule inhibition. However, recent epigenetic manipulation of the BET bromodomain protein BRD4 by the compound JQ1 has shown exciting promise in inhibiting c-Myc in murine models of multiple myeloma. Because bromodomain proteins serve as regulatory factors for c-Myc, this indirect approach may alter gene expression. Another mechanism by which Myc promotes lymphomagenesis is by suppressing the transcription of tristetrapolin, which functions as a tumor suppressor. Normal gene expression is tightly controlled by mRNA turnover, which is in turn tightly regulated by adenylate-uridylate-rich element (AU)-binding proteins (AUBP) that recognize AU-rich elements within transcripts. Tristetrapolin is an example of an AUBP that is suppressed in cancers with Myc involvement and restoring tristetrapolin impairs Myc-induced lymphomagenesis and abolishes the malignant state. Both of these strategies represent novel epigenetic targeting of MYC+ tumors that could potentially be combined with chemotherapy.

Bcl-2 is a druggable target that is expressed in both GCB and ABC DLBCL, albeit through different mechanisms. Although some older studies found an association between bcl-2 expression and poor outcome in DLBCL, later studies have shown a more complex association. The mechanism of bcl-2 overexpression has been related to its prognostic relevance in DLBCL. Gascoyne et al showed that bcl-2 overexpression was only associated with a poor outcome in the absence of a t(14;18), which indicates that the mechanism of expression and not the protein itself is more relevant to prognosis. This becomes more understandable when considering the relationship of bcl-2 expression to the molecular subtype of DLBCL. In GCB DLBCL, bcl-2 expression is associated with t(14;18), which is only found in GCB DLBCL, whereas in ABC DLBCL, bcl-2 overexpression is associated with gene amplification or NF-κB transcriptional activation. In this latter case, bcl-2 expression may primarily be a surrogate biomarker for ABC DLBCL and may not in itself be an important therapeutic target. More recently, Gascoyne et al published a study showing that the concurrent protein expression of MYC and BCL-2 had an adverse outcome, whereas expression of either alone did not portend a worse outcome with R-CHOP. Although this study is only correlative, it provides additional evidence for testing inhibitors of BCL-2, such as navitoclax or ABT-199, and Myc.

PMBL

There is a virtual absence of prospective studies in PMBL, which has led to conflicting findings and a lack of treatment standards. Nonetheless, several observations have emerged from the literature. First, most patients with PMBL do not achieve adequate tumor control with standard immunochemotherapy, necessitating routine mediastinal radiotherapy. Second, even with radiotherapy, which has serious late-term side effects, 20% of patients have disease progression. Third, more aggressive chemotherapy is associated with an improved outcome. Due to the widespread use of R-CHOP chemotherapy, it has become a de facto standard for PMBL. Most strategies also incorporate consolidation radiotherapy to overcome the inadequacy of immunochemotherapy. The most accurate assessment of R-CHOP and radiotherapy comes from a subset analysis of PMBL patients in the Mabthera International Trial Group study of R-CHOP-based treatment. In 44 patients, 73% of whom received radiotherapy, the EFS was 78% at 34 months. These results indicate that patients who receive R-CHOP–based treatment, the majority being young and female, will confront the potentially serious long-term consequences of radiotherapy. Retrospective studies suggest that PMBL has a better outcome with more dose-intense regimens. Dose intensity is important in nodular sclerosis Hodgkin lymphoma, a closely related disease. Based on evidence that dose intensity is important in PMBL, Dunleavy et al assessed DA-EPOCH-R, a dose-intense regimen, without radiotherapy in PMBL. In a recent report of 51 patients with untreated PMBL, the EFS and overall survival were 93% and 97%, respectively, at the median follow-up of 5 years. Only 2 patients required consolidation radiation treatment and no patients died of PMBL. These results suggest that DA-EPOCH-R obviates the need for radiation in most PMBL patients, thus eliminating the risk of radiation-induced malignancies and heart disease. This is particularly important given that PMBL patients are typically young and often women and are at increased risk of breast cancer. Although the outcome of PMBL is excellent with regimens such as DA-EPOCH-R, it would be important to further reduce the toxicity and length of treatment. Therefore, targeted agents will be important to test. JAK2 is a potentially important target for PMBL.

Summary

Although DLBCL remains curable in advanced stages, up to one-third of patients will ultimately fail initial therapy and the efficacy of salvage options are diminished in the rituximab era. Anthracycline-based chemotherapy and rituximab have been historic breakthroughs in the management of DLBCL, with notable effects on survival. DLBCL is a heterogeneous disease composed of molecular subtypes that are as different from one another as they are from other aggressive lymphomas. This is reflected in their different mechanisms of pathogenesis and druggable targets. We have
entered the “molecular era” of defining DLBCL, when we must identify and target oncogene and non-oncogene addictions within distinct molecular subsets of DLBCL. Numerous small molecules are at various stages of development and demonstrate promise. To realize the goal of personalized precision therapy for DLBCL, it is essential that clinical trials be conducted within the molecular subsets of DLBCL.

Disclosures
Conflict-of-interest disclosure: The author declares no competing financial interests. Off-label drug use: ibrutinib and bortezomib for DLBCL.

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