New Strategies in Diffuse Large B-cell Lymphoma: Translating Findings from Gene Expression Analyses into Clinical Practice

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Background

Diffuse large B-cell lymphoma (DLBCL) is the most commonly occurring lymphoma in the United States (1). With standard chemotherapy, DLBCL, even when it presents in advanced stages, is a curable disease (2). Over the past decade, significant improvements in survival have been reported in the treatment of DLBCL, due largely to the routine incorporation of rituximab in standard doxorubicin-containing chemotherapy regimens (3, 4). In a population-based registry from Vancouver, British Columbia, Canada, the observed improvement in outcome after the introduction of rituximab was substantial: The 2-year progression-free survival rate for patients with DLBCL increased from 51% to 69% in the postrituximab era. Similarly, The 2-year overall survival estimate increased from 52% to 78% in the postrituximab era in this registry (5). Therefore, despite the improvements observed with rituximab, approximately one third of patients with advanced-stage DLBCL will still be refractory to therapy or will ultimately relapse, and the vast majority of patients with relapsed disease will die of lymphoma. Therefore, novel therapeutic approaches beyond rituximab are needed.

Historically, clinicians and investigators have relied on prognostic schemes that incorporate clinical risk factors to predict patients with DLBCL who are at high risk for disease progression, relapse, and death. The International Prognostic Index (IPI) for lymphomas was developed in the 1990s and remains the most robust clinical prognostic index for aggressive lymphomas (6). In a pooled analysis using the IPI, adults with aggressive non-Hodgkin lymphoma (mainly DLBCL), who had been treated with doxorubicin-containing chemotherapy were evaluated for clinical features predictive of outcome (6). The derived model had 5 features (age, tumor stage, serum lactate dehydrogenase concentration, performance status, and number of extranodal disease sites), and it identified 4 risk groups with predicted 5-year overall survival rates of 73%, 51%, 43%, and 26%, respectively. Although the IPI was derived from data obtained before the routine use of rituximab, a recent analysis of German clinical trials in the R-CHOP era confirmed that the IPI remains prognostic for determining event-free, progression-free, and overall survival and that the high-risk group still has a relatively poor outcome with a 3-year progression-free survival rate of 55% and 3-year overall survival rate of 59% (7). These findings emphasize the heterogeneous clinical behavior of DLBCL in the rituximab era.

Clearly, these IPI factors are clinical surrogates for the biological heterogeneity of the tumor and host in DLBCL, which leads to differential clinical behavior and outcome. Gene expression profiling is a powerful genomics technique that uses DNA microarrays to measure the expression of thousands of genes simultaneously, resulting in a molecular profile of RNA in a biopsy specimen (8). In cell-of-origin studies, investigators from the Leukemia/Lymphoma Molecular Profiling Project used unsupervised gene expression profiling (i.e., employing a pattern-recognition algorithm) to compare DLBCL samples with potentially normal counterparts (9). Their results indicated the existence of at least 3 distinct subtypes of DLBCL: activated B-cell (ABC), germinal center B-cell, and primary mediastinal. Two common oncogenic events in DLBCL, bcl-2 translocation and c-rel amplification, were detected only in the germinal-center B-cell–like subgroup (10). Activation of the NF-kB signaling pathway is a key feature of the ABC-like...
subgroup but not of the other subgroups (11). Many cases of primary mediastinal lymphoma contain a highly expressed gene fusion involving the major histocompatibility complex class II transactivator (CIITA), which affects survival in this subtype of DLBCL (12). In further support of the concept that these subtypes are really different diseases, the ABC lymphomas have an inferior prognosis, which seems to be an even more powerful predictor of outcome than the IPI, in patients treated with CHOP-like regimens (8).

Gene expression profiling also provides prognostic information in the rituximab era. Currently, an intergroup trial coordinated by Cancer and Leukemia Group B is randomizing patients to R-CHOP versus R-EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin) and incorporating gene expression analysis to validate outcome differences between germinal-center and ABC DLBCL (ClinicalTrials.gov NCT 00118209). In an updated analysis from the aforementioned Lymphoma/Leukemia Molecular Profiling Project (9), which included tumor biopsies from more than 200 patients with DLBCL treated with R-CHOP, a multivariate model showed the influence of the microenvironment on outcome (13). The prognostically favorable stromal-1 signature reflected extracellular matrix and histiocytic infiltration. The less favorable stromal-2 signature seems to be an angiogenic switch in which development of lymphoma is accompanied by angiogenesis, perhaps induced in part by surrounding macrophages.

Shipp and colleagues (14) also performed a gene expression analysis in diagnostic tumor specimens for patients with DLBCL treated with R-CHOP, a multivariate model showed the influence of the microenvironment on outcome (13). The prognostically favorable stromal-1 signature reflected extracellular matrix and histiocytic infiltration. The less favorable stromal-2 signature seems to be an angiogenic switch in which development of lymphoma is accompanied by angiogenesis. These studies have not only provided robust prognostic information but also yielded several important novel targets for therapeutic intervention (15). The remainder of this review discusses the clinical progress of some of these targeted approaches and looks forward to an era in which specific subgroups of patients with DLBCL can receive rationally targeted therapy.

On the Horizon

Development of novel agents based on gene expression analyses

NF-κB. As mentioned above, constitutive activation of the NF-κB pathway has been observed in the ABC subset of DLBCL, which seems to confer an inferior prognosis. Several inhibitors of NF-κB and IκBα are currently under development with the goal of improving treatment for this group of patients. Bortezomib is a proteasome inhibitor that blocks degradation of phosphorylated IκBα, subsequently inhibiting NF-κB activity. This agent is approved for treatment of multiple myeloma and relapsed/refractory mantle cell non-Hodgkin lymphoma, and patients with elevated NF-κB measured by immunohistochemistry of mantle cell lymphoma showed a trend toward better outcome when treated with this agent (16, 17). As a single agent, bortezomib confers very limited activity in DLBCL (18). However, Dunleavy and colleagues (19) conducted an intriguing trial in which patients with relapsed DLBCL were treated initially with bortezomib alone, followed by a combination of bortezomib and doxorubicin-containing chemotherapy (R-EPOCH). In this study, single-agent bortezomib also had no activity in DLBCL, but when combined with chemotherapy, it showed a significantly higher response (83% versus 13%; P = 0.001) and median overall survival (10.8 versus 3.4 months; P = 0.003) in ABC subtypes of DLBCL compared with the germinal-center subtype, respectively, as determined by gene expression analysis. Because the NF-κB pathway is a potent inhibitor of apoptosis induced by chemotherapeutic agents, it seemed that adding bortezomib to chemotherapy had synergy in the ABC subgroup in this trial.

Additional studies have confirmed the safety and potential efficacy of combining bortezomib with R-CHOP chemotherapy (20). In a phase II study of R-CHOP and bortezomib, the overall response rate was 88% and the 2-year progression-free survival rate was 64% (21). No differences were noted in outcome between germinal-center and non–germinal-center subtypes of DLBCL when immunohistochemistry was used to define the gene expression profile. On the basis of these promising preliminary results, a randomized phase II trial is underway to compare standard R-CHOP with R-CHOP plus bortezomib in patients with non–germinal center DLBCL (ClinicalTrials.gov NCT00931918). This represents the largest study in de novo DLBCL to limit enrollment to a particular subtype defined by gene expression data.

Additional strategies are in development that may improve upon these results. For example, combinations of bortezomib and other proteasome inhibitors, such as carfilzomib, with histone deacetylase inhibitors have shown particular promise in the laboratory against the ABC subtype of DLBCL and currently are in phase I trials (22). In addition, small-molecule Bcl-2 antagonists seem to promote bortezomib-mediated mitochondrial injury and lethality in DLBCL cells of both germinal-center and ABC subtypes in vitro; thus, these 2 agents may prove to be a rational therapeutic combination in the future (23).

B-cell receptor signaling. The B-cell receptor (BCR) is present on both normal and most malignant B cells, including DLBCL. Engagement of the BCR provides important survival signals, and interruption of the B-cell survival signal can lead to B-cell death (24). Elegant studies performed with siRNA to inhibit BCR expression have shown that constitutive signaling by BCR is critical for the survival and proliferation of human B-cell lymphomas (25). The
primary role of BCR signaling in these cells seems to be activation of spleen tyrosine kinase (Syk; ref. 26), which in turn leads to several downstream events that promote cell survival, including activation of Bruton tyrosine kinase (BTK), phosphatidylinositol 3 kinase (PI3K), and Akt, as shown in Fig. 1 (27).

A subset of DLBCL, which can be identified using gene expression analysis, seems particularly dependent upon BCR survival signals, even in the absence of antigen engagement, referred to as tonic signaling (28). Pharmacologic inhibition of Syk showed activity against this BCR-dependent subset of DLBCL in vitro (29). An additional rationale for inhibiting the BCR signal as a therapeutic modality comes from data indicating that the ABC type of lymphoma uses the signaling adaptor CARD11 for constitutive NF-κB pathway activity and survival (30). Moreover, approximately one fifth of ABC DLBCL cases have a mutated residue of CD79B, which results in increased surface BCR expression and attenuated Lyn kinase, a feedback inhibitor of BCR signaling. This pathway, therefore, seems to have particular importance in subsets of DLBCL.

Fostamatinib disodium is an orally available Syk inhibitor that is under development for rheumatoid arthritis (31). A phase I/II trial of this agent in a variety of lymphoma subtypes has been completed, and clinical responses were seen in over 20% of heavily pretreated, refractory DLBCL patients (32). Gene expression analyses were not performed in this trial; however, in the preclinical evaluation of Syk inhibition, only the subset DLBCL cell lines and primary tumors with absent surface IgG or IgM or with lower levels of cell-surface IgG, had ineffective BCR signaling and did not respond to this agent. Therefore, in the future, it may be possible to utilize IgM or IgG expression as a surrogate of BCR dependency in choosing patients for this therapeutic approach.

Other potential targets downstream of the BCR include BTK and PI3K. PCI-32765 is an orally available inhibitor of BTK, and a phase I study of this agent in various lymphoma subtypes was recently completed (33). The authors reported that 29% of patients with DLBCL responded to treatment with this agent at various dose levels, and phase II trials using it as a single agent and in combination with rituximab and chemotherapy are planned. Gene expression analysis was not used to evaluate patients in this trial. CAL-101, an inhibitor of PI3K, is also orally available and under development for various lymphoma subtypes (34). Although no responses in DLBCL have been reported yet, preclinical studies of combinations involving this drug seem promising, and it is likely the same subset of patients who respond to inhibition of Syk and BTK will be sensitive to this agent as well (35). On the basis of the low toxicity profile of CAL-101, which should facilitate its combination with standard chemotherapy, as well as early single-agent efficacy signals, it can be concluded that members of the PI3K pathway represent an extremely promising target in DLBCL. Combinations of these agents may yield the best results in appropriate subsets of patients defined by gene expression analyses (36).

**PKCβ gene.** As mentioned above, gene expression analysis has suggested that the PKCβ gene is almost uniquely overexpressed in fatal/refractory DLBCL compared with cured DLBCL (14). PKCβ expression was also associated with poor outcome and shortened survival in a large independent series of primary DLBCL patient samples (37). PKCβ is downstream from multiple signaling pathways, including the BCR and NF-κB (38, 39), as shown in Fig. 1. PKCβ also seems to have an effect on tumor angiogenesis through VEGF signaling, which is associated with the poor-prognosis stromal-2 gene expression signature. Therefore, inhibition of PKCβ has the potential to favorably affect multiple subgroups of high-risk patients defined by gene expression profiling. Enzastaurin is a selective inhibitor of PKCβ that has significant activity against DLBCL in vitro. A phase II study of single-agent enzastaurin in relapsed DLBCL has been completed, and the results show a subset of patients who responded and enjoyed very prolonged progression-free survival (40). Of importance, there was minimal toxicity, even in the setting of prolonged exposure to the drug. No gene expression studies were
performed as part of this study, but immunohistochemistry studies suggested that PKCβ was expressed in virtually all patients in the study. The authors suggested that future studies should incorporate evaluations of PKCβ activity and PKCβ protein expression as correlative studies in patients treated with enzastaurin. On the basis of these favorable results, a randomized phase III trial comparing enzastaurin with placebo as first remission maintenance treatment in high–clinical risk DLBCL was undertaken (ClinicalTrials.gov NCT00332202). This trial has been completed and is awaiting analysis. In addition, preliminary results from a randomized phase II study comparing R-CHOP with R-CHOP plus enzastaurin were recently presented (41). These results suggest an improvement in progression-free survival in the high–clinical risk subgroup of patients treated with enzastaurin. Preclinical studies have shown activity by combining enzastaurin with other targeted therapies, including bortezomib (42). It is hoped that adequate tissue samples will be available from these and future clinical studies to determine whether a high-risk subgroup defined by gene expression profiling, rather than the IPI, may selectively benefit from this promising agent.

Angiogenesis and the microenvironment

As previously mentioned, gene expression analyses in tumor biopsies from patients treated with rituximab and chemotherapy has revealed the importance of the microenvironment. In particular, the stromal-2 signature suggests that the presence of tumor-associated angiogenesis and macrophages portends a poor prognosis. Additional data in support of this finding include the observation that a high pretreatment serum VEGF concentration is associated with poor outcome in non-Hodgkin lymphoma, even when R-CHOP is used as initial therapy (43, 44). Bevacizumab is a recombinant, humanized, monoclonal antibody that recognizes all isoforms of VEGF. The Southwest Oncology Group conducted a trial of single-agent bevacizumab in relapsed/refractory DLBCL. Although there was limited single-agent activity, baseline urine VEGF and plasma vascular cell adhesion molecule-1 levels correlated with survival, and a small number of patients had prolonged stability of disease (45). It is not known whether these patients had the stromal-2 signature. A small phase I trial of bevacizumab with R-CHOP suggested safety and a marginal positive correlation between VEGF level and response (46). On the basis of these results, the Southwest Oncology Group subsequently performed a multicenter phase II trial of R-CHOP with bevacizumab in patients with newly diagnosed DLBCL. The study did not meet its prespecified endpoint, and there seemed to be increased toxicity (e.g., cardiac dysfunction and gastrointestinal perforation) from bevacizumab compared with historical controls of patients treated with R-CHOP alone (47). A subsequent phase III trial comparing R-CHOP with R-CHOP plus bevacizumab was also stopped prematurely because of toxicity concerns (ClinicalTrials.gov NCT00486759). Gene expression profiling was not incorporated in either of these studies.

A more promising agent that may favorably influence the microenvironment and target angiogenesis in DLBCL is lenalidomide. This immunomodulatory agent has pleiotropic effects, including antiangiogenic effects, in the treatment of lymphoma (48). A phase II trial of lenalidomide monotherapy in relapsed/refractory aggressive lymphomas (more than half of the patients had DLBCL) showed significant clinical activity in a subset of patients, with an overall response rate of 35% and minimal nonhematological toxicities (49). No gene expression profiling was performed in this trial; however, a subsequent study retrospectively evaluated clinical outcomes of patients with germinal-center B-cell-like versus nongerminal-center B-cell-like (52% response rate) versus germinal-center B-cell-like (9% response rate) patients. Numerous trials are exploring lenalidomide in combination with R-CHOP therapy as an upfront treatment and as a single agent or with rituximab as maintenance treatment for patients with newly diagnosed DLBCL. For example, a phase I/II study has determined that lenalidomide can be safely given at a dose of 25 mg daily on days 1 to 10 of R-CHOP therapy, with high complete response rates observed (51). It is hoped that these trials will incorporate analysis of gene expression–defined subgroups of patients to determine the impact of this therapy on subsets of DLBCL.

Conclusions

Although DLBCL is highly curable, a substantial number of patients still succumb to this disease. Recent biologic insights from gene expression studies have confirmed that biologic heterogeneity explains this highly variable clinical

Table 1. Novel rationally targeted agents for DLBCL derived from gene expression analyses

<table>
<thead>
<tr>
<th>Drug</th>
<th>Gene expression subgroup (reference)</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bortezomib</td>
<td>ABC (9)</td>
<td>NF-κB</td>
</tr>
<tr>
<td>Enzastaurin</td>
<td>Fatal/refractory (14)</td>
<td>PKCβ</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>Stromal 2 (13)</td>
<td>VEGF; angiogenesis</td>
</tr>
<tr>
<td>Lenalidomide</td>
<td>Stromal 2 (13)</td>
<td>Angiogenesis</td>
</tr>
<tr>
<td></td>
<td>ABC (9)</td>
<td>Pleiotropic targets</td>
</tr>
<tr>
<td>Fostamatinib</td>
<td>BCR-dependent (28)</td>
<td>Syk; BCR signaling</td>
</tr>
<tr>
<td></td>
<td>ABC (9)</td>
<td>NF-κB through CARD11</td>
</tr>
<tr>
<td>PCI-32765</td>
<td>BCR-dependent (28)</td>
<td>BTK; BCR signaling</td>
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outcome. Of more importance, using this promising technology, investigators will be able to identify subgroups of patients who may benefit from specific novel, rationally targeted therapies (Table 1). It is imperative that future clinical trials involving this disease use this technology in correlative studies to define these subgroups of patients and the impact of novel treatment approaches. Although immunohistochemistry algorithms that have some correlation to gene expression profiling are available, all of these algorithms are imperfect at present, and in the setting of discovery, it is necessary to use gene expression profiling to accurately determine DLBCL subtypes (52). Evolving technology will soon allow such assays to be performed on routinely obtained paraffin-embedded tissues at a reasonable cost (53, 54). Particularly in an age of novel therapies that show intriguing signals of activity, I expect we will use gene expression profiling, or an accurate surrogate, to define the best therapeutic approach for individual patients in the clinic in the near future.

Disclosure of Potential Conflicts of Interest

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