Aggressive Non-Hodgkin Lymphoma

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Diffuse Large B-Cell Lymphoma: Risk Stratification and Management of Relapsed Disease

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The clinical factors described by the International Prognostic Index (IPI) provide a model for risk stratification in diffuse large B-cell lymphomas (DLBCLs). However, there is variability in outcome within IPI risk groups, indicating the biological and clinical heterogeneity of these diseases. Studies of gene expression profiling (GEP) in DLBCL are uncovering biological heterogeneity with prognostic significance. Various gene expression signatures with predictive value independent of the IPI are now recognized. Immunophenotypic features of DLBCL have also been shown to have prognostic value. The use of fluorodeoxyglucose–positron emission tomography (FDG-PET) scanning may provide additional predictive information when used at diagnosis or soon after initiation of treatment. Future prognostic models in DLBCL are likely to incorporate functional imaging, immunophenotype and GEPs as well as clinical data in risk stratification and choice of treatment.

Treatment of relapsed DLBCL remains a major problem. High-dose therapy (HDT) and stem cell transplantation (SCT) has been shown to produce superior overall survival (OS) compared with conventional dose salvage therapy in patients with relapsed, chemosensitive DLBCL. However, only 20% to 30% of patients are cured by this approach, and the effectiveness of HDT and SCT in patients treated with rituximab-based combinations as first-line therapy is unknown. Although new transplant techniques including non-myeloablative allogeneic SCT are being investigated, their role is unclear. New treatment strategies are needed for these patients. The use of molecular techniques such as GEP is identifying many potential new therapeutic targets in DLBCL including histone deacetylase, HLA-DR, bcl-2, bcl-6, mTOR and TRAIL.

Risk Stratification in Diffuse Large B-cell Lymphoma

Clinical prognostic factors in DLBCL

The clinical prognostic factors described in the International Prognostic Index (IPI) (**Table 1**) have been used in risk stratification for patients with diffuse large B-cell lymphoma (DLBCL) for more than a decade.[1] The age-adjusted IPI (aaIPI) has also been used extensively, particularly in studies adopting intensive treatment approaches such as high-dose therapy (HDT) and stem cell transplantation (SCT). A stage-modified IPI has also been proposed for patients with limited-stage disease. Although the IPI has proved valuable for stratification of patients in clinical trials, there is variability in outcome within the individual risk groups. Additionally, there is little evidence that treatments 'tailored' to specific IPI risk groups have improved outcome. The failure of first remission HDT and SCT to improve survival in poor risk disease is one such example.[2] The failure of these clinical risk factors to reliably predict response to specific therapies in part reflects the inherent biological heterogeneity of DLBCL and highlights the need for more precise, patient-specific and biologically based risk factors. The use of appropriately timed functional imaging may prove valuable in this respect.

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Table 1. Five-year relapse-free and overall survival rates according to the International Prognostic Index (IPI) and age-adjusted IPI (adapted from reference [1]).

Risk Group	Number of Adverse Factors*	5-Year Relapse-Free Survival (%)	5-Year Overall Survival (%)
International Prognostic Index			
Low	0 or 1	70	73
Low-intermediate	2	50	51
High-intermediate	3	49	43
High	4 or 5	40	26
Age-adjusted International Prognostic Index			
Low	0	86	83
Low-intermediate	1	66	69
High-intermediate	2	53	46
High	3	58	32

* Adverse risk factors for IPI are: stage III or IV disease, age > 60 years, elevated lactate dehydrogenase (LDH), ECOG performance status ≥ 2, ≥ 2 extranodal sites
# Adverse risk factors for age-adjusted IPI are: stage III or IV disease, elevated LDH, ECOG performance status ≥ 2

Potential prognostic value of functional imaging using FDG-PET after or during therapy for DLBCL

The use of fluorodeoxyglucose–positron emission tomography (FDG-PET) to predict outcome after completion of first-line therapy for DLBCL has been investigated by several groups. Spaepen et al reported results for 96 patients with aggressive non-Hodgkin lymphoma (NHL) evaluated by PET scanning at the completion of therapy.[3] Of 67 patients with negative PET scans, 80% remained in clinical complete response (CR) with a mean follow-up of 730 days. The median time to relapse for the remaining 20% was 316 days. Of 29 patients with positive PET scans, all relapsed with a median disease-free interval of only 105 days. In a similar study Mikhaeel at al have reported a positive- (PPV) and negative-predictive value (NPV) of 100% and 82%, respectively, for PET scanning used at the completion of therapy.[4]

Although these data suggest that post-treatment functional imaging has predictive value, its clinical utility is less clear since there is no evidence that additional or intensified treatment at this point can improve outcome. Some groups have therefore investigated whether functional imaging during therapy can predict response and survival, allowing early change of therapy in patients with a predicted poor outcome. Early functional imaging might be a more accurate predictor of outcome since it may uncover persistent metabolic activity in resistant clones, which respond more slowly to chemotherapy than sensitive ones. Some small published series have shown that persistent FDG uptake after 2 to 4 cycles of chemotherapy predicts for subsequent progression-free survival (PFS) in NHL. In these studies, patients who are PET negative have a 0% to 16% probability of relapse, compared with 87% to 100% of PET positive patients.

More recently, Kostakoglu et al have investigated the use of FDG uptake after the first cycle of chemotherapy in patients with NHL and Hodgkin lymphoma and compared this with PET scans performed at the completion of chemotherapy.[5] At both time points, a positive FDG-PET was associated with shorter PFS compared with a negative PET. The false negative rate was higher at the completion of therapy compared with after one cycle (35% vs 15%, respectively). The PPV was higher after one cycle compared with after completion of therapy (90% vs 83%), as was the NPV (85% vs 65%). In a follow-up study, this group has reported similar results when using a more refined PET method. Of 10 patients with persistent FDG uptake after one cycle of therapy, 9 relapsed with a median PFS of only 2 months. Of 17 patients with negative uptake after one cycle, none has relapsed ($P = < 0.0001$). Early PET had an estimated sensitivity and NPV of 100%, a specificity of 94%, a PPV of 90% and an overall accuracy of 96%.

These results suggest a possible role for early functional imaging as a predictive factor in DLBCL. New prospective trials in DLBCL should now incorporate functional imaging as an endpoint to evaluate its utility in more precisely defined and uniformly treated patient populations.

Despite these encouraging preliminary data, the clinical utility of functional imaging remains unclear. Although it may reliably detect disease resistance early in the course of therapy, there is no evidence to suggest that an early change of therapy in poorly responding patients improves survival. Previous studies of 'early' HDT and SCT in 'slow responders' have failed to show a survival advantage for transplantation.[6] Using dynamic dosing regimens such as dose-adjusted EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, rituximab), early FDG-PET appears less predictive (personal communication, WH Wilson, MD, PhD), suggesting that it's utility may be regimen-dependent. There is continued uncertainty regarding the interpretation of PET scans in NHL. The criteria for 'positive' versus 'negative' scans based on standard uptake values (SUVs) have been inconsistent in different studies. Additionally, availability of functional imaging, expense and third party reimbursement remain problematic in some centers.

Molecular and immunohistochemical prognostic factors in DLBCL

Recent studies using gene expression profiling (GEP) in DLBCL have identified patterns of gene expression, as well as individual genes that appear to have important prognostic significance, related to underlying tumor biology. These observations have been used to develop risk groups based on small numbers of genes or on immunohistochemical stains. In addition, the expression of many individual proteins detected by immunohistochemistry has been shown to have prognostic significance (**Table 2**).

Analysis of the expression of thousands of genes in DLBCL using cDNA and oligonucleotide microarrays has

Table 2. Examples of individual immunophenotypic features with reported prognostic significance in diffuse large B-cell lymphomas (DLBCL).

Immunophenotype	Impact on Prognosis
bcl-2 expression	adverse
bcl-6 expression	favorable
HLA class II expression	favorable
Mutated p53	adverse
High proliferative rate defined by Ki-67	adverse
Tumor-infiltrating lymphocytes	favorable
CD5 expression	adverse
MUM-1 positive	adverse
Cyclin D2 positive	adverse

been performed by several groups and has been shown to correlate with clinical outcome. In studies using the lymphochip cDNA microarray in 44 patients with DLBCL, Alizadeh et al demonstrated that OS was higher in patients who had high levels of expression of genes characteristic of normal germinal center B-cells (GCB), compared with those with an activated B-cell-like (ABC) expression profile.[7] Using oligonucleotide microarrays, Shipp et al described a 13 gene predictive model in 77 patients with DLBCL uniformly treated with CHOP-based chemotherapy.[8] A subsequent study by Rosenwald et al described a 17-gene predictive model, based not only on the cell of origin of the tumor, but also on the host immune response and the proliferative rate of the tumor[9] (**Figure 1**; see Color Figures, page 547). Both of these predictive models were independent of the IPI. However, the microarrays used in these studies were different, as were the techniques used for developing predictive models, such that there was no overlap between the genes identified in these two studies.

Although microarray techniques have advanced understanding of the biology of lymphoid malignancy and have allowed the prognostic groups identified by the IPI to be further refined, their clinical utility is limited by the requirement for fresh or optimally cryopreserved samples, and by high costs. A simplified 6-gene predictive model has subsequently be developed by Lossos et al using quantitative RT-PCR in 66 patients with DLBCL treated with CHOP-based chemotherapy.[10] In this model 3 genes (*LMO2, BCL6* and *FN1*) were correlated with prolonged survival and 3 (*BCL2, CCND2* and *SCYA3*) were correlated with shorter survival. Tissue micorarray (TMA) technology has recently provided further prognostic information in DLBCL. This technique allows simultaneous, high throughput immunohistochemical analysis of protein expression in multiple specimens on a single slide, allowing the identification of protein surrogates for genes identified as being dysregulated in GEPs. These can be performed on routine formalin-fixed and paraffin-embedded clinical samples.[11]

Hans et al have reported results from a TMA containing 152 DLBCL cases, of which 142 had been previously evaluated using cDNA microarrays.[12] Sections were stained for CD10, bcl-6, MUM1, FOXP1, cyclin D2 and bcl-2. Expression of bcl-6 and CD10 was associated with a favorable outcome compared with MUM1 or cyclin D2 expression. Cases were classified as GCB-like or ABC-like based on expression of bcl-6, CD10 and MUM1 according to the criteria shown in **Table 3**. The 5-year OS for the GCB group was 76% compared with 34% for the non-GCB group. The OS rates reported were very similar to those reported for the same cases classified using GEPs. In multivariate analysis high IPI score and non-GCB phenotype were independent predictors of OS. Zinzani et al have confirmed the adverse prognostic significance of the non-GCB phenotype in 68 patients with nodal DLBCL treated with the MACOP-B (methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin) regimen.[13] Although there was not complete concordance with the Hans study in terms of the antigens defining GCB or non-GCB phenotype the OS for these subgroups was comparable in both studies, confirming the potential for TMAs to determine possible immunohistochemical surrogates for GEP studies.

Table 3. Tissue microarray criteria for germinal center B-cells (GCB) versus non-GCB derivation of diffuse large B-cell lymphomas (DLBCL). (Adapted from reference [12].)

	CD10	Bcl6	MUM1	5-year Event-Free Survival (%)	5-year Overall Survival (%)
GCB	+ (–)	+ (–)	–	63	76
Non-GCB	–	–	+	36	34

Summary

Emerging results from GEPs and TMAs demonstrate the power of these techniques as prognostic tools in DLBCL. However, many uncertainties exist that will need to be resolved before these techniques gain widespread use in risk stratification. One of the largest potential limitations of the GEP and TMA studies to date is that these are based on patient samples obtained before the widespread use of rituximab in the treatment of DLBCL. Several studies have demonstrated that prognostic factors in DLBCL can be modified by therapy. The adverse prognostic significance of bcl-2 expression in DLBCL can be overcome by the addition of rituximab to chemotherapy, using CHOP[14] and dose-adjusted EPOCH.[15] This regimen has also been reported to modify the adverse prognostic significance of ABC phenotype as determined by GEP.[16] In a recent study from the Eastern Co-operative Oncology Group (ECOG) Winter et al have shown that the adverse prognostic effect of lack of bcl-6 expression in DLBCL for patients treated with CHOP is abolished when rituximab is added to chemotherapy.[17] New studies are therefore in progress based on material from patients with DLBCL treated uniformly with CHOP-rituximab.

The availability of snap frozen material for GEP studies remains a limitation likely to prevent the widespread application of GEPs in routine clinical practice. In this respect TMAs are likely to represent a more accessible method for detecting protein surrogates for genes detected by microarray analysis. Based on emerging data from TMAs correlated with GEPs, it is likely that the use of a relatively small number of immunohistochemical stains may provide adequate information for risk stratification.

These factors will require prospective evaluation in the context of clinical trials before they become part of routine clinical practice. Until then, the IPI should still be considered the standard system for risk stratification in this disease, particularly since its value as a prognostic model appears to be maintained for patients in the relapsed setting as well as those who are previously untreated.

Management of Relapsed DLBCL

The role of high-dose therapy and autologous stem cell transplantation

The PARMA trial established HDT and autologous SCT (ASCT) as standard therapy for relapsed DLBCL that is still sensitive to conventional dose salvage chemotherapy.[18] However, the results of this trial and their relevance to present management of DLBCL should be re-evaluated. Inclusion criteria for this study included age < 60 years, a CR to previous therapy, and absence of bone marrow or central nervous system involvement. Additionally, multiple histologic subtypes of aggressive NHL were included, not all of which were DLBCL. Of the initial 215 patients entered on study, only 109 were randomized, with most of the remaining patients excluded because of failure to respond to salvage therapy with DHAP (dexamethasone, cytarabine, cisplatin). All subsequent survival analyses were restricted to randomized patients only, with no intent-to-treat analyses performed. The importance of intent-to-treat analyses in this context is illustrated by the data summarized in **Table 4**, showing response rates to second-line chemotherapy for 'transplant eligible' patients treated with second-line regimens, and the event-free survival (EFS) following ASCT. Response rates to second-line therapy should not be regarded as a suitable endpoint in studies designed to assess the effectiveness of SCT. The original publication of the PARMA study does not provide statistical power calculations for the trial. Since the trial was stopped prematurely because of low accrual it is unclear that it was adequately powered to reliably detect significant differences in OS or DFS.

The relevance of this study in the present context is unclear. Improvements in supportive care, including the use of peripheral blood progenitor cells (PBPCs), have extended the use of SCT approaches to older patients, typically up to 70 or 75 years old. Most centers will offer transplantation to patients who achieved a partial as well as complete response to their initial chemotherapy, and the use of PBPCs has diminished the requirement for an uninvolved bone marrow at the time of harvesting. Therefore, HDT and ASCT is now being used in a different, less defined patient population from those included in the PARMA trial. There are no trials that demonstrate a survival advantage for SCT in this extended patient group.

The emergence of new, more effective first-line therapies for aggressive NHL also raises questions about the most effective salvage strategies. The addition of rituximab to CHOP and other first-line regimens for aggressive NHL has been shown to improve response and survival rates in patients with DLBCL.[21-23] Similar improvements in response and survival rates have been reported with dose-dense regimens, including CHOP-14.[24] It is unclear whether HDT and ASCT will prove to be an effective salvage strategy for patients who relapse after these regimens. For example, a recent report of dose-adjusted EPOCH-R as primary therapy in DLBCL has shown a 2-year PFS of 83%, with an almost identical OS indicating the high activity of this regimen, and the inability to salvage relapsing patients with high dose strategies (WH Wilson, MD, PhD, personal communication). A retrospective comparison of outcomes following ASCT for patients receiving first-line CHOP or CHOP-rituximab has been reported.[25] No difference in EFS or OS was observed according to initial treatment in this study, although only 103 patients were analyzed. The potential benefit of HDT and ASCT in patients with relapsed DLBCL is, therefore, once again unclear and requires re-evaluation.

Recent studies in stem cell transplantation

The emphasis of recent clinical research in this area has been twofold. Many investigators have attempted to develop more effective second-line regimens in order to maximize the number of patients in CR prior to SCT. Other investigators have attempted to identify prognostic factors

Table 4. Results of selected studies of pretransplant salvage therapy for aggressive NHL. Response to second-line therapy and outcome according to intent-to-treat analysis.

Regimen (reference)	*n*	Response Rate to Second-Line Chemotherapy	Number Transplanted (%)	Event-Free Survival
Mini-BEAM (19)	102	43%	38 (37%)	22% at 3 years
ICE (20)	163	66%	96 (59%)	35% at 3 years
DHAP (18, 25)	215	58%	55 (26%)*	24% at 3 years
R-ICE (26)	36	78%	25 (69%)	54% at 2 years*

* randomized trial—remaining patients randomized to continued DHAP therapy
Abbreviations: mini-BEAM, carmustine, etoposide, cytarabine, melphalan; ICE, ifosfamide, carboplatin, etoposide; DHAP, dexamethasone, cytarabine, cisplatin; R-ICE, rituximab + ICE

that will limit the use of SCT approaches to those patients most likely to benefit, allowing remaining patients to be offered alternative, often experimental therapies.

Second-line regimens

Commonly used second-line regimens used prior to ASCT for relapsed DLBCL include DHAP, ESHAP (etoposide, methylprednisone, cisplatin), mini-BEAM (carmustine, etoposide, cytarabine, melphalan) and ICE (ifosfamide, carboplatin, etoposide). These regimens produce CR rates of 25% to 35%. The addition of rituximab to ICE (R-ICE) increases the CR rate to 53% compared with 27% for patients treated with ICE in a previous study.[26] The PFS after transplantation was noted to be slightly longer in patients treated with R-ICE compared with historical patients receiving ICE (54% vs 43% at 2 years) although this did not reach statistical significance. The overall response rates did not differ between ICE and R-ICE and OS was also the same for both groups. None of the patients in these two series had received rituximab as a component of initial therapy. The effectiveness of adding rituximab to second-line therapy for patient previously treated with rituximab remains unclear. Current randomized trials evaluating second-line regimens have mostly included rituximab, even for patients who have previously been treated with this agent. Such studies include the NCI Canada LY 12 study in which patients with relapsed DLBCL will be randomized to receive rituximab GDP (gemcitabine, dexamethasone, cisplatin) or rituximab-DHAP as a second line regimen prior to SCT, and the CORAL study in Europe, in which a similar patient group are randomized between rituximab DHAP and R-ICE. At present, the optimal second-line regimen is unclear, and the benefit of the inclusion of rituximab in second-line therapy for patients previously exposed to this agent is also unclear.

Prognostic factors for patients with relapsed DLBCL

Early studies of HDT and SCT demonstrated the importance of chemosensitivity as a predictive factor for outcome after transplantation. Other favorable factors identified in many studies include initial remission duration of greater than 12 months and the absence of bulky disease at the time of SCT. The predictive value of the age-adjusted IPI has been demonstrated in a follow-up of patients treated on the PARMA trial.[27] The aaIPI at the time of relapse was available for 204 of the original 216 patients and was highly predictive of response to DHAP with an OR rate of 77% for patients with an aaIPI score of 0, falling to 42% for those with a score of 3. The aaIPI score was also predictive of OS for the entire cohort of patients. For randomized patients, the aaIPI was predictive of OS for those receiving DHAP, but the prognostic significance of the aaIPI was lost in those undergoing ASCT. Of note, in subset analysis, there was no difference in PFS or OS according to randomized arm for patients with aaIPI score 0, but a highly significant difference for those with aaIPI scores of 1 to 3. In a similar study, Hamlin et al reported outcome for a homogeneous population of 150 patients with relapsed or refractory DLBCL receiving ICE chemotherapy followed by HDT and ASCT.[28] They confirmed the predictive value of the aaIPI, showing that patients with a score of 2 or 3 at the time of relapse, when analyzed by intent to treat, had 4 year PFS and OS rates of only 16% and 18%, respectively, compared with 70% and 74% for patients with an aaIPI score of 0.

In the absence of prospective randomized trials, it is difficult to conclude that HDT and SCT offers a definite survival advantage to low-risk patients, although both these studies show poor results in patients with high aaIPI scores, suggesting that these patients should be offered alternative strategies.

The potential use of TMAs to predict outcome in relapsed patients has been investigated in a single study in which tissue for TMAs was available for 88 of 186 patients with relapsed or refractory DLBCL uniformly treated with ICE chemotherapy prior to SCT.[29] The TMA included immunohistochemical markers distinguishing between GCB and non-GCB origin. The aaIPI at relapse was shown to reliably predict OS after SCT. No difference in survival after SCT was observed for patients with GCB or non-GCB phenotype according to TMAs. At present, there are very few published data from studies of GEPs at the time of relapse, partly due to a shortage of appropriately frozen tissue specimens. The predictive value of GEP signatures at relapse is under investigation.

At present HDT and ASCT remains the standard of care for patients with relapsed DLBCL following CHOP or similar chemotherapy provided the disease is sensitive to second-line chemotherapy. Although there are few data to confirm the benefit of this approach in patients relapsing after rituximab-based therapy, it is likely to remain the standard. However, for those patients with high aaIPI scores at the time of relapse, and for those with chemorefractory disease at the time of relapse, other approaches are required. Therapies under investigation for these patients, as well as those whose disease relapses after HDT and ASCT include allogeneic stem cell transplantation, radiolabeled monoclonal antibodies and various novel targeted therapies.

Allogeneic SCT in relapsed DLBCL

Current data on the role of allogeneic SCT and reduced intensity allogeneic SCT for patients with poor-risk DLBCL are very limited. Comparative studies of autologous and allogeneic SCT for aggressive NHLs have failed to show a survival advantage for allogeneic SCT, despite the observation of lower relapse rates in patients undergoing allogeneic SCT compared with those undergoing ASCT.[30] This lower relapse rate has usually been offset by the higher regimen-related mortality associated with allogeneic transplantation. At present, there is no clear evidence of a clinically significant graft-versus-lymphoma effect in DLBCL, and patients should only receive this therapy in the con-

text of a research study.

Although allogeneic SCT is now being used increasingly in patients with DLBCL who relapse after ASCT, there are few data at present to confirm its effectiveness. A recent study analyzed outcomes for 114 patients with various histologic subtypes of NHL who relapsed after ASCT and received myeloablative allogeneic SCT.[31] The authors reported a 52% disease progression at 3 years, with a regimen-related mortality of 22%. Three-year OS and PFS was 33% and 25%, but with longer follow-up to 5 years, these figures fell to 24% and 5%. No analysis was performed with respect to NHL subtype, and the outcome for patients with aggressive NHL is therefore not available from this study. However, the failure to demonstrate curative potential in this highly selected group suggests that this approach should also be restricted to patients in prospective clinical trials.

Radiolabeled monoclonal antibodies

Both ^{131}I tositumomab (Bexxar) and ^{90}Y-ibritumomab tiuxetan (Zevalin) have been shown to be active agents for patients with indolent and transformed CD20-positive B-cell lymphomas, but only limited experience exists with the use of these agents in DLBCL. A multicenter phase II study in Europe has assessed the role of ^{90}Y ibritumomab tiuxetan in 104 patients with relapsed or refractory DLBCL who were not eligible for HDT and ASCT, mainly because of advanced age.[32] The overall response rate was 44% and was noted to be higher in patients who had not had prior therapy with rituximab, compared with those who had been previously treated with rituximab and chemotherapy as their primary treatment. Median PFS was around 6 months for patients who were rituximab naïve, compared with only about 2 months for those previously treated with rituximab. Recent studies have explored the potential including these agents in high-dose regimens used with ASCT. Most studies to date have included patients with various histologic subtypes of NHL and have shown that both ^{131}I tositumomab and ^{90}Y ibritumomab tiuxetan can be combined with standard high-dose chemotherapy regimens without apparent additional toxicity or prolongation of engraftment kinetics. A recent study from Vose et al has explored the use of ^{131}I tositumomab plus BEAM in 23 patients with refractory or multiply relapsed B-cell NHL, most of whom had DLBCL. They reported an overall response rate of 65%.[33] At a median follow up of 38 months, the PFS and OS rates were 39% and 55% respectively. This approach will be compared with rituximab and BEAM for patients with relapsed or refractory DLBCL in the recently opened BMT CTN 0401 trial.

New therapeutic targets in DLBCL

Histone deacetylase (HDAC) inhibitors are among several classes of drug under active investigation in relapsed DLBCL. The degree of acetylation of histones in the nucleosome is a major determinant of the regulation of many genes. Deacetylation of histones results in condensed chromatin structure and repression of gene transcription. Inhibitors of HDAC include suberoylanilide hydroxamic acid (SAHA), which has been shown to induce differentiation and/or apoptosis in various tumor cell lines and has demonstrated clinical activity in heavily pretreated patients with NHL. These agents probably influence multiple pathways. Preclinical data suggest that these agents may be capable of upregulating expression of MHC class II antigens in DLBCL, potentially rendering these tumors more susceptible to immune surveillance. Previous studies have shown that lack of MHC class II expression is an adverse prognostic factor in DLBCL.[34] They may also exert some of their activity in DLBCL through bcl-6. This gene is thought to be important in the pathogenesis of DLBCL and has been shown to be anti-apoptotic in tumor cells, possibly through inhibition of transcription of the p21WAF-1 gene. In the presence of HDAC inhibitors, p21WAF1 transcription results in growth inhibition, apoptosis and differentiation. Various HDAC inhibitors are now in early phase clinical trials in DLBCL and results are awaited.

Mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase involved in the regulation of cell growth in response to multiple nutrients. It has an important role in many cellular functions. It is phosphorylated via the PI3/AKT pathway and plays a role in the regulation of apoptosis. It also appears to be involved in the regulation of angiogenesis through effects on production of VEGF. Clinical studies of various agents which target mTOR are now underway, including CCI-779.

Targeting of angiogenesis has also been investigated in the Southwest Oncology Group S0108 study of the anti-VEGF monoclonal antibody, bevacizumab, in patients with relapsed DLBCL.[35] A clinical non-progression rate of 25% was observed for the 51 patients in this study, with a median time to progression of 5 months (range 4 to 18 months). Prognostic factors defined by molecular techniques such as GEPs are likely to yield future therapeutic targets. For example, protein kinase C β (PKC-β) has been identified as a predictor of poor response to chemotherapy in DLBCL.[8] This serine/threonine kinase modulates BCR signaling and activation of the NF-κB survival pathway in B-cells. This finding has recently been confirmed in a tissue microarray study of 200 cases of DLBCL,[36] and the potential for PKC-β as a therapeutic target has been validated by recent in vitro studies.[37] Clinical trials of a PKC-β inhibitor in relapsed/refractory DLBCL are now in progress.

Summary

Emerging data from GEPs and TMAs suggest that these techniques may provide more accurate definition of risk groups in DLBCL than is possible with the IPI. These factors will need to be defined in patients receiving rituximab-based combination chemotherapy. Such studies are in progress. Until results are available, the IPI should remain the major tool for risk stratification for prospective clinical

trials. For those high-risk patients with refractory or relapsing disease after first line therapy, prognosis remains poor and new treatment strategies are required. Although HDT and ASCT remains the standard approach, only a minority of patients appear to benefit, and the effectiveness of this approach requires re-evaluation in the context of new first line therapies. Prospective studies are required to evaluate the effect of other transplant techniques. Identification of new targets for therapy using GEPs is defining new agents which may prove highly effective in this disease. Prospective evaluation of these agents in studies which include correlative biologic endpoints is ongoing.

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