Non-Hodgkin Lymphoma

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Optimal Use of Prognostic Factors in Non-Hodgkin Lymphoma

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The management of non-Hodgkin lymphoma is complicated by wide heterogeneity within recognized subtypes. Patients with supposedly similar diagnoses can have remarkably varied clinical presentations, molecular profiles and clinical outcomes. Reliable prognostic markers could allow the identification of patient subsets that may benefit from alternate approaches. Historically, a large number of clinical and molecular prognostic factors have been elucidated. However, the recent introduction of new therapies such as monoclonal antibodies has revolutionized treatment practices and greatly improved outcomes. This has called into question the value of previously recognized prognostic factors that need to be revalidated in the era of immunochemotherapy. It would appear that the commonly used clinical indices (IPI and FLIPI) retain predictive capacity, although they may have limited ability to identify a very poor outcome group. Currently there are no molecular markers that have been revalidated and shown to retain significance in the setting of current treatment practices for diffuse large B-cell lymphoma or follicular lymphoma. The biologic insights provided by molecular studies should allow for more targeted therapies to be developed, which will increase treatment choice and the possibility of tailored therapy in the future. It is imperative that future steps forward be made in the context of well-designed clinical trials with prospective correlative studies of clinical and biologic markers. This will allow us to continuously assess outcome predictors in the context of treatment change and to rationally design tailored treatment algorithms.

The management of patients with non-Hodgkin lymphoma (NHL) is a complex endeavor. More than 30 different subtypes of NHL are recognized within the WHO classification and, in addition, marked heterogeneity exists within subtypes.[1] Ongoing advances in molecular biology will likely allow refinement of the classification with recognition of newer entities and homogenization of subtypes. However, at present physicians are faced with the challenge of treating patients with supposedly similar diagnoses, but markedly disparate presentations, molecular profiles and clinical outcomes.

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Efforts toward the elucidation of prognostic markers have had two primary goals. The first goal is to provide an accurate prediction of survival to facilitate doctor-patient discussions and foster a realistic expectation of outcome, to guide choice of initial treatment, and to allow appropriate stratification on clinical trials to ensure uniform reporting of outcomes and cross-trial comparisons. The second goal is to identify unique biologic subsets of patients that may allow for the rational identification of therapeutic targets that can be exploited in a tailored therapy approach. Although many individual prognostic factors have been reported and multiple prognostic models proposed, in order to be clinically useful several conditions must be met (**Table 1**).

The prognostic information required must be obtainable from samples that are readily accessible and the technology used must be uniformly available. Although this is usually achievable for clinical factors it can limit the utility of biologic markers. The requirement for fresh or snap-frozen tissue and the complexity of the procedure have

Table 1. Criteria for clinical utility of prognostic markers or models.

- Test sample is readily accessible
- Uniformly available technology
- Standardized methodology
- Reproducible
- Independent from other recognized prognostic markers
- Predicts a wide range of clinical outcomes or characterizes a molecular profile that would benefit from a specific targeted therapy
- Validated prospectively in an independent patient population treated with current standard of care
- Alternate treatments available

constrained the use of techniques such as gene expression profiling or PCR-based approaches reliant on RNA samples, whereas techniques based on immunohistochemistry have wider applicability for routine patient care. Standardization of methodology with accepted criteria defining cutoff values that are reproducible is essential for a test to be universally applied. The marker should provide prognostic information that is independent from other recognized factors. The range of outcomes predicted must be sufficiently wide to justify modification of treatment or the marker should characterize a molecular profile that would benefit from a specific targeted therapy. The relevance of the proposed prognostic marker or model must be prospectively validated in an independent population of patients treated with the current standard of care. Finally, alternative treatment approaches must be available.

It is important to recognize that risk assessment is a moving target. Introduction of new therapies can significantly alter the relevance of previously recognized prognostic factors by virtue of their mechanism of action. Therefore, clinical progress necessitates reevaluation of existing prognostic markers and models to ensure their continued applicability. The current review will focus on the two most common subtypes of NHL, diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL), and explore the utility of recognized prognostic factors in the current climate of treatment change.

Diffuse Large B-Cell Lymphoma

DLBCL is the most common subtype of NHL, accounting for approximately 30% of all newly diagnosed cases and more than 80% of aggressive lymphomas.[1] DLBCL represents a heterogeneous entity. Multiple morphologic variants are recognized within the WHO classification, a variety of molecular and genetic abnormalities are variably present, and patients exhibit a wide range of clinical presentations and outcomes. The CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) chemotherapy regimen has been the mainstay of therapy for several decades, since attempts to improve outcomes with more intensive chemotherapy failed to show additional benefit.

The era of monoclonal antibodies has transformed treatment practices for aggressive lymphoma. The Groupe d'Etude de Lymphome d'Adultes (GELA) reported the first randomized controlled trial demonstrating the benefit of adding rituximab, a chimeric IgG1 monoclonal antibody targeting CD20, to CHOP chemotherapy (R-CHOP) for the treatment of elderly patients (age ≥ 60 years) with newly diagnosed DLBCL. A 5-year update of this trial demonstrates that the benefit seen with the addition of rituximab has been maintained over time, indicating an improvement in the cure rate for this patient population (5-y OS 58% vs 45%, $P = 0.0073$).[2]

Three additional randomized controlled trials have confirmed this benefit in select groups of patients with DLBCL. The US Intergroup trial[3] and the RICOVER-60 trial[4] evaluated the use of rituximab and chemotherapy in elderly patients, while the MInT trial[5] investigated its use in young patients (age ≤ 60 years) with a good prognostic profile. Results of a population-based study further demonstrated the value of the addition of rituximab to chemotherapy in an unselected population of patients with DLBCL in the province of British Columbia.[6]

Although the adoption of R-CHOP as the new standard of care has led to improved outcomes for this curable lymphoma, patients who fail first-line therapy continue to pose a difficult challenge. Despite our improved understanding of the diversity of DLBCL and increased number of treatment options, most clinicians continue to treat this entity with a single management strategy. The question of whether reliable prognostic markers exist and should be used to guide treatment choice deserves consideration.

Clinical prognostic factors in DLBCL

The International Prognostic Index (IPI) has become the primary clinical tool used to predict outcome for patients with aggressive NHL.[7] Based on the number of negative prognostic features present at the time of diagnosis (age > 60 years, stage III/IV disease, elevated lactate dehydrogenase (LDH) level, Eastern Cooperative Oncology Group (ECOG) performance status ≥ 2, more than one extranodal site of disease), four discrete outcome groups were identified with a 5-year overall survival ranging from 26% to 73%.

The clinical trials testing R-CHOP have yielded limited information regarding the utility of clinical prognostic factors in patients with DLBCL treated with immunochemotherapy. The prediction of outcome following R-CHOP is complicated by the fact that the benefit of rituximab may not be equally translated across all patient subgroups. In the GELA trial, when patients were stratified according to the age-adjusted IPI (aaIPI), low-risk patients seemed to experience a greater benefit from the addition of rituximab than high-risk patients.[2]

A multivariate analysis of base-line prognostic factors in the GELA trial found an elevated beta$_2$-microglobulin level (more than 3 mg/L) and the presence of more than one extranodal site of disease to be negative prognostic factors

in terms of overall survival.[2] The MInT trial found bulky disease and the presence of at least one aaIPI factor to be predictive of event-free survival (EFS) on multivariate analysis.[5] Using these two predictors, patients could be separated into a favorable group (non-bulky, aaIPI = 0) that had a significantly better 3-year EFS than the unfavorable group (bulky, aaIPI = 1, or both) (89% vs 76%, $P = 0.016$), but the difference in 3-year overall survival was not significant (98% vs 91%, $P = 0.08$).

The clinical information derived from the randomized controlled trials does not distinguish a group with sufficiently poor outcome to warrant treatment stratification. Since these trials were confined to select subpopulations (either elderly or young patients with a favorable prognosis), the utility of the IPI in the era of immunochemotherapy could not be determined. To assess the applicability of the IPI, we performed a retrospective analysis of an unselected population of patients with newly diagnosed DLBCL treated with R-CHOP in the province of British Columbia.[8] Using the Lymphoid Cancer Database of the BC Cancer Agency, we identified 365 patients with a median age of 61 years. Although the IPI remains predictive in patients treated with R-CHOP, it no longer distinguishes four outcome groups (**Table 2**). Redistribution of the IPI factors into a Revised IPI (R-IPI) provides a more accurate prediction of outcome and distinguishes three separate outcome groups with 4-year overall survival ranging from 55% to 94%. Although the R-IPI allows identification of a poor outcome group, the IPI factors can no longer be used to identify a group of patients with less than a 50% chance of survival. These results should be validated prospectively in an independent patient population.

Molecular prognostic markers in DLBCL

The heterogeneity of DLBCL is highlighted by the variable expression of a variety of molecular aberrations, some of which have been shown to be predictive of outcome. A full examination of all biologic markers reported to be prognostic in patients with DLBCL is beyond the scope of this manuscript. However, several excellent reviews have been recently published.[9-11] **Table 3** lists some of the molecular prognostic markers reported in DLBCL.

Although studies of individual biomarkers have improved our understanding of the pathogenesis of DLBCL, many studies have yielded conflicting results. Reasons for these discrepancies include the retrospective nature of most studies, small patient sample size, lack of uniformity in technique and failure to control for other simultaneous biologic processes that may be confounding outcomes. Importantly, these markers need to be revalidated in patients who have been treated with immunochemotherapy.

Bcl-2 is an anti-apoptotic protein that is important in normal B-cell development and differentiation. Bcl-2 overexpression has been reported in approximately 40-60%

Table 2. Outcome according to International Prognostic Index (IPI) factors in 365 patients treated with R-CHOP in British Columbia.

Risk Group	# Factors	% Patients	4-year PFS (%)	4-year OS (%)
Standard IPI				
Low	0,1	28	85	82
Low-Intermed	2	27	80	81
High-Intermed	3	21	57	49
High	4,5	24	51	59
Revised IPI (R-IPI)				
Very Good	0	10	94	94
Good	1,2	45	80	79
Poor	3,4,5	45	53	55

*updated from Sehn[8]

Table 3. Molecular prognostic markers in diffuse large B-cell lymphoma.

Prognostic Marker	Effect on Outcome	Mechanism
Bcl-2	Unfavorable	Anti-apoptosis
Bcl-6	Favorable	Transcriptional repressor
CD-10	Favorable?	Neutral endopeptidase
CD-5	Unfavorable?	B-cell differentiation
HGAL	Favorable	Germinal center phenotype
FOXP1	Unfavorable	Transcription factor
MUM1	Unfavorable	Transcription factor
Mutation p53	Unfavorable	Cell cycle regulation
Cyclin D2/D3	Unfavorable	Cell cycle regulation
Skp2	Unfavorable	Cell cycle progression
Survivin	Unfavorable	Anti-apoptosis
PKC-β	Unfavorable	B-cell signaling
CD-21	Favorable	B-cell differentiation
low ICAM-1	Unfavorable	Lymphocyte trafficking
sICAM-1	Unfavorable	Lymphocyte trafficking
Endostatin	Unfavorable	Angiogenesis
sVEGF	Unfavorable	Angiogenesis
MMP-9	Unfavorable	Promotes metastases
Caspase 8 inhibition	Favorable	Apoptosis signaling
Caspase 9 inhibition	Unfavorable	Apoptosis signaling
nm23-H1	Unfavorable	B-cell differentiation
sIL-10	Unfavorable	Immune response regulator
loss MHC class II	Unfavorable	Immune surveillance
GCB versus ABC	Variable	Molecular subtype

*Table modified from Lossos[9], Gascoyne[11] (refer for full list of references)
Abbreviations: HGAL, human germinal center associated lymphoma; Skp2, s-phase kinase-associated protein 2; ICAM-1, intracellular adhesion molecule-1;,sICAM-1, soluble ICAM-1; sVEGF serum vascular endothelial growth factor; MMP-9, matrix metalloproteinase-9; sIL-10, serum IL-10; MHC, major histocompatibility complex; GCB, germinal center B-cell like; ABC, activated B-cell like

of patients with DLBCL and has been associated with poorer survival. *In vitro* studies have shown that rituximab induces downregulation of Bcl-2 protein expression and by this mechanism may abrogate resistance to chemotherapy. The significance of Bcl-2 overexpression was reevaluated in patients treated with R-CHOP in the GELA trial.[12] In contrast to patients treated with CHOP alone, no correlation between Bcl-2 overexpression and survival was seen in patients treated with R-CHOP, implying that the addition of rituximab had overcome its negative influence. Other investigators have also reported that the addition of rituximab to chemotherapy has eliminated the prognostic significance of Bcl-2 overexpression in DLBCL.[13,14]

Bcl-6 protein expression, a marker of germinal center derivation, has been shown to predict for a favorable outcome in DLBCL. A prospective correlative study performed in conjunction with the US Intergroup Trial examined the prognostic value of Bcl-6 protein expression in patients treated with R-CHOP.[15] In patients treated with CHOP, outcomes were superior for Bcl-6 positive patients relative to Bcl-6 negative patients. Whereas, outcomes for patients treated with R-CHOP were not influenced by Bcl-6 status. The addition of rituximab to CHOP appears to have eliminated the prognostic significance of Bcl-6 protein expression in patients with DLBCL.

Gene expression profiling in DLBCL

Gene expression profiling studies have attempted to characterize the specific aberrations in tumor cells by measuring the differences in mRNA expression on a genome-wide scale. These studies have confirmed that molecularly distinct subgroups exist within DLBCL and have distinct outcomes after treatment with anthracycline-based chemotherapy.[16-19] At least two major subtypes have been identified, one with a gene expression profile similar to normal germinal center B-cells (GCB) and the other mimicking activated peripheral blood B-cells (ABC).[16,17] Patients with the GCB profile have a significantly better overall survival independent of IPI score after treatment with CHOP-type regimens (5-y OS 60% vs 35%, $P < 0.001$).[17]

Due to lack of a standardized commercially available test and the requirement for fresh or snap-frozen tissue specimens, gene expression profiling is not yet a practical tool for risk assessment in routine patient care. Several investigators have used the information derived from gene array studies to create prediction models based on more amenable techniques such as immunohistochemistry or PCR-based methods.[20-23] Hans and colleagues used three markers (CD10, BCL6, and MUM1) to assign patients to GCB versus non-GCB categories and demonstrated a better correlation with outcome than predicted by gene array.[21] However, in a similar study by Colomo and colleagues no association with outcome was observed.[20] Using PCR techniques, Lossos and colleagues evaluated 36 genes found to be predictive of outcome in gene array studies and created a prediction model from the six candidates found to be most influential (LMO2, BCL6, FN1, CCND2, SCYA3 and BCL2) yielding three independent risk groups.[23]

The predictive value of gene expression profiling and the models noted above must be reevaluated in patients treated with R-CHOP. Rituximab has been shown to selectively improve outcomes in Bcl-6 negative patients,[15] and therefore should preferentially benefit the ABC subgroup. In an interesting recent report, Bcl-2 protein expression has been shown to be a negative predictor of outcome in the ABC subgroup, but not the GCB subgroup.[24] Since rituximab has been shown to ameliorate the negative prognostic impact of Bcl-2 overexpression, this may also preferentially benefit the ABC group and may eliminate the difference in outcome previously noted between GCB and ABC subtypes.

PET scanning as a prognostic indicator in DLBCL

Fluorine-18-fluorodeoxyglucose (18FDG) positron emission tomography (PET) scanning is a more sensitive and specific imaging modality than CT scanning for aggressive NHL. Early restaging PET scans (early PET) performed after one to four cycles of therapy have been shown to be predictive of outcome.[25-27] In a prospective trial, Spaepen and colleagues assessed the utility of early PET performed after 3-4 cycles of doxorubicin-based chemotherapy in 70 patients with aggressive NHL.[25] None of the 33 patients with a positive early PET achieved a durable remission, whereas 84% of patients with a negative early PET remain in remission.

Haioun and colleagues assessed the utility of early PET scanning following two cycles of anthracycline-based chemotherapy (associated with rituximab in 41%) in 90 patients with aggressive NHL.[27] The 2-year EFS (82% vs 43%, $P < 0.001$) and 2-year overall survival (90% vs 61%, $P = 0.006$) were significantly better in patients who were PET negative compared with PET positive, respectively, and PET was a stronger predictor than the IPI. These same investigators compared the prognostic value of early PET scanning with phenotypic profile (GCB versus non-GCB) obtained by immunohistochemistry.[28] While the value of early PET scanning was confirmed, no prognostic value of phenotype was seen.

More studies are required to explore the optimal use of early PET scanning in DLBCL. Appropriate timing must be established, since PET scans performed too early might lead to premature abandonment of curative therapy. The timing with respect to chemotherapy administration must also be established since treatment-related effects may lead to falsely positive results. Given its potential predictive power and its apparent independence from the treatment selected, early PET scanning will likely become one of the predominant prognostic tools used to guide treatment decisions in DLBCL in the future.

Are there useful prognostic markers in DLBCL?

The addition of rituximab to CHOP chemotherapy has resulted in a marked improvement in outcome and has altered what was previously understood regarding risk assessment in DLBCL. The IPI (or R-IPI) remains a useful tool for outcome prediction, but no longer distinguishes a subgroup of patients with less than a 50% chance of survival. Given the excellent outcomes seen, care must be taken not to inappropriately deviate from established curative therapy or to add needless toxicity. Currently, in the era of R-CHOP there are no molecular markers validated to be predictive of outcome for DLBCL. Previously recognized molecular markers need to be reassessed, since several markers have already been shown to no longer be prognostic. Early PET scanning promises to be a valuable tool which will allow patients to be treated with standard therapy initially and selectively switched to alternate therapy if poor response is demonstrated, but requires further evaluation.

Follicular Lymphoma

Follicular lymphoma (FL) is the second most common lymphoma, representing approximately 70% of all indolent lymphomas and 22% of all new cases of NHL.[1] Most patients present with advanced-stage disease at diagnosis and cannot be cured by conventional therapy. Median survival is 8-10 years, but marked heterogeneity in outcome is observed. While some patients with slow-growing disease can be followed for decades without need for treatment, other patients exhibit a rapid disease course requiring early intervention. Transformation to aggressive lymphoma occurs at a rate of 3% per year and generally portends a poor prognosis.[29]

There is no universally accepted standard front-line therapy for FL. A broad range of therapeutic options is available and historical studies have not shown a survival benefit of one regimen over another. Recently, several randomized controlled trials have confirmed a marked improvement in event-free survival with the addition of rituximab to chemotherapy in previously untreated patients with FL and have suggested a possible benefit in terms of overall survival,[30-32] making immunochemotherapy the new standard of care. The availability of newer therapies appears to be changing the natural history of FL.[33,34] This has prompted a reevaluation of treatment paradigms and has reopened the debate of whether watch and wait strategies should be abandoned and whether poorer risk patients can be identified that may benefit from alternate approaches.

Clinical prognostic factors in FL

The Follicular Lymphoma International Prognostic Index (FLIPI) has become a widely used tool for risk assessment of FL.[35] Using five adverse prognostic factors (age > 60 years, Ann Arbor stage III/IV, hemoglobin < 120 g/L, > 4 nodal areas, elevated serum LDH) low, intermediate and high risk groups were identified with 10-year overall survival rates of 71%, 51% and 36%, respectively. A comparison of prognostic indices in FL was recently performed in an independent patient population and confirmed the predictive capacity of the FLIPI.[36] Interestingly, the IPI, which is an easier index to apply, appeared to be as predictive as the FLIPI but identified a slightly lower proportion of patients in the high risk category.

In view of the recent shift to upfront immunochemotherapy, the FLIPI must be revalidated to ensure that it remains predictive and to update expected outcomes. This question has been preliminarily explored by Buske and colleagues who evaluated the prognostic value of the FLIPI in patients treated on a prospective trial of CHOP versus R-CHOP.[37] With a short median follow-up of 20 months, a true assessment of overall survival (the primary endpoint of the FLIPI) was not possible. A significant difference in time-to-treatment failure (TTF) was noted between patients in the high risk group compared with the intermediate and low risk groups (2-year TTF 67% versus 90% and 92%, respectively, $P = 0.0002$), suggesting that the FLIPI may retain its predictive capacity to identify a poorer risk group. However, one limitation of the FLIPI has been its inability to identify a very poor outcome group, since even the high-risk group had a 5-year overall survival of greater than 50%, and this will undoubtedly be even higher in patients receiving rituximab.

Cytologic grade in FL

Three grades of FL are recognized by the WHO based on the proportion of centroblasts seen in neoplastic follicles. Grade 3 has been further subclassified into grade 3a where centrocytes are still present and grade 3b which contains solid sheets of centroblasts. The correlation of clinical grade to clinical outcome is an unresolved debate. This is in part due to the poor reproducibility of histologic grade among hematopathologists, the low frequency or exclusion of FL grade 3 in many clinical trials, and to variations in treatment practices for the different grades (anthracyclines commonly used for FL grade 3). There is general consensus that FL grades 1 and 2 behave indolently and have similar outcomes, whereas FL grade 3 appears to behave more aggressively. Reports have been conflicting regarding the potential curability of FL grade 3 with anthracycline-based therapy. It has been suggested that FL grade 3a forms part of a spectrum of indolent lymphoma with grades 1 and 2, and grade 3b may be similar to DLBCL,[38] but a recent report of FL patients treated with an anthracyline-based regimen found no difference in outcome between grades 3a and 3b.[39] Within this same paper, the authors further explored the predictive value of diffuse areas within FL grade 3, and concluded that patients with a diffuse component of ≤ 50% had a more favorable outcome than those with a diffuse component of > 50%. The relevance of this finding is unclear since the WHO criteria dictates that any area of DLBCL within a FL indicates transformation to an aggressive phase and should be reported as a separate diagnosis. Currently, it is difficult to recommend alternate treatment strategies based on FL grade, but given the molecular simi-

larities of FL grade 3b to DLBCL it would seem prudent to treat these patients in keeping with this entity.

Molecular prognostic markers in FL

Several excellent reviews of the biology, molecular pathogenesis and prognostic markers in FL have been recently published, and cannot be presented here in full detail.[40-42] **Table 4** lists some of the molecular prognostic markers reported in FL.

The hallmark genetic event in the development of FL is the t(14,18) translocation which juxtaposes the BCL2 oncogene into the IGH heavy chain locus, leading to the constitutive expression of Bcl-2 anti-apoptotic protein. This early molecular event occurs in 85% of FL grades 1 and 2, and although critical for lymphomagenesis is by itself insufficient to produce FL. Follicular lymphoma lacking the t(14,18) translocation does occur and is more commonly seen in FL grade 3, but many of these cases overexpress Bcl-2 protein by alternate mechanisms. The growth advantage provided by the overexpression of Bcl-2 likely allows the acquisition of further genetic events that largely occur as a series of chromosomal gains and losses and can be detected by routine cytogenetics as a heterogeneous collection of complex karyotypes. It is unclear to what extent these random genetic events account for the clinical heterogeneity of the disease. The mean number of alterations is highly variable at diagnosis, with higher numbers of alterations tending to correlate with a higher grade of FL. Although the acquisition of various mutations (such as c-myc and loss of p53) have been associated with the development of transformation, the complexity of the FL karyotype at diagnosis does not appear to correlate with the risk of developing subsequent transformation.[43] Recurring cytogenetic events that have been noted to correlate with an unfavorable outcome include a variety of chromosomal gains (+7, +12q13-14, +18q) and chromosomal losses (del6q, –9p21, –17p13).[41] Translocations involving BCL6 occur infrequently in FL grades 1 and 2, but were noted in 18% of cases of FL grade 3a and 44% of FL grade 3b,[38] and have been shown to correlate with a risk of transformation.[44]

Immunohistochemistry is a valuable tool for the diagnosis of FL, and several studies have correlated the level of expression of various proteins with clinical outcome. The level of expression of MIB-1 (Ki-67) provides a measure of proliferative rate, and has been shown to correlate with FL grade but has limited prognostic significance. Although most patients with FL overexpress Bcl-2 protein, higher levels of expression have been correlated with a worse outcome. In contrast, higher levels of expression of germinal center markers including CD-10, Bcl-6 and PU.1 have been correlated with a favorable outcome.[45,46] The presence of more than 15 $CD68^+$ macrophages per high power field has also been shown to predict for a poor outcome.[47]

Table 4. Molecular prognostic markers in follicular lymphoma.

Prognostic Marker	Effect on Outcome	Mechanism
Chromosomal gains +7, +12q13-14, +18q	Unfavorable	Dominant oncogenes
Chromosomal losses Del6q, –9p21, –17p13	Unfavorable	Loss tumor suppressor gene
BCL-6 translocation	Unfavorable	Genomic instability
Bcl-2 expression	Unfavorable	Anti-apoptotic
Bcl-6 expression	Favorable	Germinal center phenotype
CD10 expression	Favorable	Germinal center phenotype
PU.1	Favorable	Germinal center phenotype
Macrophage content	Unfavorable	Modulation by microenvironment
MDM2 expression	Unfavorable	Functional p53 loss
Bcl-X_L	Unfavorable	Anti-apoptotic
Cyclin B1	Favorable	Cell cycle progression
Immune response (IR-1 versus IR-2)	Variable	Modulation by microenvironment
81-gene predictor	Variable	Reflects tumor behavior

*Table modified from Gascoyne[41] (refer for full list of references), additional reference Torlakovic[46]

Gene expression profiling in FL

One of the first gene array studies reported in FL compared the gene expression of purified malignant B-cells to normal germinal center B-cells and noted the differential expression of 65 genes.[48] Although this study provided some insight into the biology of FL, a correlation with outcome was not performed. Moreover, since cells from the microenvironment were intentionally excluded differences in cellular interactions could not be appreciated. FL cells reside within a microenvironment that closely resembles the normal germinal center and are intimately associated with follicular dendritic cells, helper T cells and macrophages. It is believed that interactions between these cells modulate the growth and survival of FL cells.

The importance of this phenomenon has been highlighted by the gene array–based prediction model created by Dave and colleagues.[49] Gene expression profiling was performed on whole biopsy specimens from 191 patients with untreated FL. Two signatures of gene expression were identified that best correlated with survival prediction. The "immune-response 1" (IR-1) signature included genes encoding for T-cell markers and genes that are highly expressed in macrophages, and predicted a favorable outcome. The "immune-response 2" (IR-2) signature included genes that are preferentially expressed in macrophages, dendritic cells or both, and predicted an unfavorable outcome. When patients were grouped into quartiles based on their survival-predictor scores which reflected the signature expression levels within their biopsies, the median survivals

ranged from 3.9 years to 13.6 years. The predictive capacity of the gene expression model was independent of the IPI. Interestingly, the gene expression signatures were shown to reflect the biologic characteristics of the nonmalignant cells within the tumor.

In a separate study by Glas and colleagues, whole tissue biopsies from 80 patients with FL were used to develop a gene expression profile of 81 genes that could predict for indolent versus aggressive behavior at diagnosis or relapse.[50] The FL stratification profile contained genes involved in cell cycle control, DNA synthesis, and genes reflecting increased metabolism that were upregulated in the aggressive phase of the disease, as well as genes derived from the reactive infiltrate of T cells and macrophages that were up-regulated in the indolent phase of the disease. The 81-gene profile provided a more accurate prediction of clinical behavior than either histologic grade or the IPI.

Are there useful prognostic markers in FL?

The advent of immunochemotherapy has led to a marked improvement in outcome for FL, but a curative strategy has yet to be identified. Early indications suggest the FLIPI clinical index remains prognostic but may not identify a significantly poor risk group to warrant treatment stratification. Although molecular studies have provided invaluable insights into the biology of FL, if we attempt to apply the criteria for a prognostic marker outlined in **Table 1** to any of the molecular markers for FL listed above, none could be considered clinically useful. In addition, the value of these molecular prognostic markers has not been reevaluated in patients treated with current practices. Therefore, at this time the use of molecular markers for treatment stratification of FL cannot be advocated.

Conclusions

Substantial clinical progress in lymphoma mandates that we reevaluate the clinical and molecular factors predictive of outcome. We have seen that several validated prognostic markers no longer retain significance in the era of immunochemotherapy. It is also possible that markers previously found to be irrelevant may now have prognostic value. The biologic insights provided by molecular studies should allow for more targeted therapies to be developed, which will increase treatment choice and the possibility of tailored therapy in the future. It is imperative that future steps forward be made in the context of well-designed clinical trials with prospective correlative studies of clinical and biologic markers.[51] Results from these studies should be reported with sufficient rigor to allow appropriate interpretation.[52] This will enable us to continuously assess outcome predictors in the context of treatment change, and to rationally design tailored treatment algorithms.

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