Prognostic factors in follicular lymphoma: new tools to personalize risk

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Follicular lymphoma (FL) is the most common indolent lymphoma, and it has a long median overall survival (OS). However, the recent discovery of clinical and biological prognostic biomarkers in FL is shedding light on FL heterogeneity and the need for a precise and risk-stratified individual approach at diagnosis and relapse. Many FL patients who are asymptomatic with indolent disease can be vulnerable to the toxicity, emotional distress, and financial burden of overtreatment. Yet a subset of FL patients develop chemoresistance to standard chemoimmunotherapy, experience transformation to aggressive lymphoma and rapid progression, and represent the population most in need of novel therapies and curative approaches. Novel biomarkers that incorporate both clinical and genetic determinants of poor risk are being developed with the hope of identifying high-risk patients at diagnosis in order to offer biologically rational targeted therapies.

Learning Objectives

- To review clinical prognostic indices in FL and learn about novel prognostic markers predicting poor risk
- To understand how prognostic markers in FL help predict heterogeneity and how this may shape our approach to patients

Introduction

As the most commonly diagnosed indolent non-Hodgkin lymphoma (NHL) in the Western world, follicular lymphoma (FL) represents ~35% of all NHL cases and affects an estimated 3 in 100 000 men and women in the United States and 2 in 100 000 in Europe.[1,2] FL patients have a median age of 65 years, and most present with advanced-stage disease. However, ~30% to 40% of patients are found to have early-stage disease at the time of diagnosis, and between 5% and 10% are younger than age 40 years.[3-5] Fortunately, the past several decades have witnessed promising improvements in FL outcomes based largely on superior therapies at all stages, including the anti-CD20 monoclonal antibody rituximab, which has had an impact on the natural history of the disease. Thus, the median overall survival (OS) for FL patients diagnosed in the modern chemo-immunotherapy era approaches 20 years.[6]

The historically excellent outcomes observed in most FL patients have afforded clinicians and patients security in selecting from several effective therapies that will work in the vast majority of individuals. The long natural history of the disease coupled with similar survival rates regardless of the treatment approach selected raise important questions about what the unmet needs in FL truly are. Should all FL patients still be approached in the same way? Who are the patients at highest risk of death? How can we identify patients who would benefit most from aggressive vs no therapy? What is the most effective way to measure the impact of our interventions? Rates of complete response (CR) or estimates of progression-free survival

(PFS) may no longer be suitable end points for clinical trials in which most patients have durable remissions, and in the era of novel targeted therapeutics that offer clinical benefit for long periods of time, CR and PFS may have fleeting relevance. Thus, the development and refinement of novel prognostic markers will play an important role in refining FL risk.

Despite the aforementioned gains in survival, FL remains a highly heterogeneous entity with various outcomes. In some patients, the disease exhibits transformation or aggressive and chemotherapy-resistant behavior, whereas in other patients, the disease is indolent with durable remissions after treatment. The ability to risk stratify patients into one of these two categories at diagnosis will become increasingly important for predicting outcomes and selecting therapy. It will also help minimize the toxicity of overtreatment, minimize financial burden, increase the quality of life for good-prognosis patients, facilitate the application of novel therapeutics that address genetic determinants of disease in poor-risk patients, and improve morbidity and mortality. Discovering the biologic rationale to explain the heterogeneity of poor-risk patients would have a meaningful impact that could influence the next generation of treatments for patients with FL, both at diagnosis and upon progression.

Patient-specific variables that influence FL behavior include the burden of tumor, tumor histology and grade, and intratumoral/microenvironmental markers. Clinical prognostic indices such as Follicular Lymphoma International Prognostic Index (FLIPI) and FLIPI-2 scores identify FL patients with more aggressive disease and shorter responses to treatment,[7-9] but they do not incorporate biological data influencing FL evolution, and alone, they are insufficient to account for observed survival differences in individual patients.

Early gene expression studies established the importance of the tumor microenvironment (TME) in prognosis, and next-generation sequencing studies identified numerous mutational events occurring

Table 1. Molecular prognostic factors in FL associated with prognosis

Factor	Role/function	Mutation/alteration (if applicable)	Consequence and prognostic impact	Reference
Microenvironment	Gene signatures were developed from whole-genome microarrays of frozen lymph node biopsies of untreated FL patients yielding IR1 and IR2	NA	IR1: overexpression of genes enriched for T cells; favorable, RR of death, 0.15; 95% CI, 0.05-0.46. IR2: overexpression of genes expressed in follicular dendritic cells and macrophages; unfavorable, RR of death, 9.35; 95% CI, 3.02-28.90.	10
MLL2	Histone methyltransferase; acts as a tumor suppressor	Inactivating mutation; found in 89% of FL	May contribute to FL tumorigenesis	43
CREBBP	Histone acetyltransferase; functions as transcriptional co-activator; mutation found in ~32% of FL	Inactivating mutation removes or inactivates coding domains	Failure to acetylate BCL6 and p53; constitutive activation of bcl-6 and decrease in p53 activity	44
EP300	Histone acetyltransferase; functions as transcriptional co-activator; mutation found in ~9% of FL			
ARID1A	Histone linker, chromatin modifier, and tumor suppressor	Inactivating mutation; found in 11% of FL	Impaired DNA repair; correlates with longer FFS	43,51,52
MEF2B	Histone methyltransferase; cooperates with CREBBP and EP300; activates transcription of BCL6	Inactivating mutation; found in 7% to 15% of FL	Enhanced transcriptional activity of MEF2B deregulates BCL6 expression and contributes to lymphomagenesis	43
EZH2	Histone methyltransferase; promotes normal germinal center development	Activating, gain of function mutation; occurs in ~7% to 30% of FL. Seems to be an early event in FL pathogenesis	Causes elevated H3K27 trimethylation, promotes follicular hyperplasia, contributes to pathogenesis of lymphoma	43,48,53,54
BCL2	Antiapoptotic protein; present at both diagnosis and at transformation in FL	Gain of function mutations; affecting 16% to 65% of FL	Increased risk of transformation and poor survival	33

CI, confidence interval; FFS, failure-free survival; IR, immune response; NA, not applicable; RR, relative risk.

at various time points within the FL genome, also influencing outcomes[10-13] (Table 1). Until recently, however, we have been unable to apply biological predictors of risk to the clinical setting. A growing body of recent literature has uncovered novel predictors of outcome by using more precise approaches to identify poor-risk FL patients both at diagnosis and at the time of relapse.[14,15] Validated strategies on how best to incorporate these prognostic tools into clinical use remain unmet needs in the management of FL.

Prognostic factors that personalize risk
Clinical predictors

IPI, FLIPI, and FLIPI-2. The International Prognostic Index (IPI) was among the first clinical indices to identify predictive markers of survival in aggressive lymphoma.[16] The International Non-Hodgkin Lymphoma Prognostic Factor Project was a multi-center international collaboration that studied more than 2000 patients with intermediate- or high-grade lymphomas treated with anthracycline-containing chemotherapy regimens in the pre-rituximab era.[9] The resulting model incorporated age >60 years, elevated serum lactate dehydrogenase, Eastern Cooperative Oncology Group performance status ≥2, stage III or IV disease, and >1 extranodal sites of disease. The IPI successfully identified 4 risk groups with predicted 5-year OS rates of 73%, 51%, 43%, and 26%. However in a large multivariate analysis of FL patients treated on 2 prospective clinical trials, the Groupe d'Etude des Lymphomes de l'Adulte (GELA) group found that although the IPI did predict for OS in FL, few patients fell into the high-risk IPI category, suggesting that a more robust prognostic index was required for FL.[17] Thus, the FLIPI emerged from a multicenter collaboration that included more than 4000 patients to refine assessment of risk in FL and identify those with the most aggressive disease and shortest remission durations.[7] The index uses >4 nodal sites, elevated serum lactate dehydrogenase, age >60 years, advanced stage, and hemoglobin <12 g/dL to predict OS and assigned 1 point for each characteristic. They found 3 risk groups that predicted 5- and 10-year OS. A score of 0 to 1 was low risk with a 5-year OS of 91% and a 10-year OS of 71%; a score of 2 was intermediate risk, with a 5-year OS of 78% and a 10-year OS of 51%; and a score of 3 or greater was high risk, with a 5-year OS of 52% and a 10-year OS of 36%. These results suggested that the FLIPI was better able to identify high-risk patients than the IPI (Table 2).

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Table 2. Comparison of FLIPI, FLIPI-2, and m7-FLIPI

Index	Risk group	No. of factors	Prognostic impact OS	%	FFS	%	Reference
FLIPI	Low	0-1	5 y	91			7
			10 y	71			
	Intermediate	3-5	5 y	78			
			10 y	51			
	High	2	5 y	53			
			10 y	36			
FLIPI 2	Low	0	3 y	91			8
			5 y	80			
	Intermediate	1-2	3 y	69			
			5 y	51			
	High	3-5	3 y	51			
			5 y	19			
M7-FLIPI	Low				5 y	68-77	15
	High				5 y	22-38	

FLIPI: >4 nodal sites, lactate dehydrogenase above normal, age ≥60 y, stage III to IV, Hgb <12 g/dL; FLIPI 2: lymph node mass >6 cm, age ≥60 y, bone marrow involvement, Hgb <12 g/dL, β_2-microglobulin elevation; M7-FLIPI: FLIPI factors with mutational status of 7 genes: *EZH2, ARID1A, MEF2B, EP300, FOX01, CREBBP,* and *CARD11.* Hgb, hemoglobin.

The utility of the FLIPI has been corroborated since rituximab therapy became standard practice in treating lymphoma, and FLIPI retained its prognostic significance in patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) and other chemoimmunotherapy approaches.[18] In a large registry study, the National Lymphocare Study (NLCS) validated the FLIPI in ~2000 patients across the Unites States and found that approximately one-third of patients fell into each risk group. Median OS had not been reached after nearly 5 years of follow-up, and 2-year OS rates for low-, intermediate-, and high-risk patients were 98%, 94%, and 87%, respectively.[19] The FLIPI-2 was designed as a predictor in the rituximab era and included some of the same parameters used in the original FLIPI as well as novel markers including elevated β_2-microglobulin, largest nodal mass measuring >6 cm, and bone marrow involvement.[8] Three-year OS rates for patients in the low-, intermediate-, and high-risks groups were 99%, 96%, and 84%, respectively. Currently, however, the FLIPI seems to be the most commonly used predictor of survival in FL but not necessarily a tool to use for selecting therapy.

Disease burden and response to therapy. Although the FLIPI helps predict the course of individual patients with FL, Groupe d'Etude des Lymphomes Folliculaires (GELF) criteria uses clinical features to influence the decision to initiate treatment.[20] Patients with low-tumor-burden FL, as designated by GELF and other prognostic indices, who do not meet criteria for therapy may be offered an observation strategy because of the outstanding survival in the absence of therapy. A large British study of more than 300 low-tumor-burden FL patients in the pre-rituximab era compared observation to chlorambucil chemotherapy and found no difference in OS.[21] A later international study in low-tumor-burden FL compared observation to rituximab alone with rituximab followed by rituximab maintenance and also found no difference in OS or rates of transformation. However, rituximab did improve quality of life, and 88% of patients in the rituximab group did not require therapy at 3 years compared with 46% of patients undergoing observation, suggesting that rituximab lengthened time to next therapy.[22] The multicenter RESORT study evaluated single-agent rituximab followed by re-treatment at time of progression vs maintenance rituximab in low-tumor-burden patients. Similarly,

excellent survival was observed but without improvement in quality of life or time to treatment failure.[23]

FL patients with high tumor burden by GELF criteria are frequently offered treatment with chemoimmunotherapy to minimize symptoms of disease. Moreover, randomized clinical trials in high-tumor-burden FL demonstrate OS advantages when chemoimmunotherapy such as R-CHOP and rituximab plus cyclophosphamide, vincristine, and prednisone (R-CVP)[24,25] are used as part of first-line therapy, and there is a PFS advantage when rituximab maintenance is used.[26]

Given the favorable outcomes observed in most FL patients independently of FLIPI score or GELF criteria at diagnosis, what are other prognostic markers available to help discern the variability in clinical course? A pooled analysis of data from 3 prospective multicenter studies in high-tumor-burden FL that used positron emission tomography (PET) scans to assess response after 6 cycles of treatment suggested that PET-negative status was predictive of improved PFS and OS.[27] PET scans of 246 patients underwent central PET review and were scored independently according to the Deauville 5-point scale.[28] The authors found that 4-year PFS for patients with a positive PET scan (Deauville score of 4 or higher) after therapy was 23% compared with a 4-year PFS of 63% for those with a negative PET scan. Similarly, negative PET scans were associated with improved OS compared with positive PET scans after therapy completion (95% vs 87%), although both groups did well overall (Table 3).

Other efforts are ongoing to investigate surrogates for FL survival. A recent international collaboration led by Sargent et al[29] sought to establish whether CR to first-line treatment could be used to predict PFS. The Follicular Lymphoma Analysis of Surrogacy Hypothesis (FLASH) investigators performed a meta-analysis of 13 randomized trials of first-line treatment in FL and included more than 3000 patients treated with a variety of regimens. They found that CR rate at 30 months was able to predict PFS and proposed this as a possible end point for clinical trials in FL. However, in an unselected cohort of patients, many of whom will have excellent outcomes with or without therapy, it is unclear whether CR rate as a proxy for PFS will adequately capture clinical benefit in FL.

Table 3. Clinical factors associated with prognosis

Factor	Prognostic impact	PET scan	Deauville score	Reference
Early disease progression after first-line chemoimmunotherapy (within 2 y) with R-CHOP, R-CVP, R-fludarabine	OS of 34% to 50% in early-relapse group; OS of 90% in reference group without early relapse			14
Early disease progression after first-line chemoimmunotherapy (within 1 y)	SMR, 3.90; 95% CI, 2.89-5.25; $P < .001$			31
PET response	4 y PFS, 63%; OS, 95%	Positive	4 or 5	27

SMR, standard mortality ratio.

Early disease progression. One of the most important unmet needs in FL risk stratification involves approaching patients at the time of disease progression. Until recently, little was known about the role of remission duration after first-line therapy and what in particular predicts for shorter or longer duration of response. Timing of disease progression and its influence on patient survival had not been previously studied, possibly because of widespread acceptance of FL as a largely indolent disease in which patients experience the benefits of sequential treatment therapies independently of when progression occurs.

Several studies suggest that disease recurrence within 2 years of first-line treatment of FL occurs consistently in as many as 20% of patients, independent of maintenance rituximab.[26,30] Our group investigated whether time to progression after diagnosis in FL patients receiving first-line chemoimmunotherapy was a prognostic factor impacting survival outcomes. In a pivotal study, we performed an analysis of the NLCS, a prospective longitudinal registry study enrolling more than 2700 patients with FL between 2004 and 2007 at academic and community sites in the Unites States. Patients were observed for nearly 10 years. Previous analyses from this registry established that the most frequently used first-line chemoimmunotherapy regimen for FL was R-CHOP.[4] From this registry, we sought to identify FL patients at high risk for death after treatment with R-CHOP to determine whether early disease progression predicted for inferior survival.

We evaluated 588 patients with stage II, III, or IV FL treated with R-CHOP chemotherapy in the first-line setting. After a median follow-up of 7 years, those with disease progression within 2 years of first-line treatment (early relapse) had very poor outcomes, with 5-year OS of 50% compared with 5-year OS of 90% for patients in the reference group who did not experience early relapse (Figure 1). Cox regression analysis demonstrated that early relapse was associated with markedly reduced OS with a hazard ratio of 7.17 when compared with the reference group; even after adjustment for FLIPI score, early progression carried an increased risk of death with a hazard ratio of 6.44. Moreover, two-thirds of the deaths in the entire NLCS cohort occurred in the early-relapse group. These findings were validated in an independent cohort of more than 100 patients from the University of Iowa/Mayo Clinic, in whom the 5-year OS was 34% for patients with early progression compared with 94% in the reference group. A separate analysis of patients treated with R-CVP and R-fludarabine showed similar results, supporting time to progression as an independent adverse prognostic marker independent of therapy.

These robust and reproducible findings in other studies[31] demonstrate that early relapse after diagnosis in patients treated with first-line chemoimmunotherapy is an extremely powerful prognostic indicator of outcome in FL and should be used to stratify risk at the time of relapse. Similar results were seen in a subsequent analysis from the Mayo Clinic that evaluated 1-year PFS. Accordingly, 12-year PFS (PFS-12) and PFS-24 have been proposed as surrogate novel end points for poor OS in clinical trials.

The clinical implications of this are exceedingly relevant to the design of future clinical trials targeting relapsed FL and should aim to address this population with novel or more aggressive therapies. Whether the impact on early relapse of novel regimens that do not use chemotherapy is sustained will require validation in future studies. Importantly, the question of identifying high-risk early-relapsing patients at the time of diagnosis will be paramount to changing the natural history of FL. Understanding the biological mechanisms underlying early disease progression to help personalize initial therapy should in parallel discover molecular drivers of good-risk late-relapsing or never-relapsing patients who may benefit from less aggressive treatment at diagnosis, given their excellent outcomes.

Biological predictors

Histologic grade. FL evolves in a nodal growth pattern after malignant transformation of germinal center B cells. It is characterized by the t(14;18)(q32;q21) translocation resulting in constitutive overexpression of the antiapoptotic *BCL2* gene.[32] Present in 85% of patients with FL, t(14;18) is necessary but insufficient for lymphomagenesis, and protein levels of BCL2 are not biological predictors of disease course. Exome sequencing suggests that *IGH-BCL2* is a founder translocation that gives rise to a premalignant tumor precursor.[13] Coding sequence mutations in *BCL2* have been

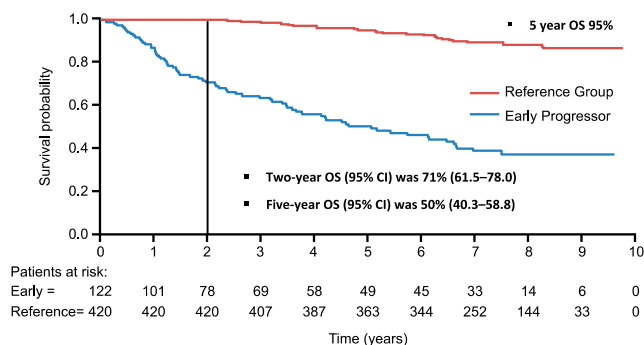- 122 patients were classified as early progressors (n=110 PD and n=12 non-PD death within 2 years)

- 5 year OS 95%
- Reference Group
- Early Progressor
- Two-year OS (95% CI) was 71% (61.5–78.0)
- Five-year OS (95% CI) was 50% (40.3–58.8)

Patients at risk:

	0	1	2	3	4	5	6	7	8	9	10
Early =	122	101	78	69	58	49	45	33	14	6	0
Reference=	420	420	420	407	387	363	344	252	144	33	0

Figure 1. Poor survival in FL patients with early relapse after R-CHOP therapy.

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found to occur in a subset of FL, and in 1 series, they were independently associated with higher risk of transformation and increased risk of death as a result of lymphoma.[33] Polymerase chain reaction amplification of the t(14;18) translocation to establish minimal residual disease after first-line therapy is highly sensitive and has prognostic importance in a number of studies.[34] Whether depth of remission by minimal residual disease truly helps identify patients with favorable vs unfavorable risk is actively being studied in numerous clinical trials.

The intratumoral number of large centroblasts per high-power field establishes FL grade, with increasing number of centroblasts correlating to clinical aggressiveness. Grade 1 to 3a FL is considered indolent with indistinguishable clinical outcomes, whereas grade 3b disease may be cytogenetically distinct, with infrequent BCL2 and BCL6 translocations and behavior akin to that found in diffuse large B-cell lymphoma (DLBCL), and is usually approached as such therapeutically.[35] The largest retrospective series on FL grading evaluated 505 diagnostic samples from FL patients that underwent blinded central review. Most patients were classified as low grade (75% grade 1 to 2), 20% were grade 3a, and only 5% were grade 3b. Patients with FL grades 1 to 3a had similar rates of OS at approximately 12 years without a plateau and did not seem to benefit from first-line anthracycline. Patients with grade 3b disease were more likely to be treated with anthracyclines in the first-line setting; their disease behaved similarly to DLBCL, and patients had the possibility of cure and 5-year OS of 43%.[36]

Impact of the tumor microenvironment on FL risk. Multiple complex pathways influence outcomes in the molecular biology of FL. Gene expression profiling has demonstrated that among these, the interaction between the FL neoplastic cell and its microenvironment is critical. In a pivotal study by Dave et al,[10] 2 distinct gene signatures were constructed by using whole-genome microarray analysis from frozen lymph node biopsies in treatment-naïve FL patients in the pre-rituximab era. These unique gene signatures were based on molecular features from nonneoplastic tumor-infiltrating cells rather than malignant FL cells and were associated with significant differences in prognosis of survival ranging from 3.9 to 13.6 years, depending on which genes were expressed. The immune response 1 signature was associated with favorable outcomes and included expression of genes enriched from T cells. The immune response 2 signature was associated with poor outcomes and included genes expressed in follicular dendritic cells and macrophages, reinforcing the important interrelatedness of the tumor with the host immune response. Later gene expression profiling studies have recapitulated the importance of nonneoplastic surroundings in FL outcome,[37] and several have also evaluated the possibility of immunohistochemistry surrogates of immune response by identifying the number of tumor-associated macrophages in a specimen. However, they have revealed methodological differences in assessment of immune infiltrating cells, with various results depending on whether rituximab was part of therapy or not.[38,39] Thus, evaluating the TME is not part of the current armamentarium used in predicting FL risk.

The presence of CD4$^+$ regulatory T cells and T follicular helper cells (Tfh) in the TME is also prognostically important. T-cell subset analyses have revealed that these individual cell populations affect the patient's antitumor response by favoring tumor growth or not. However, the prevalence, distribution, function, and exact clinical relevance of these T-cell subsets in FL are the subject of ongoing research. FOXP3-expressing regulatory T cells are recruited by the FL tumor, recognize tumor antigens, and are capable of suppressing other antitumor effector cells. This has been implicated in FL tumorigenesis and risk of transformation, particularly when present in a perifollicular distribution.[40] The Tfh cells of the TME express programmed death 1 (PD1), but the contribution of these PD1-positive Tfh cells in FL prognosis has been controversial. Recent data suggest that PD1 expression actually emerges from distinct CD4$^+$ T cells that contribute to different patient outcomes.[41] Emerging data regarding the efficacy of immune checkpoint inhibitors in solid tumors with a high mutational load[42] are inspiring investigation of mutated FL as being especially vulnerable to these therapies. These are areas of ongoing research and will be of particular importance in FL, in which the interaction of immune pathways in the tumor as well as the TME naturally either support or suppress lymphomagenesis.

Mutational landscape and molecular prognostic markers. Large-scale genome-wide profiling studies have provided invaluable insight into the genetic diversity of FL. Data emerging from these studies reveal that alterations in genes largely involved in epigenetic regulation and modification of chromatin dominate the FL mutational landscape. Histone tails are subjected to various types of posttranslational modification that include methylation, acetylation, and ubiquitylation, which impact histone affinity for DNA and accessibility of chromatin for gene expression. Morin et al[43,45] sequenced tumor and matched normal DNA from NHL in 117 samples and 10 cell lines. They identified 651 genes that were somatically mutated in NHL, and after validation, they found that 109 of them were somatically mutated in multiple patients. Among them, inactivating somatic mutations in histone methyltransferase *MLL2* were found to be the most frequently occurring and were found in 89% of FL. *MLL2* is one of a family of 6 histone-specific methyltransferases and is thought to be a tumor suppressor in DLBCL and FL. Inactivating mutations in *MLL2* are considered early drivers of FL tumorigenesis.[43]

CREBBP and *EP300* are related histone acetyltransferases that function as transcriptional coactivators in various cell signaling pathways. Pasqualucci et al[44,48] performed next-generation whole-genome sequencing and genome-wide single nucleotide polymorphism array analysis of multiple NHLs including FL. Sequencing analysis of 46 FL patients discovered mutations that removed or inactivated important coding domains in *CREBBP* and *EP300* in ~32% and 40% of patients with FL, respectively. These were not distributed among other NHL types, suggesting a specific pathogenic role in FL. *CREBBP* and *EP300* mutations caused a failure to acetylate Bcl-6 and p53. The resultant constitutive activation of the Bcl-6 oncoprotein and decrease in p53 tumor suppressor activity then cooperate in lymphomagenesis.[44]

Other important epigenetic modifiers in FL include monoallelic and inactivating mutations in histone linker *ARID1A*, which regulates DNA repair and has been identified in 11% of FL, along with mutations in histone methyltransferase *MEF2B*, which occurs in ~7% to 15% of FL. In contrast to loss of function or inactivating mutations in some epigenetic modifiers, gain of function mutations in the polycomb group methyltransferase *EZH2* gene occur in about one-third of FL patients, and like MLL2, are thought to be early mutational events that cooperate with BCL2 to promote tumorigenesis.[45]

The vast repertoire of these mutations underscores the importance of epigenetic modulation as a fundamental principle in FL pathogenesis contributing to its heterogeneous phenotype and may hold promise

toward the design of novel therapeutics. Despite these advances in identifying influential molecular drivers in FL, there are currently no accurate markers associated with or predictive of good-risk indolent FL or poor-risk early-relapsing FL at an individual patient level. Moreover, the challenge lies in incorporating these data into meaningful clinical use. In an effort to improve upon risk stratification in FL, Pastore et al[15] created the m7-FLIPI as a clinicopathologic prognostic tool incorporating clinical factors and genetic alterations to assign risk.

The m7-FLIPI emerged from a retrospective analysis that studied two cohorts (a training cohort from the German Low-Grade Lymphoma Study Group [GLGLSG] and a validation cohort from the British Columbia Cancer Agency [BCCA]) of patients treated with R-CHOP or R-CVP as part of a clinical trial (GLGLSG) or standard of care (BCCA). Patients included in these cohorts had grade 1 to 3a FL, with symptomatic advanced-stage disease requiring therapy. They analyzed the coding sequences of 74 genes with established recurrent mutations in FL and performed univariate and multivariate analyses for all genes mutated in 5 or more patients; they then correlated these with the FLIPI score. The m7-FLIPI ultimately included high-risk FLIPI score, poor Eastern Cooperative Oncology Group performance status, and nonsilent mutations in 7 genes known to be dysregulated in FL: *EZH2, ARID1A, EP300, FOXO1, MEF2B, CREBBP*, and *CARD11*, which were validated in the BCCA cohort. m7-FLIPI validated FLIPI and proved to be superior to it and to a model that included gene mutations only. It identified high-risk and low-risk groups of patients more robustly than FLIPI alone (although not significantly much better than FLIPI-2). Interestingly, approximately 50% of patients classified as high risk using the traditional FLIPI score were re-classified as low risk using the m7-FLIPI, and they had outcomes similar to those with low-risk FLIPI. Similarly, the high-risk m7-FLIPI group represented 22% of the validation cohort and had 5-year failure-free survival (FFS) of 25%, whereas 5-year FFS was 46% in the high-risk group identified by standard FLIPI alone. Although FFS was their primary end point, OS differences were also observed. Patients with high-risk m7-FLIPI had inferior outcomes, with 5-year OS ranging from 42% to 65% compared with patients with low-risk m7-FLIPI who had 5-year OS ranging from 84% to 89%.

In an effort to biologically characterize early-relapsing patients at diagnosis, Vindi et al[46] conducted a secondary analysis of the m7-FLIPI to study its predictive utility for early progression. Whereas high-risk m7-FLIPI patients represented a high proportion of early-relapsing patients, 50% of early-progressing patients were classified as low-risk m7-FLIPI. This suggests that the m7-FLIPI may be inadequate for differentiating FL patients most vulnerable for death, which highlights a need to further explore and better characterize the molecular foundation of early-relapsing patients.

This clinicogenetic risk model is the first to incorporate molecular data into a clinical prognostic index. The authors used genomic DNA from formalin-fixed paraffin-embedded tissue, which can be used to perform gene expression assays in the future without the need for fresh or frozen tumors, which makes this an appealing technology and one that could be used as a predictive biomarker in future clinical trials. The m7-FLIPI still requires validation prospectively, and its feasibility and reproducibility should be established on a large scale as well as in the setting of novel therapeutics, which may abrogate some of the detrimental effects of genomic alterations through modification of signaling pathways.

Transformation

Diffuse, higher-grade FL may be a harbinger of occult transformation to an aggressive NHL, which has profound implications on its natural history. No specific mechanisms have been attributed to histologic transformation, although detailed genetic analyses suggest continuous tumor evolution. Prognostic biomarkers to predict when or if transformation occurs would be highly relevant to clinical practice and would help risk stratify treatment for individual patients.

Genomic analyses in FL suggest that transformation occurs in a nonlinear fashion with early driver mutations not found in the subsequent transformed FL but rather in an earlier common progenitor. A whole-exome and whole-genome sequencing study by Okosun et al[47] studied 10 paired diagnostic and transformed FL specimens and found mutations in both diagnostic and transformed specimens with high degrees of semblance, supporting the presence of an ancestral clone. Mutations were enriched for genes involved in chromatin regulation. Accumulations of mutations in cell cycle genes such as cyclin-dependent kinases and p53, epigenetic regulators such as *MLL2, EZH2*, and *CREBBP*,[43,45,47,48] and alterations in the tumor microenvironment[49] have also been implicated as important contributors in histologic transformation. Previously, transformation had been thought to carry dismal outcomes, but recent data from the rituximab era suggest that survival is improving with R-CHOP–based therapy, with median OS of 5 years.[50]

Recognizing that this model of continuous tumor evolution leads to FL transformation would permit clinical investigators to target the underlying mechanisms that cause continued genetic instability. This could lead to novel therapeutics with the potential to have an impact on the progression of FL.

Applying prognostic tools for a precision approach

Rapid progress in the development of novel therapeutics for FL and a better understanding of FL biology and outcomes have provided unique opportunities to impact treatment for patients with the greatest unmet needs. In the case of FL, the most meaningful advance will be the ability to differentiate patients at the time of diagnosis who have high-risk biology and are vulnerable to early relapse and death from the low-risk patients who will have good outcomes. Prognostic markers that can risk stratify patients with high-risk FL biology and predict histologic transformation, early relapse, and chemotherapy resistance will permit rational design of clinical trials that could significantly improve outcome for the 20% of FL patients in whom the majority of deaths will occur. This has the potential to change the natural history of FL as a disease and permit the adoption of novel treatment paradigms for the remaining 80% of patients with excellent outcomes.

At the time of relapse, we do not have adequate predictive biomarkers to inform who is more likely to benefit from what type of therapy. One attempt to answer this question will emerge from a planned prospective study from the National Clinical Trials Network in early-relapsing FL patients. Patients who experience relapse within 2 years of chemoimmunotherapy induction will be randomly assigned to 1 of 3 novel treatment regimens. Tissue specimens from diagnosis and relapse will be collected to explore the underlying biology of these patients, with the goal of establishing innovative biomarkers to identify the high-risk patients at diagnosis. This will then facilitate the future use of rational targeted therapies more precisely.

FL can no longer be approached as a one-size-fits-all disease. The emergence of important new clinical and biologic prognostic biomarkers is revealing the extent of the heterogeneity of FL. The time has arrived for a patient to receive a nuanced, precise, and risk-stratified approach to treatment at diagnosis, relapse, and time of transformation. However, the best way to combine and apply our clinicopathologic tools for individuals remains to be determined.

Efforts should persist in the rational design of clinical trials that risk stratify FL at both diagnosis and relapse, study molecular markers predicting poor outcome, and evaluate novel end points in this disease.

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