Prognostic Factors in Follicular Lymphoma

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ABSTRACT

Follicular lymphoma (FL) is one of the most common types of non-Hodgkin’s lymphoma. It is usually diagnosed at an advanced stage, for which many treatment options exist, however, no curative standard therapy has been identified. The outcome is highly variable with a median survival of approximately 10 years. The life expectancy of patients with FL has been extended with the use of rituximab, a monoclonal antibody targeting the CD20 antigen on FL cells, but there remains a group of patients who fail to respond to chemoimmunotherapy and die early of their disease. Transformation of FL to an aggressive histology is an important event with high morbidity and mortality. The Follicular Lymphoma International Prognostic Index has become the clinically useful prognostic tool, but gives only a rough estimate of expected outcome. There is a need for useful biomarkers for prediction of the disease course of single patients to individualize therapy, especially in the new era of chemoimmunotherapy.

INTRODUCTION

Follicular lymphoma (FL) constitutes approximately 20% of all newly diagnosed lymphoma cases, making it the second most common subtype of non-Hodgkin’s lymphoma worldwide and the most common subtype seen in North America. It is characterized by an indolent clinical course, typical morphology, and the presence of a chromosomal translocation, t(14;18)(q32;q21) or variant in 85% of patients. This chromosomal aberration results in the juxtaposition of the immunoglobulin heavy chain gene (IGH) on chromosome 14 with the BCL2 oncogene on chromosome 18 leading to constitutive and therefore inappropriate, expression of the BCL2 protein. Overexpression of BCL2 confers relative resistance to apoptosis, thus giving the cells a survival advantage that may facilitate the acquisition and retention of secondary genetic abnormalities.

FL is a heterogeneous entity with some patients developing progressive or transformed disease early and 15% dying within 2 years from diagnosis, while others remain alive for decades without need for treatment. This variability in outcome underscores the necessity to gain further insight into the biology and clinical behavior of the disease to enable individualized therapy.

TREATMENT AND CLINICAL COURSE

The median age at diagnosis of FL is 59 years with a male to female ratio of 1:1.7. FL is typically diagnosed in the advanced stages, with only 26% to 33% of patients presenting with stage I to II disease. With conventional chemotherapy, even if combined with radiotherapy, advanced-stage FL is incurable. It is characterized by an indolent course with patients developing slowly progressive lymphadenopathy over many years, with or without constitutional symptoms (eg, fever, weight loss, drenching night sweats). Temporary, spontaneous regressions occur in 20% of patients managed without initial therapy. Historically, median survival has ranged from 6 to 10 years.

Transformation to an aggressive lymphoma occurs at a rate of 3% per year and is associated with substantial morbidity and mortality. Transformation is defined as the development of a more aggressive histology lymphoma, most commonly diffuse large B-cell lymphoma (DLBCL), that is thought to be clonally related to the original FL.

Limited-stage FL treated with external-beam radiotherapy results in prolonged remission in 30% to 50% of patients and an apparent plateau on the disease-free survival curve after 15 to 20 years. Two randomized studies showed that deferred initial treatment in asymptomatic advanced-stage patients does not compromise long-term outcome or risk of transformation. FL is highly sensitive to antineoplastic agents early, but grows increasingly resistant with successive lines of therapy.

The chimeric anti-CD20 monoclonal antibody rituximab has significant activity alone and in combination with chemotherapy.
Biomarkers in Follicular Lymphoma

Multiparameter Indices

The International Prognostic Index (IPI), originally developed for aggressive lymphoma, identifies four risk groups based on age, tumor stage, serum lactate dehydrogenase (LDH) level, performance status, and number of extranodal sites of disease. The IPI also reliably identifies risk groups among FL, but only classifies a small proportion of patients into the highest-risk category.

Three prognostic indices have been developed specifically for FL. The Italian Lymphoma Intergroup (ILI) index identifies three risk groups with 10-year survival rates of 65%, 54%, and 11% based on six clinical parameters (advanced age, male sex, number of extranodal sites of disease, B symptoms, serum LDH level, and erythrocyte sedimentation rate).

In 2004, the Follicular Lymphoma International Prognostic Index (FLIPI) was published resulting from a multicenter effort. It includes five parameters: age (> 60 years), stage (III-IV vs I-II), anemia (hemoglobin < 120 g/L), number of involved nodal areas (> 4 vs ≤ 4), and serum LDH (elevated v normal), and classifies patients into three groups with 10-year OS rates of 71%, 51%, and 36%, respectively. The FLIPI is predictive in patients treated with immunochemotherapy, but it only classifies a small proportion of patients into the highest-risk category.

Recently, the FLIPI-2 index was published incorporating beta-2 microglobulin, lymph node size larger than 6 cm, bone marrow involvement, anemia, and age older than 60 years (Table 1). All of these clinical indices are robust, easy to use in clinical practice, and of value for stratification in clinical trials; however, marked variations in outcome remain within each risk group.

Table 1. Adverse Factors in the FLIPI and the FLIPI2 Indices

<table>
<thead>
<tr>
<th>FLIPI(25)</th>
<th>FLIPI2(26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt; 60 years</td>
<td>Age &gt; 60 years</td>
</tr>
<tr>
<td>Stage III-IV</td>
<td>Bone marrow involvement</td>
</tr>
<tr>
<td>Anemia (Hb &lt; 120 g/L)</td>
<td>Anemia (Hb &lt; 120 g/L)</td>
</tr>
<tr>
<td>Number of involved nodal areas &gt; 4</td>
<td>Nodes &gt; 6 cm</td>
</tr>
<tr>
<td>LDH &gt; ULN</td>
<td>p2-microglobulin &gt; ULN</td>
</tr>
</tbody>
</table>

Abbreviations: FLIPI, Follicular Lymphoma International Prognostic Index; Hb, hemoglobin; LDH, lactate dehydrogenase; ULN, upper level of normal.

Biomarkers in Blood and Bone Marrow

There are conflicting reports regarding the prognostic impact of bone marrow (BM) involvement by FL. BM involvement was associated with decreased survival in several investigations including those of the ILI and the FLIPI. In contrast, others have not found BM involvement in itself to affect survival, but rather the histologic pattern or degree of marrow involvement. Importantly, BM status at the time of diagnosis has been included in the FLIPI-2.

The characteristic t(14;18)(q32;q21) generates a BCL2-IGH fusion gene, which can be used as a marker of disease detectable by polymerase chain reaction (PCR). This technique is highly sensitive and can routinely detect one translocation-positive cell in 10⁶ cells in peripheral blood (PB) or BM. PCR has been used for detecting minimal residual disease (MRD) after therapy. Three different breakpoints within the BCL2 gene have been identified in FL. However, the value of molecular monitoring in FL has not yet been established.

Currently, the implication of the presence of t(14;18)-positive cells detected by PCR remains unknown. Not all PCR-positive patients relapse, and some convert to PCR negativity without therapy. Furthermore, t(14;18)-positive cells have been found in patients in long-term remission after therapy for FL and BCL2 translocations have been detected by PCR in PB from healthy individuals and from autopsies from patients without lymphoma.

Other Biomarkers in Blood

Biomarkers readily measurable from the PB that have been correlated with outcome in FL are summarized in Table 2. Beta-2 microglobulin has reached wide acceptance as a prognostic marker and is included in FLIPI-2 but is not universally available in retrospective cohorts. LDH is a robust prognostic factor in several types of lymphoma; however, in FL it is elevated in only 20% of patients. Serum angiogenic factors have been analyzed in heterogeneous groups of patients with lymphoma with partly conflicting results.

Interestingly, polymorphisms leading to high expression of tumor necrosis factor may influence outcome in non-Hodgkin’s lymphoma, pointing to the importance of host factors.

FL is a neoplasm of germinal center B cells that mimic the architecture of normal secondary lymphoid follicles. Benign and malignant follicles contain a heterogeneous mixture of non-neoplastic cells including T cells, benign B cells, follicular dendritic cells (FDC), and macrophages. The majority of patients with FL manifest two major types of neoplastic B cells: small centrocytes and larger centroblasts. Histologic grading of FL is based on the relative proportions of these two cell types.

Histologic Grade

The current WHO classification recognizes three histologic grades (grades 1 to 3) of FL. Grading of FL is poorly reproducible.
among pathologists, calling into question studies that report prognostic impact based solely on this variable. Similarly, subtle architectural variations, such as the presence of diffuse areas, have not been well annotated in most FL studies. Immunostaining for FDCs has been infrequently performed, making it difficult to accurately determine true follicle formation in most reported studies. Several studies have suggested a correlation between grade and survival in FL. It correlates with the number of large cells and thus with histologic grade and with OS in univariate analysis but not in multivariate analysis.

**Immunophenotype: Neoplastic Cells**

**Proliferation index.** The proliferation index has prognostic value in FL and correlates with the number of large cells and thus with histologic grade and with OS in univariate analysis but not in multivariate analysis.

**BCL2 and BCL2 family proteins.** The family of BCL2-related proteins plays a central role in the surveillance of mitochondrial integrity by balancing between pro- and antiapoptotic members. Only a few of these proteins have been studied in relationship to prognosis in FL and no consistent correlation has been found. As a possible exception, a high number of MCL1-positive centroblasts has been correlated with poor OS. Similarly, expression of the long RNA isoform of BCLX has been correlated with prognosis in FL; however, expression of the protein did not.

Using reverse-phase protein microarrays, when microdissected follicles from reactive lymph nodes were compared with follicles with FL, most proapoptotic and antiapoptotic proteins except for BCL2 were present at comparable levels in BCL2-positive and BCL2-negative tumors with activation of the AKT/BAD signaling pathway. Gulmann et al also used a proteomic approach to analyze apoptotic pathways in FL. They found phosphorylation of AKT to be frequent in FL suggesting that it may act as an antiapoptotic agent along with other proapoptotic proteins. BCL2. High ratios of BCL2/BAK and BCL2/BAX were associated with early death from disease. The expression patterns of other proapoptotic members of the BCL2 family have largely unknown clinical impact. Future studies on the role of BCL2 family proteins in FL are relevant of distinguishing between FL grade 3A and 3B remains unclear. Identifying areas of diffuse disease, likely a harbinger of transformation, may be more clinically relevant.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Effect on OS</th>
<th>Other Effects</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute lymphocyte count</td>
<td>Longer if ↑ 52</td>
<td>Poor response and short TTF if ↑ 15,53</td>
<td></td>
</tr>
<tr>
<td>Beta-2 microglobulin</td>
<td>Shorter if ↑ 54</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shorter if ↑ 55</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shorter if ↑ 56</td>
<td>Shorter FFP if ↑</td>
<td>FL grade 3</td>
</tr>
<tr>
<td></td>
<td>No effect↑</td>
<td>Shorter FFP in first relapse if ↑ 57</td>
<td>FL grade 3</td>
</tr>
<tr>
<td>LDH</td>
<td>Shorter if ↑ 24,25,33</td>
<td>Poor response and short PFS if ↑ 58</td>
<td>Stage I-II</td>
</tr>
<tr>
<td>Albumin</td>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>Shorter if ↑ 24,25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td>Shorter if ↑ 58</td>
<td>Associated with other poor prognosis factors</td>
<td>Only 13% FL</td>
</tr>
<tr>
<td>FGF</td>
<td>Shorter if ↑ 60</td>
<td>Strong prognostic factor</td>
<td>Only 14% FL</td>
</tr>
<tr>
<td>VEGF + FGF</td>
<td>Shorter if ↑ 61</td>
<td>Combination independent of IPI</td>
<td>Only 14% FL</td>
</tr>
<tr>
<td></td>
<td>No effect↑</td>
<td></td>
<td>30% FL</td>
</tr>
<tr>
<td>Endostatin</td>
<td>Shorter if ↑ 63</td>
<td>Associated with VEGF ↑</td>
<td>Only 16% FL</td>
</tr>
<tr>
<td>TNF</td>
<td>Shorter if ↑ 64,65</td>
<td>Shorter FFS if ↑</td>
<td>Combined with receptor levels; 40% indolent lymphoma</td>
</tr>
<tr>
<td>TNF + sCD23</td>
<td>sICAM-1</td>
<td>Shorter if ↑ 67</td>
<td>Indolent lymphoma</td>
</tr>
<tr>
<td></td>
<td>Correlated to advanced disease and B-symptoms</td>
<td>Minority FL</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: FL, follicular lymphoma; OS, overall survival; TTF, time-to-treatment failure; FFP, freedom from progression; LDH, lactate dehydrogenase; PFS, progression-free survival; VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor; IPI, International Prognostic Index; TNF, tumor necrosis factor; sICAM-1, soluble inter-cellular adhesion molecule-1.
needed as they may identify patients who will benefit from novel BH3 mimetics, small molecules that specifically target BCL2 proteins.87,88

**Germinal center–related markers.** BCL6 encodes a zinc finger transcription factor involved in germinal center formation. CD10 is a membrane metalloendopeptidase expressed in the germinal center. In FL, these two markers of germinatal center origin are expressed in more than 95% and 75% of cases, respectively.99 Although their prognostic significance is established in DLBCL,90 their relevance in FL is less clear. Using immunohistochemical scoring systems to combine the percentage of positive malignant cells and their intensity of expression, the expression level of BCL6 has been correlated with favorable prognosis in FL.91 PU.1 is an ETS–domain transcription factor essential for the development of myeloid and B lymphoid cells. Constitutive PU.1 expression inhibits the earliest B-cell development, and low levels of PU.1 expression in hematopoietic progenitor cells are instrumental in promoting B-cell fate determination.92 Torlakovic et al93 studied the clinical impact of proteins associated with the germinal center in FL and found PU.1 protein expression, but not CD10 or BCL6, to be a favorable marker of OS independent of the FLIPI.99

**Clinical impact of proteins associated with the germinal center in FL**

**Patient predictors of outcome**

**Cell cycle regulators.** TP53 mutations have been reported in FL at diagnosis and are associated with an inferior survival.94 The presence of mutations leading to overexpression of the TP53 protein has been associated with transformation.95,96 Expression of TP53 protein is only moderately correlated with the presence of mutations (64%) and similarly with the expression of key regulators of TP53, such as CDKN1A or MDM2.97 The latter have also been shown to correlate with transformation.98 A gene expression profiling study of 57 patients with FL established a set of 14 genes that were highly expressed in patients with a favorable response to CHOP; however, only expression of CYCLIN B1 mRNA and protein level had prognostic impact independent of the FLIPI.99

Others. SOCS3 is a cytokine suppressor that inhibits cytokine signaling by Janus kinase (i.e., the JAK-STAT pathway). Overexpression of SOCS3 has been reported to be an independent unfavorable prognostic factor in FL.100 Overexpression of Ying-Yang 1, a zinc-finger protein regulating ILA gene expression, has also been linked with shorter survival in FL.101

**Cytogenetics and Molecular Genetics**

**Classical karyotyping.** Cytogenetic analysis of FL reveals a wide range of recurrent, nonrandom chromosomal alterations. FL is characterized by the overexpression of the antiapoptotic protein BCL2 as a result of the t(14;18)(q32;q21) or its rare variants t(2;18) and t(18;22). t(14;18) is present in approximately 85% of grade 1 and 2 FL and although it is considered insufficient on its own to cause FL, it provides these B cells with a survival advantage leaving them prone to accumulate additional genomic abnormalities.102 However, in a study of FL cases lacking the t(14;18), Horsman et al103 found distinct patterns of recurrent chromosomal alterations. In another report, FLs lacking the t(14;18) were less likely to express CD10 or BCL2, were more likely to arise at extranodal sites and had a better OS. Recently, Katzenberger et al104 described t(14;18)–negative FL characterized by a predominantly diffuse growth pattern, presenting clinically with large but localized inguinal tumors. The majority of FL patients without a t(14;18) have a deletion in 1p36.

The malignant cells in FL have an average of four to six different cytogenetic changes in addition to the BCL2 translocation.105,106 Only 5% of FL have t(14;18) as the sole detectable abnormality. The most common abnormalities are break points in chromosome 1, deletions in the long arm of chromosome 6 (6q−), trisomy 7 (+7), trisomy 12 (+12), presence of a derivative of chromosome 18 (der(18)), and duplication of X (+X).106

Early studies suggested that the number of abnormalities and presence of certain alterations (6q−, +7, +X, +21) were associated with an inferior survival.107 However, as karyotypic complexity increases after therapy,108 there is an inherent selection bias if biopsies

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**Fig 1.** Biomarkers impacting survival or transformation in follicular lymphoma (FL). Some biomarkers are controversial, resulting from treatment heterogeneity. Larger circle: features of nonmalignant cells. Inner circle: tumor cells. IR, immune response; FDC, follicular dendritic cells; IF, interfollicular; F, follicular; MVD, microvessel density; VEGF, vascular endothelial growth factor; EGF, endothelial growth factor; TNF, tumor necrosis factor; aUPD, acquired uniparental disomy; SNPs, single nucleotide polymorphisms.
taken at the time of progression or relapse are used for analysis. Tilley et al.\textsuperscript{109} reviewed 66 FL biopsies taken at the time of diagnosis and found deletion 6q to be associated with an inferior survival. H"oglund et al.\textsuperscript{110} and colleagues\textsuperscript{113} analyzed 124 samples of FL using chromosomal comparative genomic hybridization (CGH) and reported a number of recurrent alterations. In a subset analysis of 82 patients, loss of chromosomal material at 6q25-27 was a strong independent predictor of inferior survival. Cheung et al.\textsuperscript{114} identified two genomic regions, deletions in 1p36.22-p36.33 and 6q25, and 6q26, were associated with inferior survival. Ho\textsuperscript{110} and colleagues analyzed 124 samples of FL using microarray platforms with spotted DNA probes (array CGH). Viardot and colleagues\textsuperscript{115} reviewed 66 FL biopsies taken at the time of diagnosis and found no association between the number or type of cytogenetic abnormalities with clinical outcome.\textsuperscript{111}

**Comparative genomic hybridization.** Comparative genomic hybridization (CGH) can be performed by hybridizing tumor DNA against normal chromosomes (chromosomal CGH) or by using microarray platforms with spotted DNA probes (array CGH).\textsuperscript{112} Viardot and colleagues\textsuperscript{115} analyzed 124 samples of FL using chromosomal CGH and reported a number of recurrent alterations. In a subset analysis of 82 patients, loss of chromosomal material at 6q25-27 was a strong independent predictor of inferior survival. Cheung et al.\textsuperscript{114} identified two genomic regions, deletions in 1p36.22-p36.33 and 6q25-27, to be highly associated with transformation and inferior OS in patients with FL using array CGH. Recently, Schwaenen et al.\textsuperscript{115} reviewed 210 karyotypes from FL biopsies taken at diagnosis and found no association between the number or type of cytogenetic abnormalities with clinical outcome.\textsuperscript{111}

**Single nucleotide polymorphism arrays.** Copy-neutral loss of heterozygosity, undetectable by previously available methods, can be identified using single nucleotide polymorphism (SNP) arrays. This phenomenon, termed acquired uniparental disomy (aUPD), results from mitotic recombination or nondisjunction and has been described in FL.\textsuperscript{112,116} In a recent report, a number of recurring aUPDs were described in FL, of which aUPD on 1p36 correlated with shortened OS and aUPD on chromosome 16 was predictive of transformation.\textsuperscript{117}

### Table 3. Morphologic, Immunophenotypic, and Gene Expression Biomarkers in FL

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Impact on Survival</th>
<th>Risk of Transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphology</strong></td>
<td>Therapy-dependent, increasing grade associated with worse OS; effect possibly mitigated by doxorubicin-containing regimens</td>
<td>No effect</td>
</tr>
<tr>
<td>Tumor cells</td>
<td>No effect</td>
<td>Typically already present in grade 3B</td>
</tr>
<tr>
<td>Histologic grade\textsuperscript{70,74}</td>
<td>Controversial</td>
<td>Increased</td>
</tr>
<tr>
<td>Grade 3A v 3B\textsuperscript{25,55,70,71,76,77}</td>
<td>Controversial</td>
<td>No effect</td>
</tr>
<tr>
<td>Architecture (diffuse)\textsuperscript{26,71}</td>
<td>Controversial</td>
<td>Not studied</td>
</tr>
<tr>
<td>Proliferative rate\textsuperscript{22,78,79,133,174,175}</td>
<td>Controversial</td>
<td>Increased; associated with FDC disruption</td>
</tr>
<tr>
<td>Microenvironment</td>
<td>Unfavorable if immature phenotype of FDCs</td>
<td>No effect</td>
</tr>
<tr>
<td>Microvessel density\textsuperscript{129-131}</td>
<td>Controversial</td>
<td>Not studied</td>
</tr>
<tr>
<td>Macrophages\textsuperscript{63,127,135-137}</td>
<td>Controversial</td>
<td>Controversial</td>
</tr>
<tr>
<td>FDC\textsuperscript{152,135-178}</td>
<td>Controversial</td>
<td>Not studied</td>
</tr>
<tr>
<td>CD4\textsuperscript{+} T cells\textsuperscript{83,102,136,142,149,177}</td>
<td>Favorable</td>
<td>Not studied</td>
</tr>
<tr>
<td>CD8\textsuperscript{+} T cells\textsuperscript{136,142,148,147}</td>
<td>Unfavorable</td>
<td>Not studied</td>
</tr>
<tr>
<td>Regulatory T cells\textsuperscript{136,140-142,177}</td>
<td>Controversial</td>
<td>Not studied</td>
</tr>
<tr>
<td>Single gene (RNA and/or protein)</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>BCL6 and CD10\textsuperscript{91}</td>
<td>High ratio favoring BCL-2 associated with early death</td>
<td>Not studied</td>
</tr>
<tr>
<td>BCL2 and BCL2/BAX or BAX\textsuperscript{80,86}</td>
<td>High MCL-1 associated with worse outcome</td>
<td>Not studied</td>
</tr>
<tr>
<td>MCL\textsuperscript{91}</td>
<td>High BCL-2 associated with inferior survival</td>
<td>Not studied</td>
</tr>
<tr>
<td>BCLX\textsuperscript{82}</td>
<td>Expression of MUM1 may be associated with inferior survival</td>
<td>Not studied</td>
</tr>
<tr>
<td>MUM1\textsuperscript{110,181}</td>
<td>Variable; IR-1 associated with favorable OS, while IR-2 predicts for inferior OS</td>
<td>Not studied</td>
</tr>
<tr>
<td>PU.1\textsuperscript{93}</td>
<td>Expression of MUM1 may be associated with inferior survival</td>
<td>Not studied</td>
</tr>
<tr>
<td>SOCS3\textsuperscript{100}</td>
<td>Variable; IR-1 associated with favorable OS, while IR-2 predicts for inferior OS</td>
<td>Not studied</td>
</tr>
<tr>
<td>YY.1\textsuperscript{101}</td>
<td>Variable; IR-1 associated with favorable OS, while IR-2 predicts for inferior OS</td>
<td>Not studied</td>
</tr>
<tr>
<td>Multigene</td>
<td>No effect; strongly predictive of immediate clinical behavior</td>
<td>No effect</td>
</tr>
</tbody>
</table>

Abbreviations: FL, follicular lymphoma; OS, overall survival; FDC, follicular dendritic cells; IR, immune response signature.
colleagues\textsuperscript{121} confirmed these findings and identified a second polymorphic site related to the duration of response (FcRIIA 131 histidine/arginine). Similarly, Ghielmini et al\textsuperscript{122} reported that FcRIIA V/V was a predictive factor for event-free survival in rituximab monotherapy in FL. In contrast, Maloney et al and others\textsuperscript{123,124} did not find that FcRIIA or RIIA polymorphisms correlated with outcome in a study of R-CHOP.

Recently, Cerhan et al\textsuperscript{125} analyzed the impact of immune response SNPs in FL. Germine DNA was analyzed from patients with FL and a final set of four prognostically relevant immune response SNPs was identified (interleukin [IL] \textsuperscript{-8}, IL2, IL12B, and IL1RN). An outcome predictor was built using clinical and demographic factors combined with the four deleterious SNPs, which identified three risk groups with 5-year OS estimates of 96\%, 72\%, and 58\%, respectively. These patients were treated in an era before the use of rituximab. Although these four genes strongly predict outcome in patients with FL, none of them has been shown to be associated with the risk of developing FL\textsuperscript{125,126}. Together with the gene expression profiling (GEP) data below,\textsuperscript{127} these results suggest that the composition and functional status of the immune cells in the tumor microenvironment of FL may largely be driven by the genetics of the host.

**Non-Neoplastic Cells of the Microenvironment**

Lymph nodes involved with FL contain an admixture of non-neoplastic T cells, FDCs, macrophages, and other cells. A renewed interest in the microenvironment in FL followed the Leukemia Lymphoma Molecular Profiling Project (LLMPP) study in 2004\textsuperscript{127} in which GEP of whole-section frozen lymph nodes from 191 cases of FL demonstrated that the tumor microenvironment was the most important predictor of patient outcome. 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 some genes that are highly expressed in monoocytes/macrophages, and predicted a favorable outcome. The IR-2 signature included genes preferentially expressed in macrophages or dendritic cells, and predicted an unfavorable outcome. When patients were grouped into quartiles based on their survival-predictor scores, median survival rates ranged from 3.9 years to 13.6 years. These data highlighted the dominant prognostic role of the microenvironment in FL and suggested that the critical determinants of outcome might already be present at diagnosis.

After the LLMPP study, Glas et al\textsuperscript{128} performed GEP in a series of patients with FL who were grouped according to outcome and transformation. They defined an 81-gene predictor that correlated with immediate clinical behavior but not with long-term survival or risk of transformation. Interestingly, the genetic profile of patients with early transformation exhibited an activated status of T cells, FDCs, and macrophages characterized by genes that were present in the favorable IR-1 signature of the LLMPP study. Differences in experimental design, patient selection, gene expression platforms, and data analysis may explain the different conclusions between these two studies.

**Microvessel density.** Despite evidence for a vital role of angiogenesis in supporting tumorigenesis,\textsuperscript{129} some reports have suggested that increased vessel density correlates with better prognosis in FL.\textsuperscript{130} Intriguing results by Streubel et al\textsuperscript{111} demonstrated that endothelial cells of the microvasculature share the BCL2 rearrangement characteristic of FL, implying an intimate relationship between the two.

**FDC meshwork “immaturity.”** In addition to presenting antigens, FDCs comprise the scaffolding of both benign and malignant follicles, their dendritic processes providing structural support for B cells and non-neoplastic cells. An immature FDC phenotype is typified by expression of low-affinity nerve growth factor and CNA,\textsuperscript{42} accompanied by absence of mature FDC markers such as CD21, CD35, CD23, and CXCL13. This phenotype typifies undifferentiated fibroblastic cells and has been reported to correlate with progression

### Table 4. Molecular Genetic Biomarkers in FL

<table>
<thead>
<tr>
<th>Gene</th>
<th>Impact on Survival</th>
<th>Risk of Transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCL2\textsuperscript{185,188}</td>
<td>No clear survival difference between t(14;18)-positive FL and t(14;18) negative; suggestion that BCL2 breakpoint may affect survival, but unconfirmed by later studies</td>
<td>Somatic mutations of BCL2 gene on the translocated allele may rarely underlie transformation</td>
</tr>
<tr>
<td>MYC\textsuperscript{161-163,170}</td>
<td>Translocations of MYC rarely encountered at diagnosis; tend to confer an inferior survival</td>
<td>Infrequently associated with transformation, but when found they are associated with markedly inferior post-transformation survival</td>
</tr>
<tr>
<td>CDKN2A/B\textsuperscript{160,187}</td>
<td>Tumor suppressor genes on chromosome 9p21; only rarely found at diagnosis and not definitively associated with survival</td>
<td>Paired sample studies clearly show loss of CDKN2A/B through deletion, mutation, or hypermethylation is associated with histologic transformation</td>
</tr>
<tr>
<td>TP53\textsuperscript{95-97}</td>
<td>Loss of TP53 tumor suppressor only rarely seen at diagnosis; mutation is closely correlated with protein expression in FL</td>
<td>Loss of TP53 was the first gene implicated in transformation of FL; LOH not typically associated with 17p13 deletion</td>
</tr>
<tr>
<td>MDM2\textsuperscript{97,99}</td>
<td>None</td>
<td>Expression correlated with transformation, but not obviously correlated with TP53 gene status; found within the amplified region of chromosome 12q13-14 frequently associated with transformation</td>
</tr>
<tr>
<td>BCL6\textsuperscript{194,198}</td>
<td>No clear affect on survival in FL</td>
<td>Postulated to increase risk of transformation, but studies documenting the presence of translocations were based exclusively on inverse LD-PCR</td>
</tr>
<tr>
<td>CCNB\textsuperscript{199}</td>
<td>Increased expression of cyclin B1 associated with improved survival after CHOP chemotherapy</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: FL, follicular lymphoma; LOH, loss of heterozygosity; LD, long-distance; PCR, polymerase chain reaction; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone.
and/or transformation, but not in all studies. These changes correlate with loss of normal T-cell infiltration within follicles and might reflect a reduced cross-talk between these two cell types. Disruption of the tight FDC meshwork that characterizes FL has been associated with early transformation.

Macrophages. Tumor-associated macrophages have been described in a number of cancers, with increased numbers of benign macrophages typically associated with inferior survival. Farinha and colleagues demonstrated that the small subset of FL cases with high macrophage content experienced markedly decreased OS and PFS independent of the IPI. These data appear consistent with the LLMPP GEP study, with high macrophage content being a surrogate for the IR-2 signature. Alvaro et al reported opposite results finding that increased macrophages were associated with indolent clinical behavior. In a related study, these same authors found that 30% of macrophages express STAT1 protein and that presence of this subset of cells was associated with inferior survival. In two recent trials, a high macrophage content was associated with poor survival after chemotherapy, but not if combined with rituximab.

Macrophage plasticity has been well described, as these cells can be broadly separated into helper (M1) and healer (M2 or activated) cell types. In most tumors, tumor-associated macrophages are polarized to a M2 phenotype and appear to create a trophic environment that favors the tumor cells. A pan-macrophage marker (CD68) cannot capture these distinctions and may explain the discordant results.

T-cell subsets. T cells comprise a majority of the non-neoplastic cells in FL biopsies and play an important role in FL. Subsets include helper CD4-positive T cells, cytotoxic CD8-positive T cells, CD97-positive follicular helper T cells, and immunosuppressive regulatory T (Treg), but the exact role played by these subsets is not completely understood. Studies investigating the roles of CD4-positive and CD8-positive T cells in FL utilize different methodologies and have conflicting results. Tregs are a subset mostly with a CD4-positive CD25-positive forhead box protein P3-positive immunophenotype that serve a critical role in regulating CD4 and CD8 effector functions by suppressing proliferation and cytokine production of these cells. In epithelial malignancies, Tregs have been implicated in creating an immunosuppressed microenvironment that allows the tumor cells to escape the host immune response. Some investigators have reported that an increased number of Tregs was associated with favorable clinical behavior in FL, while others claim that the T-cell distribution may be more relevant. Recently, a low number of tumor-infiltrating programmed cell death 1-positive was associated with transformation and with inferior survival. Still, the precise role of T-cell subsets in FL biology remains unclear. The clinical impact of these cells in the microenvironment may be largely influenced by the characteristics of the patient and the treatments received. Specific therapies may have different effects on neoplastic cells versus cells within the microenvironment. Moreover, the role of host genetics influencing the immune microenvironment has only recently been explored.

Transformation

Histologic transformation is often heralded by a sudden change in clinical behavior, such as a rise in serum LDH or development of unusual extranodal sites of disease. It is a dominant clinical event, associated with shortened survival and relative resistance to therapy. The reported frequency varies dramatically ranging from 5% to 60%
of patients with FL. Since risk of transformation is time dependent, approximately 3% per year, this variation probably reflects differing durations of follow-up. The length of time that patients are followed and the rigor with which biopsies are obtained at progression heavily affect the reported frequency. Biomarkers that predict for survival do not necessarily correlate with those that predict risk of transformation.

An association between increased FLIPI score and frequency of transformation has been noted. Morphologic features associated with transformation risk include the presence of diffuse areas and disruption of the FDC meshwork. The presence of grade 3B FL and increased intrafollicular CD4-positive T cells have also been linked to early transformation.

Studies using paired samples (FL and subsequent DLBCL) have demonstrated that transformation is a molecularly heterogeneous event, including loss of TP53, loss of the CDKN2A tumor suppressor, or acquisition of MYC translocations, leading to upregulation of MYC or its target genes. Other molecular alterations include mutations of the coding region of BCL2 and translocations involving BCL6. Cytogenetic studies of paired samples have revealed candidate chromosomal alterations, including +7, +12q13-14, and -6q16-21. Lastly, a number of small studies using gene expression profiling of paired samples have been published. These demonstrate the molecular heterogeneity underlying transformation, implicating upregulation of p38 MAP KINASE, up-regulation of MYC and its target genes, and a generic increase in the mitotic machinery.

Recent data suggest that FL may comprise two major patient subgroups; those destined to develop transformation and those who will not. Distinguishing between these two clinical risk groups might have major implications for therapy.

**Translation Into the Clinic**

A summary of factors associated with outcome in FL is shown in Figure 1. As presented in Tables 2, 3, and 4, consensus on which biomarkers to use in clinical practice and how these might impact treatment decisions is still lacking. Most prognostic markers were studied before the introduction of rituximab and require validation in the current era of chemoimmunotherapy. At this time, disease behavior and robust indices, such as the FLIPI and FLIPI-2, remain the basis for clinical decision making. The key elements in the interaction of host immunogenetics, tumor microenvironment, and therapy in FL are still not well understood (Fig 2). The inclusion of correlative science into clinical trials should help to determine the precise role of biomarkers in predicting survival and transformation risk in FL. Prospective randomized clinical trials will be instrumental in defining future prognostic factors in FL, as uniform treatment groups can be compared (Fig 3). If patients who respond poorly to standard therapy can be identified and the mechanism(s) underlying their inferior outcome determined, these patients could reasonably be considered candidates for novel therapies with the potential for improved outcome avoiding toxicity from ineffective therapy.
Conception and design: Thomas Relander, Pedro Farinha, Randy D. Gascoyne
Administrative support: Randy D. Gascoyne
Provision of study materials or patients: Randy D. Gascoyne
Collection and assembly of data: Randy D. Gascoyne

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Biomarkers in Follicular Lymphoma

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133. Mantovani A, Sica A, Sozzani S, et al: The chemokine system in diverse forms of macrophage ...
CORRECTIONS

Author Corrections


The primary institutional affiliation for David Barad was inadvertently omitted and should have been listed as The Center for Human Reproduction, New York, NY.

The authors apologize to the readers for the mistake.

DOI: 10.1200/JCO.2010.32.8278


In Table 2 and Table 4, the univariate and multivariate results (HR, 95% CI, and P values) for DFS and OS were presented for dMMR and pMMR patients who were not treated, whereas they should have been given for patients who were treated.

The authors apologize to the readers for the mistakes.

DOI: 10.1200/JCO.2010.32.8286

Journal Corrections


In Table 1, under Primary Tumors, the value for FA (KEGG) in the column “P Reduced Multivariate” was inadvertently omitted and should have been < .001.

Journal of Clinical Oncology apologizes to the authors and readers for the mistake.

DOI: 10.1200/JCO.2010.32.8252


The left-hand image in Figure 3 was identified as “Interfollicular CD4+T cells,” whereas it should have been “Intrafollicular CD4+T cells.”

Journal of Clinical Oncology apologizes to the authors and readers for the mistake.

DOI: 10.1200/JCO.2010.32.8260