From the Departments of Malignant Hematology (CB) and Hematopathology (LZ, MN) at the H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida.

Submitted July 6, 2011; accepted January 29, 2012.

Address correspondence to Celeste Bello, MD, MSPH, Department of Malignant Hematology, Moffitt Cancer Center, 12902 Magnolia Drive, FOB-3 HEM PROG, Tampa, FL 33612. E-mail: Celeste Bello@moffitt.org

No significant relationship exists between the authors and the companies/organizations whose products or services may be referenced in this article.

The authors have disclosed that this article discusses unlabeled/unapproved uses of the drugs ofatumumab, epratuzumab, and lenalidomide for follicular lymphoma.

Introduction

For the most part, follicular lymphoma (FL) is considered an indolent type of non-Hodgkin lymphoma (NHL). It comprises about 20% of all NHLs and is the most common subtype of indolent NHL. The disease is characterized by a relapsing and remitting pattern, with mainly nodal and bone marrow involvement. This results in a chronic illness, which is managed with close observation and chemotherapy when appropriate. No consensus on the optimal treatment of FL exists, and it remains essentially an incurable disease.

Much heterogeneity exists within the FL category. World Health Organization (WHO) histologic grades 1 and 2 are considered the indolent subtypes, and grade 3 is considered the more aggressive subtype (both 3a
These categories are defined by the presence and histologic appearance of centroblasts (Figs 1 and 2). Variability in the behavior of the lymphoma exists even within a specific grade. Several prognostic features have been evaluated to better categorize the disease, with varied results.

With no gold standard of therapy, several treatment regimens can be considered when determining which chemotherapy to use for patients with FL. Some of the more common regimens include cyclophosphamide-based treatments (such as cyclophosphamide, vincristine, and prednisone plus rituximab [R-CVP] and cyclophosphamide, doxorubicin, vincristine, and prednisone plus rituximab [R-CHOP]), rituximab alone or in combination chemotherapy, bendamustine, and fludarabine-based treatments. Radioimmunotherapy

Fig 1A-B. — (A) Low-powered view of abnormal follicular proliferation arranged in a “back-to-back” pattern. (B) Low-grade follicular lymphoma (< 15 centroblasts/high-powered field).

Fig 2A-D. — Follicular lymphoma grade 3. (A) Neoplastic follicles of varying size with diminished mantle zones, lacking tingible body macrophages and composed of small and large centrocytes and centroblasts, > 15/high-powered field (inset). (B) Neoplastic follicles positive for CD10, (C) BCL2, and (D) BCL6.
is also an effective treatment option in the upfront or relapsed setting. Despite these numerous treatment options, no one regimen has been shown to be superior with regard to overall survival (OS). As a result, management of patients with FL varies among clinical practices.

Several advances in identifying prognostic markers and in treating FL have been made in the past few years. This article reviews the utility of certain prognostic markers with regard to the current management of FL as well as current and emerging therapies.

Pathogenesis

FL arises from germinal center B cells of lymphoid follicles. Almost all FLs carry breaks at 18q21, with approximately 85% of them having a translocation involving chromosomes 14 and 18 [t(14;18)(q32;q21)].

This translocation results in the juxtaposition of the oncogene B-cell leukemia-lymphoma 2 (BCL-2) on chromosome 18, with the immunoglobulin heavy-chain locus on chromosome 14. This leads to the overexpression of BCL-2, which blocks apoptosis and gives the cells a survival advantage.

Although the overexpression of BCL-2 appears to play a major role in the pathogenesis of FL, it does not explain the entire pathogenesis of the disease, as demonstrated in a study showing that the t(14;18) can be detected in healthy individuals without FL. Other factors such as chronic antigen stimulation, other genetic lesions, and the tumor microenvironment may play an additional necessary role in the pathogenesis of FL.

Prognostic Factors

Over the past few years, several prognostic factors have been identified in FL. In 2004, observations from an international cooperative group study resulted in the formation of the Follicular Lymphoma International Prognostic Index (FLIPI). The following five characteristics at diagnosis were found to correlate with a poor prognosis: age > 60 years, Ann Arbor stage III–IV, hemoglobin level < 12 g/dL, more than 4 nodal areas, and serum lactate dehydrogenase (LDH) level above normal. Based on the presence or absence of these factors, patients were stratified into three risk groups (low, intermediate, or high), which correlated with OS (Table 1).

The FLIPI is an effective and easy-to-use tool to categorize patients with FL, but it has limitations, the most important being that it does not predict which patients need treatment or when they need treatment.

A more recent evaluation looked at a second prognostic index called the FLIPI-2. This study evaluated 942 patients with newly diagnosed FL who received immunochemotherapy. The following factors were found to be significant and were included in the scoring index: age > 60 years, hemoglobin level < 12 g/dL, dimension of largest involved lymph node > 6 cm, beta-2 microglobulin level > normal, and bone marrow involvement. Based on the presence of these factors, patients were classified into one of three different risk categories for relapse: low, intermediate, or high. The FLIPI-2 was found to be predictive of treatment outcomes in patients with newly diagnosed FL who received immunochemotherapy. However, like the initial FLIPI, the FLIPI-2 does not predict when to initiate treatment and which treatment to use.

Genetic alterations have been shown to predict prognosis in certain cases of FLs. Mutations involving p53 were found to correlate with survival. These mutations were rare at the time of diagnosis, occurring in only 6% of the patients tested. In this small group, patients with a variant allele of SNP rs645732 had a shorter time from diagnosis to transformation and a higher risk of transformation compared with other genotypes.

Transformation of FL to a large-cell lymphoma has also been noted with specific genetic alterations. A recent study showed that single nucleotide polymorphisms (SNPs) on chromosome 6p can predict transformation independent of other variables, including those noted in the FLIPI. In this study, patients with a variant allele of SNP rs645732 had a shorter time from diagnosis to transformation and a higher risk of transformation compared with other genotypes.

The lymphoma microenvironment has also been found to affect disease prognosis. Gene expression profiling and immunohistochemical evaluation of the nonlymphoma cells in the FL microenvironment have been shown to correlate with survival. Cells such as dendritic cells, macrophages, and T-helper/regulatory cells seem to play a major role.

In a study performed by Dave et al., gene expression profiling of the nonlymphoma cells in the microenvironment made it possible to categorize patients with FL into two distinct groups: immune-response 1 signature and immune-response 2 signature. Immune signature-1—en-
coded genes were expressed in T cells and macrophages, whereas immune signature-2–encoded genes were expressed in macrophages and dendritic cells. Patients with an increased expression of immune-response 1 appeared to have a favorable survival, with a relative risk of death of 0.15 compared with 9.35 for those with an increased expression of immune-response 2.

Another study evaluating the FL microenvironment through immunohistochemistry patterns showed that the presence of certain nonlymphoma cells correlated with more aggressive clinical features. The study found two specific patterns in the reactive microenvironment. The first pattern was mainly composed of T lymphocytes and macrophages and was significantly associated with a favorable clinical course. The second pattern was predominantly composed of CD57+ T cells (natural killer cells) and was associated with a significantly higher frequency of adverse features such as B symptoms and bone marrow involvement.

Despite these prognostic markers and tools, the decision to treat patients with FL is still based largely on comorbidities, symptoms, and patient preference. It remains a decision that is highly individualized and based mainly on clinical judgment.

### Treatment

**Frontline Treatments**

Treatment of FL has evolved over the past few decades. Several regimens have demonstrated efficacy in improving PFS and time until treatment failure. In addition, maintenance and consolidation treatments have been shown to improve outcomes in patients with low-grade FLs.

The decision to treat patients is based largely on histologic grade and the extent of disease. Generally, grade 3 FLs are treated like diffuse large B-cell lymphomas. For grades 1 and 2, the decision to treat is not as clear. For the purpose of this section of the article, treatments of FL refer to low-grade lymphomas.

For localized disease (Ann Arbor stage I-II), locoregional radiation therapy (RT) is the preferred method of treatment (Fig 3). A subset of these patients may even be cured with RT alone. A study evaluating 106 patients with localized disease who received RT had a freedom-from-treatment failure rate of 39% at 15 years. Observation is also an option in patients who may develop excessive toxicities from RT due to the location of the lymphoma or patient comorbidities.

Advanced disease (Ann Arbor stage III-IV or extensive stage II) is not curable with conventional treat-
ments. For that reason, treatment is usually reserved for patients who are symptomatic or who have bulky disease (Fig 3). Systemic chemotherapy is the preferred method of treatment for advanced disease, with RT reserved for bulky refractory areas or palliation. Determining which systemic treatment is optimal and at which dose are matters of debate.

Immunotherapy with rituximab alone or combined with chemotherapy has demonstrated good results with regard to PFS and, in some instances, OS. Single-agent rituximab in treatment-naïve patients with FL has yielded an overall response rate (ORR) of 72% to 73%, with a median time to disease progression of just over 2 years.\textsuperscript{15,16} In the relapsed setting, rituximab has yielded an ORR of 40%, with a median time to disease progression of about 18 months.\textsuperscript{17} In addition to being an effective therapy for FL, rituximab has a low toxicity profile and is tolerated well, even in elderly patients. It also seems to improve the outcomes of certain chemotherapies when added to the regimen.

Several chemotherapeutic regimens when combined with rituximab have shown significant efficacy in the frontline setting (Table 2).\textsuperscript{18-21} One example is a phase III study by Hiddemann et al,\textsuperscript{19} which showed that R-CHOP was superior to CHOP alone. In this study, 428 patients with previously untreated stage III or IV FL were randomized to receive R-CHOP or CHOP for 6 cycles; the ORR was 96% and 90%, respectively. However, the time to treatment failure was significantly better in the R-CHOP group than in the CHOP group. At a median follow-up of 18 months, 12.5% of patients in the R-CHOP group experienced treatment failure compared with 30% of the CHOP group ($P < .001$).

Another example is a study performed by Marcus et al\textsuperscript{18} comparing R-CVP with CVP alone. Patients in the R-CVP treatment group had an ORR of 81% and a median time to disease progression of 34 months compared with 57% and 15 months, respectively, for the CVP-alone group.

Several chemotherapy regimens have shown efficacy in the treatment of FL in the frontline setting, and the decision of which one to use requires a balance of risks and benefits. Regimens such as rituximab plus fludarabine, mitoxantrone, and dexamethasone (R-FND), bendamustine and rituximab (BR), R-CHOP, and R-CVP have all been shown to be effective. Since the majority of these regimens have not been compared in a head-to-head study, determining which one is superior is challenging.

The exception to this is a study done by Rummel et al\textsuperscript{20} evaluating R-CHOP vs BR. This study evaluated 549 patients with previously untreated indolent NHL or mantle cell lymphoma. Approximately 55% of these patients had FL. The ORR was essentially equal between the two groups (93.8% for BR vs 93.5% for R-CHOP). However, the PFS was significantly better in the BR group, with a median PFS of 55 months vs 35 months for the R-CHOP group ($P = .0002$). In addition, the tolerability profile was better in the BR group than in the R-CHOP group.

Radioimmunotherapy with yttrium-90 (${}^{90}\text{Y}$)-ibritumomab tiuxetan and iodine-131 (${}^{131}\text{I}$)-tositumomab has also been shown to be effective in the frontline FL setting. \textsuperscript{131}I-tositumomab was evaluated in 76 patients with previously untreated stages III and IV FL.\textsuperscript{22} After a single 1-week course of treatment, the ORR was 95%, with a 75% complete remission (CR) rate. The median PFS was 6.1 years.

In a follow-up analysis, after a median of 10 years of follow-up, the median duration of response was still around 6 years.\textsuperscript{23} However, in the 57 complete responders, the median PFS was 10.9 years. A similar study evaluating \textsuperscript{90}Y-ibritumomab tiuxetan in 59 patients with previously untreated FL is underway.\textsuperscript{24} At 1 year, the ORR was 72%, with 52% obtaining a CR. Long-term follow-up results have not been reported yet, but at a median follow-up of 23 months, the PFS was approximately 18 months.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|l|}
\hline
Trial & No. of Patients & Regimen Tested & Overall Response Rate & Median Response Duration \\
\hline
Marcus et al\textsuperscript{18} (2008) & 321 & R-CVP vs CVP & 81% R-CVP & 34 mos TTP R-CVP \\
& & & 57% CVP & 15 mos TTP CVP \\
& & & ($P < .0001$) & ($P < .0001$) \\
Hiddemann et al\textsuperscript{19} (2005) & 428 & R-CHOP vs CHOP & 96% R-CHOP & Not reported \\
& & & 90% CHOP & ($P < .01$) \\
Tsimberidou et al\textsuperscript{21} (2002) & 73 & FMD with interferon-\alpha & 97% & Not reported \\
Rummel et al\textsuperscript{20} (2009) & 549 & BR vs R-CHOP & 93.8% BR & 55 mos PFS BR \\
& & & 93.5% R-CHOP & 35 mos PFS R-CHOP \\
\hline
\end{tabular}
\caption{Summary of Studies Evaluating Frontline Chemotherapy in Follicular Lymphoma}
\end{table}

BR = bendamustine, rituximab, CHOP = cyclophosphamide, doxorubicin, vincristine, prednisone, CVP = cyclophosphamide, vincristine, prednisone, FMD = fludarabine, mitoxantrone, dexamethasone, PFS = progression-free survival, R-CHOP = rituximab-cyclophosphamide, doxorubicin, vincristine, prednisone, R-CVP = rituximab-cyclophosphamide, vincristine, prednisone, TTP = time to disease progression.
**Consolidation and Maintenance Treatments**

Consolidation treatment has been shown to be beneficial in patients with advanced stages of FL. Consolidation treatment with radioimmunotherapy in previously untreated patients with FL was evaluated in a phase III study called the First-line Indolent Trial (FIT). This study randomized patients who obtained CR or partial remission (PR) with a frontline regimen to receive further treatment with $^{90}$Y-ibritumomab tiuxetan or observation. Those who received radioimmunotherapy had a median PFS of 36.5 months vs 13.3 months for the observation group. Those who obtained CR to the initial chemotherapy had an even better response to consolidation treatment with $^{90}$Y-ibritumomab tiuxetan, with a median PFS of 54 months.

An updated analysis after a median follow-up of 5.5 years showed that the median PFS for all patients who received $^{90}$Y-ibritumomab tiuxetan improved to 49 months compared with 14 months for the control group. In addition, in this updated analysis, the median PFS had not yet been reached in those patients who received $^{90}$Y-ibritumomab tiuxetan after obtaining CR to the initial therapy. There was no difference in OS in the initial study or in the 5.5-year follow-up analysis. Only 14% of the patients in this study received rituximab as part of the frontline therapy.

On a similar note, maintenance therapy with rituximab has also been shown to be beneficial in patients with FL. The Primary Rituximab and Maintenance (PRIMA) study assessed the use of rituximab maintenance in patients with previously untreated FL. These patients received an induction regimen with chemotherapy plus rituximab. Those who obtained CR or PR were randomized to either observation or rituximab (1 dose every 2 months for 2 years). The 3-year PFS rate was found to be about 75% in the rituximab maintenance group compared with approximately 58% for the observation group.

At a median follow-up of 36 months, the median time to disease progression in the maintenance group had not yet been reached at the time the study was published.

In addition to the PRIMA study, several other studies using maintenance rituximab at varying schedules have shown similar results (Table 3). A meta-analysis published in 2009 summarizes these findings and supports the use of maintenance rituximab, with an across-the-board improvement in PFS and an improvement in OS in a pooled analysis.

**Treatment for Relapsed/Refractory Disease**

Relapsed FL does not necessarily require immediate treatment. The principles on when to initiate treatment in the frontline setting apply in the relapsed setting as well, and the majority of asymptomatic patients can be observed. When feasible, a rebiopsy should be performed to rule out transformation, especially in symptomatic patients. Since there is no standard of therapy, numerous treatment options exist, and patients who require treatment should be encouraged to enroll in a clinical trial if available. The regimens previously mentioned in the frontline setting are all options in the relapsed/refractory setting. Based on prior response to therapy, rechallenging with the initial therapy can be considered. In general, treatment of relapsed/refractory disease is based on patient and tumor characteristics and must be individualized on a patient-by-patient case.

**Stem Cell Transplantation**

The benefit of high-dose therapy followed by autologous stem cell transplant (HDT/ASCT) in FL is debatable. Several studies have shown an improvement in PFS and OS, but these studies were conducted prior to the rituximab era. A study by the Groupe d’Etude des Lymphomes de l’Adulte (GELA) evaluating HDT/ASCT after salvage chemotherapy with or without rituximab

<table>
<thead>
<tr>
<th>Trial</th>
<th>No. of Patients</th>
<th>Type of Lymphoma</th>
<th>Type of Induction</th>
<th>Response to Induction</th>
<th>Maintenance Rituximab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hainsworth et al (2005)</td>
<td>90</td>
<td>Relapsed FL, SLL</td>
<td>Rituximab</td>
<td>SD/CR/PR</td>
<td>Weekly × 4 every 6 mos × 2 yrs</td>
</tr>
<tr>
<td>Hochster et al (2005)</td>
<td>304</td>
<td>Untreated FL, SLL</td>
<td>CVP</td>
<td>CR/PR</td>
<td>Weekly × 4 every 6 mos × 2 yrs</td>
</tr>
</tbody>
</table>

CHOP = cyclophosphamide, doxorubicin, vincristine, prednisone, CR = complete remission, CVP = cyclophosphamide, vincristine, prednisone, FL = follicular lymphoma, FMC = fludarabine, mitoxantrone, cyclophosphamide, PR = partial remission, R = rituximab, SD = stable disease, SLL = small lymphocytic lymphoma.
Emerging Therapies

Monoclonal Antibodies

Due to the success of rituximab, several monoclonal antibodies are now in development for the treatment of FL. They are an especially attractive group of drugs, offering a targeted form of therapy with minimal toxicities. Although rituximab is currently the only US Food and Drug Administration (FDA)-approved monoclonal antibody for FL, the compounds discussed here are currently in development and have shown activity in FL.

Ofatumumab is a fully humanized monoclonal antibody against CD20. Previously referred to as HuMax-CD20, it targets a novel epitope of the CD20 molecule. Ofatumumab has similar antibody-dependent cellular cytotoxicity when compared with rituximab, but it delivers stronger complement-dependent cytotoxicity in vitro models.

The first in-human study evaluating patients with relapsed/refractory FL treated with ofatumumab reported modest results. This small phase I study reported an ORR of 20% to 60%. However, a small subset of patients who received rituximab in a prior treatment regimen reported an ORR of 64%. Due to these findings, a larger phase II study was performed with 116 patients with low-grade FL refractory to rituximab. Refractoriness to rituximab was defined as failure to achieve at least PR or disease progression following a response within 6 months of the last treatment with single-agent rituximab or a rituximab-containing regimen. Patients received 8 weekly infusions of ofatumumab, at either a 500-mg dose (n = 30) or a 1,000-mg dose (n = 86); dose 1 was 300 mg, while doses 2 through 8 were 500 or 1,000 mg. The ORR in the total population was 11%. In those patients whose disease was refractory to treatment with rituximab as monotherapy, the ORR was 22%. Although modest, these results demonstrated that ofatumumab is effective in some patients with low-grade FL despite prior treatment failure with rituximab.

Further studies evaluating ofatumumab alone or combined with other chemotherapeutic agents in the treatment of FL are underway. One study of interest will evaluate rituximab vs ofatumumab in relapsed FL after prior treatment with rituximab. Another planned study will evaluate the combination of bendamustine with ofatumumab vs bendamustine alone.

Another anti-CD20 monoclonal antibody is GA101. This is a humanized type II monoclonal antibody. Type II anti-CD20 monoclonal antibodies (also referred to as tositumomab-like) differ from type I (rituximab-like) in that they do not activate complement dependent cytotoxicity and more potently evoke direct programmed cell death.

In a phase II study in patients with relapsed/refractory indolent NHL, 40 patients were treated with GA101 on days 1, 8, and 22, and then every 21 days, for a total of 9 treatments. Of these 40 patients, 22 received what was referred to as a high-dose regimen. In the high-dose group, 55% had a response (CR or PR) to therapy, with half of them occurring in patients whose disease was considered refractory to rituximab. A randomized phase II study comparing GA101 and rituximab in relapsed indolent NHL is currently underway.

Epratuzumab is another monoclonal antibody in development. It is fully humanized and targets CD22, a transmembrane protein found on B cells, which is thought to play a role in activation and adhesion. Its mechanism of action appears to be through antibody-dependent cytotoxicity and also possibly through a second mechanism involving the internalization of the antibody receptor complex, which leads to activation of nonreceptor tyrosine kinases.

A small phase I/II study evaluated patients with relapsed or refractory NHL treated with epratuzumab. The subset of patients with FL had an ORR of 43% at the 360-mg/m² dose. To improve the ORR, a second study combined epratuzumab with rituximab in patients with
relapsed/refractory FL. A total of 49 patients received 4 weekly doses of both drugs. The ORR was 54%, with 10 CRs and a median duration of response of 13.4 months.

A more extended dosing regimen combining both epratuzumab and rituximab was tested in the frontline FL setting. This study evaluated 60 patients with previously untreated FL. The patients received both drugs weekly for 4 doses, then every 2 months for 4 doses. An interim analysis showed the ORR to be 84%, with 33% obtaining CR. Final results from this study are pending.

Another monoclonal antibody in development is an antibody against CD19. CD19 is a component of the B-cell receptor complex and functions to control the signaling threshold for B-cell development and humoral immunity. A few different compounds are in the pipeline, and they appear to exert their cytotoxicity through antibody-dependent cellular cytotoxicity. Ongoing clinical trials of these agents are in the early phases.

**Drugs That Alter the Microenvironment**

The immunomodulatory agent lenalidomide is the drug in this category that is farthest along in development for the treatment of FL. Lenalidomide has been approved by the FDA for treatment of myelodysplastic syndrome and multiple myeloma. Recent studies have demonstrated that it appears to have an effect on the cells in the microenvironment in NHL. The exact mechanism is not known but appears to involve the downregulation of several key cytokines as well as activation of T and NK cells.

A phase II study evaluating the combination of lenalidomide, dexamethasone, and rituximab in patients with relapsed or refractory NHL whose disease was refractory to rituximab showed an ORR of 57%. These results are encouraging, but the exact role of lenalidomide in FL is unclear. Numerous studies evaluating lenalidomide in the frontline, relapsed, and maintenance settings are underway.

Phosphatidylinositol-3-kinase (PI3K) inhibitors are also thought to interact with the microenvironment in NHL. PI3K is part of a cell-signaling pathway that promotes cell growth and survival. It appears to be commonly activated in B-cell malignancies through constitutive activation of the B-cell receptor, which is thought to be from exposure to survival factors present in the microenvironment. Lymphoma cell lines were stimulated with various cytokines and chemokines mimicking signaling from the microenvironment, resulting in phosphorylation of Akt. This phosphorylation was inhibited by the use of a PI3K inhibitor known as CAL-101 and resulted in decreased cell growth and survival.

These results suggest that PI3K may play an important role in regulating signals between malignant B cells and their microenvironment. Inhibition may enhance the effect of cytotoxic drugs by inhibiting the protective signals of the microenvironment. Further studies to evaluate the role of these drugs in FL are ongoing.

**Conclusions**

Low-grade follicular lymphoma is an indolent but incurable lymphoma. Its heterogeneous clinical course complicates decisions on which patients to treat and when. Existing prognostic scores as well as novel ones help in making treatment decisions, but for the most part, treatment is addressed on a patient-by-patient basis. Newer molecular tests have shown promise in predicting which patients will require treatment, but these tests are in the early phases of development.

When a patient is deemed in need of therapy, several effective treatment options exist. None of them has been shown to be curative, but the addition of maintenance and consolidation treatments has demonstrated an improvement in progression-free survival and has shown a trend in improvement in overall survival. As the data in these maintenance and consolidation trials mature, the effects of these agents on overall survival will become clearer.

Novel therapies have also shown promise in the treatment of follicular lymphoma. The development of new monoclonal antibodies is an expanding and promising field. These drugs provide an effective targeted therapy with minimal toxicities. In addition, novel approaches addressing the follicular lymphoma microenvironment may hold the key to improving outcomes. Continued research in these areas is paramount to our understanding and treatment of follicular lymphoma.

**References**


