prognostic factor in chronic lymphocytic leukemia. Leuk Res 2010; **34**: 892–8.

10. Haferlach C, Dicker F, Schnittger S, ***et al***. Comprehensive genetic characterization of CLL: a study on 506 cases analysed with chromosome banding analysis, interphase FISH, IgV(H) status and immunophenotyping. Leukemia 2007; **21**: 2442–51.

11. Wang L, Lawrence MS, Wan Y ***et al***. SF3BI and other novel cancer genes in chronic lymphocytic leukemia. N Engl J Med 2011; **365**: 2497–506.

12. Gribben JG, O'Brien S. Update on therapy of chronic lymphocytic leukemia. J Clin Oncol 2011; **29**: 544–50.

13. Tarn CS, O'Brien S, Wierda W, ***et al***. Long-term results of the fludarabine, cyclophosphamide, and rituximab regimen as initial therapy of chronic lymphocytic leukemia. Blood 2008; **112**: 975–80.

14. Wierda W, O'Brien S, Wen S, ***et al***. Chemoimmunotherapy with fludarabine, cyclophosphamide, and rituximab for relapsed and refractory chronic lymphocytic leukemia. J Clin Oncol 2005; **23**: 4070–8.

15. Schetelig J, Biezen van A, Brand R, ***et al***. Allogeneic hematopoietic stem-cell transplantation for chronic lymphocytic leukemia with I7p deletion: a retrospective European Group for Blood and Marrow Transplantation analysis. J Clin Oncol 2008; **26**: 5094–100.

16. Stilgenbauer S, Zenz T, Winkler D, ***et al***. Subcutaneous alemtuzumab in fludarabine-refractory chronic lymphocytic leukemia: clinical results and prognostic marker analyses from the CLL2H study of the German Chronic Lymphocytic Leukemia Study Group. J Clin Oncol 2009; **27**: 3994–4001.

17. Wierda WG, Chiorazzi N, Dearden C, ***et al***. Chronic lymphocytic leukemia: new concepts for future therapy. Clin Lymphoma Myeloma Leuk 2010; **10**: 369–78.

18. Dreger P, Corradini P, Kimby E, ***et al***. Indications for allogenic stem cell transplantation in chronic lymphocytic leukemia: EBMT transplant consensus Leukemia 2007; **21**: 12–17.

FOLLICULAR LYMPHOMA

F. De Angelis, R. Foà

Hematology Division, Department of Cellular Biotechnologies and Hematology, Univerity of Rome "Sapienza", Rome, Italy

Correspondence: rfoa@bce.uniroma1.it

Follicular lymphoma (FL) is the second most common type of non-Hodgkin lymphoma (NHL) in Western Countries, accounting for 20% of all NHL and for 70% of all indolent forms, with a median age at diagnosis of about 60 years [1–3]. Before the advent of chemotherapy, the majority of patients with FL died within 5 years. With the current therapies, the expected median survival is approximately 8–10 years [4]. About 85% of FL cases have a specific translocation t(14;18) that leads to the overexpression of the BCL2 protein, a member of a family of anti-apoptotic proteins, although other genetic alterations may be detected in this subtype of lymphoma. As defined by the WHO, FLs are characterized by a follicular growth pattern including centrocytes (small- to medium-sized cells) and centroblasts (large cells), and are graded from I to III according to the amount of centroblasts present. The clinical aggressiveness of the tumor increases with an increasing numbers of centroblasts. Grade I is defined by ⩾5 centroblasts/high power field (hpf) (follicular small cleaved), Grade II by 6 to 15 centroblasts/hpf (follicular mixed), Grade III by more than 15 centroblasts/hpf (follicular large cell). Grade III has been subdivided into Grade IIIa, in which centrocytes are present and Grade IIIb, in which there are sheets of centroblasts. Grade from I to IIIa are considered as indolent NHL subtypes, while grade IIIb behaves as an aggressive lymphoma and is treated similarly to a diffuse large B-cell lymphoma [5]. Bone marrow involvement is very common (about 70% of all cases) with paratrabecular lymphoid aggregates, although other organ involvement is uncommon. FL cells express monoclonal immunoglobulin (Ig) light chains; they are $CD19^+$, $CD20^+$, $CD10^+$, $CD22^+$ and $BCL2^+$, while they are negative for CD5 and CD23. Clonal Ig gene rearrangements are also present and most cases have extensive somatic mutations.

In recent decades, the introduction of several treatment options (single alkylating agents, combination chemotherapy with or without doxorubicin or fludarabine, total lymphoid irradiation) has improved the overall survival (OS) for patients with FL, with complete remission rates ranging from 65 to 85% [6]. Fisher *et al*. demonstrated that the introduction of the anti-CD20 monoclonal antibody Rituximab significantly improved OS [7]. The prognosis of FL at diagnosis is currently evaluated on the basis of specific indexes: the Follicular Lymphoma International Prognostic Index (FLIPI) considers five prognostic factors, including patient age, stage, number of involved nodal areas, serum lactate dehydrogenase and hemoglobin level [8]. It was developed through an international retrospective study of survival data on 4167 patients with FL diagnosed between 1985 and 1992. Currently, FLIPI is a widely accepted tool for risk assessment of FL. However, the FLIPI has been designed prior to the era of anti-CD20 monoclonal antibodies and the initial cohort does not represent the present course of the disease. More recently, a modified version of this scoring system, the FLIPI-2, was proposed by Federico *et al.* [9] on the basis of the F2 study, in which 1093 patients between January 2003 and May 2005 with a newly diagnosed FL were registered and 942 individuals receiving treatment were selected as the study population. This new prognostic score has, as a target end point, progression-free survival (PFS), considered more realistic than OS for a type of lymphoma with a median survival likelihood of 10 years.

Treatment options are stage-related: while disseminated FL is considered an incurable disease, with a trend to relapse, localized stage FL potentially has a different clinical outcome. In fact, it has been demonstrated that in 50% of cases it is possible to obtain a definitive eradication of the disease. According to the current guidelines [10, 11], stage I–II disease should not be managed with a frontline strategy of watchful waiting, radiation therapy representing the gold standard for this group of patients: a radiation dose of 30 to 36 Gy delivered in 15 to 20 fractions over 2–4 weeks is associated with local control rates of more than 95%. Despite the limited stage, *BCL2*/IgH+ positive cells could be found at diagnosis in the peripheral blood and/or bone marrow of 16 of 24 patients (66.6%) by quantitative PCR and

radiotherapy was capable of clearing blood and marrow Bcl2+ cells, a response which persisted after a median follow-up of 43.5 months [12]. No data are currently available concerning the efficacy on PFS of rituximab in localized FL, although rituximab is capable of reducing the proportion of residual Bcl2+ cells detectable in the peripheral blood and/or bone marrow of a proportion of patients following radiotherapy.

In stage III–IV disease, treatment can be safely deferred without a survival disadvantage if none of the following features occurs: systemic symptoms, high tumor burden, extranodal disease, cytopenia due to marrow involvement, spleen involvement, leukemic phase, serous effusions, erythrocyte sedimentation rate > 20 mm/h, high lactate dehydrogenase levels. A policy of watchful waiting is particularly advisable in elderly patients (> 70 years). The presumed advantage of a watchful waiting approach is that patients are spared the toxic side effects of chemotherapy. As already specified, patients with advanced and symptomatic FL are treated with the expectation that the disease will witness a relapsing and remitting course, and may require several lines of treatment during the course of the disease.

For many years, the standard first-line treatment was alkylator-based, frequently in combinations including vinca alkaloids, anthracyclines or fludarabine-based schedules, with similar OS and PFS [13, 14]. More recently, several phase III trials have confirmed the efficacy of rituximab in combination with an alkylator-containing regimen, both with and without the inclusion of anthracyclines [16, 17]. There is a suggestion that the duration of response in patients treated with rituximab and anthracycline-based therapies might be superior to that obtained with less intensive regimens utilizing alkylators; a specific randomized trial, FOLL-05, has been recently conducted in Italy with the aim of demonstrating the most effective first-line therapy in terms of OS and PFS for FL between the R-CVP, R-CHOP and R-FM schedules. Although chemotherapy in combination with rituximab has improved outcomes in the newly diagnosed setting, patients with FL almost always relapse and require a succession of therapies over many years. Patients who relapse after a first-line therapy not containing either anthracyclines or fludarabine should receive anthracycline- or fludarabine-based polychemotherapy together with rituximab; patients under the age of 65 with extended relapses after a first-line therapy containing either anthracyclines or fludarabine should be treated with high-dose therapy and autologous stem cell transplant. The same consideration should be made for first-line resistant patients [11].

With regard to new treatment options, Bendamustine is a DNA alkylating agent with novel properties, which has been studied in relapsed or rituximab-refractory FL patients [18]. The efficacy of bendamustine is probably related to its incomplete cross-reactivity with other chemotherapeutic agents. Phase II trials of bendamustine in combination with rituximab in relapsed FL have reported an ORRs of 92% and a median PFS of 23 months [19]. Ongoing studies are examining bendamustine with bortezomib, lenalidomide, temsirolimus, ofatumumab, alemtuzumab and other novel agents [20, 21]. Different maintenance strategies have been utilized in an attempt to prolong PFS in FL. Interferon (IFN) alpha has been used for several years with this aim. A meta-analysis of data from the pre-rituximab era [22] suggests that the addition of IFN as maintenance therapy for FL improves PFS, while the benefit on OS is less evident; in a recent report, pooled data from different randomized studies of the German Low Grade Lymphoma Study Group suggest that IFN maintenance prolongs remission duration also after rituximab-containing induction treatments [23]. With regard to rituximab as maintenance treatment, there has been a growing body of evidence demonstrating the clinical advantage of rituximab maintenance therapy following various induction regimens. The European Organization for Research and Treatment of Cancer (EORTC) conducted one of the pivotal Phase III trials in patients with relapsed or refractory FL (EORTC; 20891 trial). The study demonstrated the benefits of rituximab maintenance administered every three months for two years following chemotherapy or immunochemotherapy [24]. An important study was conducted using rituximab as maintenance after first-line therapy: the results of the PRIMA study [25] indicate that rituximab maintenance conferred significant PFS benefits (Hazard ratio 0.50; 95% CI: 0.39–0.64), but no effect on OS was seen. A recent meta-analysis by Vidal *et al*. [26] focused on the impact on OS of rituximab maintenance in both first line and previously treated patients has reported similar results: in fact, refractory or relapsed FL patients treated with rituximab maintenance had an improved OS, whereas previously untreated patients had no survival benefit. Ongoing studies will define the optimal maintenance duration (two years versus five years or until relapse or progression).

Although a large proportion of FL patients respond to immunochemotherapy, there is a group of patients with resistant/refractory disease for whom there is a need for new agents in an attempt to overcome the poor prognosis. There are three main groups of novel therapeutic agents, as well as other monoclonal antibodies (novel anti-CD20 antibodies such as ofatumumab and GA101 or antibodies against targets other than CD20), agents that target signal transduction pathways (e.g., proteasome inhibitors, Bcl-2 and Bcl-6 inhibitors), microenvironment modulatory drugs (immunomodulatory drugs, e.g. lenalidomide) [26–30]. Recently, in a phase I trial, GA101 was tested in 21 resistant or refractory $CD20^+$ indolent NHL patients [31]: the overall response rate was 43% (5 complete responses and 4 partial responses). The majority of reported adverse events were of grade 1 or 2. A similar experience, conducted treating 27 relapsed or refractory FL patients with ofatumumab, showed an overall response rate of 22% with a median PFS of 5.8 months [32]. Upcoming phase III studies will demonstrate if targeted therapies can further improve the management of patients with FL.

REFERENCES

1. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. Blood 1997; **89**: 3909–18.

2. Armitage JO, Weisenburger DD. New approach to classifying non-Hodgkin's lymphomas: Clinical features of the major histologic subtypes. Non-Hodgkin's Lymphoma Classification Project. J Clin Oncol 1998; **16**: 2780–95.

3. Harris NL, Jaffe ES, Diebold J, ***et al***. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee Meeting, Airlie House, Virginia, November 1997. J Clin Oncol 1999; **17**: 3835–49.

4. Horning SJ. Natural history of and therapy for the indolent non-Hodgkin's lymphomas. Seminars of Oncol 1993; **20**: 75–88.

5. Ott G, Katzenberger T, Lohr A, ***et al***. Cytomorphologic, immunohistochemical, and cytogenetic profiles of follicular lymphoma: 2 types of follicular lymphoma grade 3. Blood 2002; **99**: 3806–12.

6. Dana BW, Dahlberg S, Nathwani BN**,** ***et al***. Long-term follow-up of patients with low-grade malignant lymphomas treated with doxorubicin-based chemotherapy or chemoimmunotherapy. J Clin Oncol 1993; **11**: 644–51.

7. Fisher RI, LeBlanc M, Pres OW, ***et al***. New treatment options have changed the survival of patients with follicular lymphoma. J Clin Oncol 2005; **23**: 8447–52.

8. Solal-Celigny P, Roy P, Colombat P, ***et al***. Follicular lymphoma international prognostic index. Blood 2004; **104**: 1258–65.

9. Federico M, Bellei M, Marcheselli L, ***et al***. Follicular lymphoma international prognostic index 2: a new prognostic index for follicular lymphoma developed by the international follicular lymphoma prognostic factor project. J Clin Oncol 2009; **27**: 4555–62.

10. McNamara C, Davies J, Dyer M, ***et al***. Guidelines on the investigation and management of follicular lymphoma. Brit J Haematol 2011; **156**: 446–67.

11. Barosi G, Carella A, Lazzarino M, ***et al***. Management of nodal indolent (non marginal-zone) non-Hodgkin's lymphomas: practice guidelines from the Italian Society of Hematology, Italian Society of Experimental Hematology and Italian Group for Bone Marrow Transplantation. Haematologica 2005; **90**: 1236–57.

12. Pulsoni A, Della Starza I, Frattarelli N, ***et al***. Stage I/II follicular lymphoma: spread of bcl-2/IgH+ cells in blood and bone marrow from primary site of disease and possibility of clearance after involved field radiotherapy. Brit J Haematol 2007; **137**: 216–20.

13. Kimby, E, Bjorkholm, M, Gahrton G, ***et al***. Chlorambucil/prednisone vs CHOP in symptomatic low-grade non-Hodgkin's lymphomas: a randomized trial from the Lymphoma Group of Central Sweden. Ann Oncol 1994; **5:** S67–71.

14. Peterson BA, Petroni GR, Frizzera G, ***et al***. Prolonged single-agent versus combination chemotherapy in indolent follicular lymphomas: a study of the cancer and leukemia group B. J Clin Oncol 2003; **21**: 5–15.

15. Hagenbeek A, Eghbali H, Monfardini S, ***et al***. Phase III intergroup study of fludarabine phosphate compared with cyclophosphamide, vincristine, and prednisone chemotherapy in newly diagnosed patients with stage III and IV low-grade malignant Non-Hodgkin's lymphoma. J Clin Oncol 2006; **24**: 1590–96.

16. Hiddemann W, Kneba M, Dreyling M, Schmitz N, ***et al***. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood 2005; **106**: 3725–32.

17. Salles G, Mounier N, De Guibert S, ***et al***. Rituximab combined with chemotherapy and interferon in follicular lymphoma patients: results of the GELA-GOELAMS FL2000 study. Blood 2008; **112**: 4824–31.

18. Friedberg JW, Cohen P, Chen L, ***et al***. Bendamustine in patients with rituximab refractory indolent and transformed non-Hodgkin's lymphoma: results from a phase II multicenter, single-agent study. J Clin Oncol 2008; **26**: 204–210. Erratum in: J Clin Oncol 2008; **26**: 1911.

19. Robinson KS, Williams ME, van der Jaqt RH, ***et al***. Phase II multicenter study of bendamustine plus rituximab in patients with relapsed indolent B-cell and mantle cell non-Hodgkin's lymphoma. J Clin Oncol 2008; **26**: 4473–9.

20. Goy A, Bernstein SH, Kahl BS, ***et al***. Bortezomib in patients with relapsed or refractory mantle cell lymphoma: updated time-to-event analyses of the multicenter phase 2 PINNACLE study. Ann Oncol 2009; **20**: 520–5.

21. Fisher RI, Bernstein SH, Kahl BS, ***et al***. Multicenter phase II study of bortezomib in patients with relapsed or refractory mantle cell lymphoma. J Clin Oncol 2006; **24**: 4867–74.

22. Baldo P, Rupolo M, Compagnoni A, ***et al***. Interferon-alpha for maintenance of follicular lymphoma. Cochrane Database Syst Rev 2010; **20**: CD004629.

23. Unterhalt M, Hoster E, Dreyling M, Hiddemann W. Interferon maintenance prolongs remission duration after rituximab-containing induction in first-line advanced stage follicular lymphoma patients – a retrospective analysis of the German low-grade lymphoma study group (GLSG). Blood 2010; ASH Annual Meeting, 116, Abstract 2864.

24. van Oers MH, Klasa R, Marcus RE, ***et al***. Rituximab maintenance improves clinical outcome of relapsed/resistant follicular non-Hodgkin lymphoma in patients both with and without rituximab during induction: results of a prospective randomized phase 3 intergroup trial. Blood 2006; **108**: 3295–301.

25. Salles G, Seymour JF, Offner F, ***et al***. Rituximab maintenance for 2 years in patients with high tumour burden follicular lymphoma responding to rituximab plus chemotherapy (PRIMA): a phase 3, randomised controlled trial. Lancet 2011; **377**: 42-51.

26. Vidal L, Gafter-Gvili A, Salles G, ***et al***. Rituximab maintenance for the treatment of patients with follicular lymphoma: an updated systematic review and meta-analysis of randomized trials. J Natl Cancer Inst 2011; **103**: 1799–806.

27. Hagenbeek A, Fayad L, Delwail V, ***et al***. Evaluation of ofatumumab, a novel human CD20 monoclonal antibody, as single agent therapy in rituximab-refractory follicular lymphoma. Blood 2009; ASH Annual Meeting, 114, Abstract 935.

28. Sehn LH, Assouline SE, Stewart DA, ***et al***. A phase I study of GA101 (RO5072759) monotherapy followed by maintenance in patients with multiply relapsed/refractory $CD20^+$ malignant disease. Blood 2009; ASH Annual Meeting, 114, Abstract 934.

29. Sehn LH, MacDonald DA, Rubin S, ***et al***. Bortezomib added to CVP-R is safe and effective for previously untreated advanced stage follicular lymphoma: a phase II study by the NCIC sclinical trials group. Blood 2009; ASH Annual Meeting, 114, Abstract 407.

30. Fowler N, McLaughlin P, Hagemeister F, ***et al***. A biologic combination of lenalidomide and rituximab for front-line therapy of indolent B-cell non-Hodgkin's lymphoma. Blood 2009; ASH Annual Meeting, 114, Abstract 1714.

31. Salles G, Morschhauser F, TLamy T, ***et al***. Phase 1 study results of the type II glycoengineered humanized anti-

CD20 monoclonal antibody obinutuzumab (GA101) in B-cell lymphoma patients. Blood 2012; **119**: 5126–32.

32. Czuczman MS, Fayad L, Delwail V, ***et al***. Ofatumumab monotherapy in rituximab-refractory follicular lymphoma: results from a multicenter study. Blood 2012; **119**: 3698–704.

VIRUSES AND LYMPHOMA

G. Gaidano

Division of Hematology, Department of Translational Medicine, Amedeo Avogadro University of Eastern Piedmont, Novara, Italy

Correspondence: gaidano@med.unipmn.it

Research on oncogenic infectious agents, especially viruses, has helped us to understand the process of malignant transformation of cells because the cellular events in viral-driven transformation mirror, often brilliantly, basic cellular processes that culminate in cancer, even those not associated with viruses. Infectious agents, especially viruses, account for several of the most common malignancies — up to 20% of all cancers. Some of these cancers are endemic, with a high incidence in certain geographic locations, but sporadic/lower incidence in other parts of the world. Lymphomas arise frequently in association with viruses such as Epstein-Barr virus, human herpesvirus 8 (HHV-8), human immunodeficiency virus (HIV), human T-lymphotropic virus-1 (HTLV-1), and hepatitis C virus (HCV). Viruses may contribute to lymphomagenesis either by directly infecting the tumor clone (e.g. EBV, HHV8, HTLV-1), or via indirect mechanisms altering the host immunity (e.g. HIV) or microenvironmental interactions (e.g. HCV).

Gamma-herpesviruses. Two lymphotropic human gamma herpesviruses can cause, or predispose to, lymphoproliferative disorders: Epstein — Barr virus (EBV, formally designated as human herpesvirus 4) and Kaposi sarcoma herpesvirus (KSHV, also called human herpesvirus 8). Individuals with inherited or acquired immunodeficiency have a greatly increased risk of developing a malignancy caused by one of these two viruses. Specific types of EBV- or HHV-8 related lymphomas occur predominantly or almost exclusively in individuals with HIV infection, transplant recipients and children with primary immunodeficiency. Some of these diseases, such as Hodgkin and non-Hodgkin lymphoma resemble those occurring in immunocompetent patients, but the proportion of tumors in which EBV is present is increased. Others, like primary effusion lymphoma and polymorphic post-transplant lymphoproliferative disorders, are rarely seen outside the context of a specific immunodeficient state.

HIV. The clinical features and natural history of HIV-associated lymphomas differ greatly from those observed in the general population. The failure to improve outcomes with treatment intensification indicates the need for the introduction of new therapeutic options. HIV-associated lymphomas still represent a relevant field of clinical research. Standard methodologies for therapy in this patient population have yet to be established. However, rituximab plus chemotherapy should be offered to the majority of patients with HIV infection and diffuse large B-cell lymphoma and the feasibility of intensive aggressive chemotherapy regimens has been successfully tested in HIV-associated Burkitt lymphomas.

HTLV-I. Adult T-cell leukemia-lymphoma (ATLL) is a peripheral T-cell malignancy, closely associated with HTLV-1 infection. Clinically, ATLL is classified into four subtypes: acute, lymphoma, chronic and smoldering type. Although the prognosis of chronic and smoldering-type ATLL is relatively good, that of patients with acute- or lymphoma-type ATLL still remains extremely poor. Zidovudine/IFN-α therapy seems to be promising, although its efficacy has not yet been confirmed in well-designed prospective studies. High-dose chemotherapy with the support of autologous transplantation does not improve outcome. Allogeneic stem cell transplantation is promising and approximately 40% of aggressive ATLL patients are expected to survive long-term, although transplantation-related mortality is as high as 40–50%.

HCV. HCV is well known for its aetiological role in chronic non-A, non-B viral hepatitis, liver cirrhosis and hepatocellular carcinoma; in addition, the virus has also been implicated in a number of extra-hepatic "autoimmune" disease manifestations. A causative association between HCV and non-Hodgkin lymphoma (NHL) was postulated relatively recently and has been the subject of intense investigation, as well as some debate. On the strength of epidemiological data, emerging biological investigations and clinical observations, HCV appears to be involved in the pathogenesis of at least a proportion of patients with NHL. Morphologically, HCV-associated lymphomas represent a variety of histological subtypes including marginal zone lymphoma (splenic, nodal and extranodal), small lymphocytic lymphoma/chronic lymphocytic leukaemia, lymphoplasmacytic lymphoma and diffuse large B-cell lymphoma. Remarkably, some HCV-associated NHL appear to be highly responsive to antiviral therapy, providing some clinical evidence for this relationship, as well as the prospect for novel therapeutic intervention.

Perspectives. Some virus-related lymphomas may be difficult to treat with conventional approaches. Despite recent advancements using cytotoxic, lymphoma-specific, and adoptive therapies, the long-term outcome of patients with y-herpesvirus lymphomas occurring in severely immunocompromised patients and ATLL continues to be poor. Lytic-inducing therapies targeting NF-κB, and viral and tumor cell epigenetic mechanisms afford the advantage of exploiting the intrinsic presence of oncogenic viruses to eradicate infected tumor cells. On these grounds, novel clinical approaches targeting viral latency are currently being investigated.

REFERENCES

1. Carbone A, Gaidano G. Acquired immunodeficiency syndrome-related cancer. A study model for the mechanisms contributing to the genesis of cancer. Eur J Cancer 2001; **37**: 1184–7.