in peripheral blood and bone marrow of some patients with non-Hodgkin's lymphomas.

In 1993, the Reference Laboratory was set up as a public service on the basis of the Immunocytochemistry Department of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine with the aim of the precise diagnosis of the haematopoietic malignancies based on cytomorphology, cytochemistry, immunophenotyping and the techniques of molecular biology in accordance with FAB, WHO, EGIL, ICD-10 and ICD-O-2 classifications. The diagnostic activity of the Reference Laboratory covers 35-45% of all Ukrainian patients with acute leukemias, chronic lymphoid and myeloid leukemias, myelodysplastic syndromes, malignant lymphomas, histiocytosis, and metastatic lesions of lymph nodes and bone marrow. At present, the patients with tumors of haematopoietic and lymphoid tissues are diagnosed according to up-to-date WHO classification. We believe that only precise diagnosis of the major types of hematological malignancies to the up-to-date classification with delineation of the specific biological subtypes of hematological malignancies may represent the basis for further molecular biological and epidemiological studies. New insight into the biology of the lymphoid malignancies in the coming years might well improve our ability to evaluate patients and chose therapy.

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CHRONIC LYMPHOCYTIC LEUKEMIA

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Chronic lymphocytic leukemia (CLL) is the most common form of leukemia in Western countries with an incidence of 4.2/100,000/year [1]. The incidence increases to >30/100,000/year at an age of >80 years. The median age at diagnosis is 72 years. About 10% of CLL patients are reported to be younger than 55 years.

The guidelines for the diagnosis and treatment of chronic lymphocytic leukemia were revised by the International Workshop on CLL in 2008 (IWCLL). Criteria for CLL are as follows: the presence in the peripheral blood of 5 x 10^9/L monoclonal B lymphocytes for the at least 3 months. The clonality of the circulating B lymphocytes needs to be confirmed by flow cytometry [2]. Typical immunophenotype of CLL lymphocyte is $CD5^+$, $CD23^+$, $CD43^{+/-}$, $CD10^-$, $CD19^+$, CD20 dim, slgdim$^+$ and cyclin DI^- [3]. Bone marrow examination is not required for diagnosis and a CT scan not required for staging, but flow cytometry is crucial for correct diagnosis.

The first prognostic marker to be used in the clinical management of CLL was the Rai clinical staging system, published in 1975 [4]. This system was later followed by the Binet staging system, published in 1981 [5]. Both of these staging systems provide a basic framework for estimating prognosis and are factored into the current International Workshop on CLL guidelines for initiation of treatment [2].

Multiple factors, measured in standard clinical laboratory tests, affect the clinical course of CLL. These factors include lymphocyte count, lymphocyte doubling time, M level, sTK level, angiopoietin-2 (Ang-2) level, and soluble cluster designation markers (CD14, CD23, and CD49d). Other clinical markers that have been investigated as potential prognostic indicators include age, gender [6], lymphocyte doubling time [7], number of prolymphocytes [8], pattern of bone marrow involvement and percentage of smudge cells [9].

Approximately 80% of individuals with CLL have acquired chromosomal abnormalities within their malignant clone and can be categorized into five prognostic groups accordingly: deletion 13q (median survival, 133 months); deletion 11q (median survival, 79 months); trisomy 12 (median survival, 114 months); normal cytogenetics (median survival, 111 months); and deletion 17p (median survival, 32 months). Reciprocal chromosome translocations are described but are rare in CLL. A complex cytogenetic karyotype can be identified in ~I6% of patients and is commonly associated with poor prognostic features including CD38 expression and unmutated IgHV [10].

The outcome of patients with leukemic cells that use an unmutated IgVH gene is inferior to those patients with leukemic cells that use a mutated IgVH gene. In addition, the VH3.21 gene usage is an unfavorable prognostic marker independent of the IgVH mutational status. Leukemic cell expression of ZAP-70 and CD38 was found to correlate with the expression of unmutated IgVH genes and to predict a poor prognosis.

However, the association between expression of ZAP-70 or CD38 with the expression of unmutated IgVH genes is not absolute. It is uncertain whether leukemia-cell expression of unmutated IgVH genes or ZAP-70 predict the response to treatment or overall survival, once therapy is required. Taken together, further clinical trials are needed to standardize the assessment of these pa-

rameters and to determine whether they should affect the management of patients with CLL [2].

Recently 9 significantly mutated genes were identified that occurred in 5 core signaling pathways in which the genes play established roles: DNA damage repair and cell-cycle control (*TP53, ATM*), Notch signaling (*FBXW7, NOTCH I*), inflammatory pathways (*MYD88, DDX3X, MAPKI*), and RNA splicing/processing (*SF3BI, DDX3X*). Of these mutations, 5 of the mutated genes have been implicated in CLL for the first time [11].

Treatment of CLL ranges from periodic observation with treatment of infectious, hemorrhagic, or immunologic complications to a variety of therapeutic options, including steroids, alkylating agents, purine analogs, combination chemotherapy, monoclonal antibodies, and transplant options [12]. A metaanalysis of randomized trials showed no survival benefit for immediate versus delayed therapy for patients with early stage disease, nor for the use of combination regimens incorporating an anthracycline compared with a single-agent alkylator for advanced stage disease.

Indication for start of treatment are as follows: Binet stage C, Rai stages III or IV, Binet stage B or Rai stages I or II, with at least one of: splenomegaly, and or lymphadenopathy, when symptomatic, progressive, or massive (> 5 cm spleen, 10 cm nodes) progressive lymphocytosis (increase > 50% in 2 months or Lymphocyte Doubling Time < 6 months, AIHA or ITP unresponsive to corticosteroids, disease-related symptom (i.e., weight loss, significant fatigue, fever). Biological markers (e.g. cytogenetics, CD38, ZAP-70, IGVH mutations) are not an indication to start therapy (outside clinical trials). Response to therapy is the most important prognostic factor.

Recently substantial advances have been made in the treatment of CLL patients, most of which relate to monoclonal antibodies (MAb) alone and in combination with various chemotherapeutic drug combinations. Preferred treatment of choice (for patients with good performance status) is the combination of rituximab with fludarabine and cyclophosphamide (R-FC). Phase 2 clinical studies demonstrated that R-FC is the most effective combination to date in terms of achieving CR in CLL in previously untreated [13] and treated [14] patients.

Allogeneic stem cell transplant has been found to induce long-term disease-free survival in CLL patients with deletion 17p [15]. However, given the age of diagnosis and frequent presence of co-morbidities, transplant is not often an option for these patients. This has led to a search for non-p53 dependent agents for use in the management of CLL with deletion 17p.

Alemtuzumab, on the other hand, appears to work via a p53 independent pathway, and has demonstrated efficacy in 17p deleted or p53 mutated CLL [16]. Less effective for bulky (5 cm). 17p- patients who present with bulky lymphadenopathy remains a therapeutic challenge.

Ofatumumab, a human CD20 Mab that binds to another CD20 epitope, has shown promising results when used as a single agent in refractory CLL patients OR rate of approx 50% with a significantly longer survival in responding patients [17]. Several other MAbs are in early clinical testing or in the pipeline. In addition, a growing number of small molecules are being explored in clinical trials, providing hope for the future that CLL will be transformed into a disease that may be kept under control for very long periods of time.

For the selection of second-line treatment, the quality of first response plays a major role — if physically fit patients with refractory disease or relapse within 24 months after chemoimmunotherapy — or fludarabine-based combination therapy, the second remission should be used to proceed to an allogeneic stem cell transplant (especially indicated in very high risk [del(17p), p53 mutation] and/or refractory disease [18].

If the patient is physically unfit, the treatment should be changed to an alternative regimen. The prognosis in this group is usually poor. If relapse is later than 24 months after the first therapy, the first-line therapy should be repeated.

Oblimersen is a drug that has been studied for use in CLL. An immunotoxin known as BL22 has shown a great deal of promise in treating hairy cell leukemia (HCL) in clinical trials. A newer version of this drug, known as HA22 (CAT-8015) is now being tested for use against CLL. The Bruton's tyrosine kinase (BTK) inhibitor PCI 32765 (under development by Pharmacyclics) showed high rates of progression-free survival and low toxicity in patients with relapsed CLL, according to data presented here at American Society of Hematology (ASH) 53rd Annual Meeting. The drug is now in a phase 3 clinical trial.

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FOLLICULAR LYMPHOMA

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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