

**MATERIALS OF HEMATOLOGY  
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WORK-UP OF HEMATOLOGICAL  
MALIGNANCIES. FOCUS ON LYMPHOID  
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For about 20 years, the European School of Hematology (ESH) and the European Hematology Association (EHA) have cooperated in organizing educational activities in the field of continuous medical education designed in collaboration with the international experts. ESH has worked much to improve and harmonize the quality of education in hematology throughout Europe with the active implication of the world's most prominent hematologists and hematology organizations. In 2008, the Joint ESH-EHA Executive Committee was organized. The Committee members E. Gluckman and B. Lowenberg from ESH, W. Fibbe and R. Foa from EHA have developed a framework for continuing collaboration in the field including the workshops and training courses for hematologists. Among the scientific and educational courses on the latest developments in hematology, the hematology tutorials are of great interest for all those who are eager to improve and update their knowledge in various fields of modern hematology.

The scientific program of the tutorials comprising plenary lectures, interactively conducted clinical case study sessions and self-assessment sessions is designed to encourage interaction between the faculty and the course participants. For many years, within the framework of ESH-EHA program for continuous medical education, conferences, training courses and laboratory workshops have been organized in various countries throughout the world. The hematology tutorials involving a faculty of international experts are targeted to clinicians, biologists and students working in the field of hematological malignancies.

It was the first time when the hematology tutorial was held in Kyiv, Ukraine. The 24<sup>th</sup> Hematology Tutorial promoted a modern view of morphology, pathogenesis, diagnosis and treatment of lymphoid malignancies. The faculty of this tutorial consisted of the leading experts from different countries: Prof. R. Foa and Prof. G. Gaidano from Italy, Prof. C. Dearden from United Kingdom, Prof. S. McCann and Prof. E. Vandenberghe from Ireland, Prof. E. Kimby from Sweden, Prof. D. Gluzman and Prof. I. Kriachok from Ukraine. Among the audience comprising about 200 people were clinicians and researchers specializing in hematology arriving from various cities throughout Ukraine.

As a courtesy of ESH-ESA and personally of the faculty of the meeting, the lecturers have provided their permissions for publishing the extended abstracts of their lectures in this issue of *Experimental Oncology*.

**WHO CLASSIFICATION OF LYMPHOID  
MALIGNANCIES**

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Lymphoid malignancies are tumors of the immune system that originate from B or T lymphocytes and, rarely, from NK cells. They encompass extremely heterogeneous group of diseases based on their histological forms, biologic and molecular genetic features, sites of clinical presentation (nodal or extranodal), tumor behavior (localized or disseminated), and response to the treatment.

The history of recognition and classification of lymphoid malignancies is long, controversial and complicated. Two classification systems have been widely used until recently, the Kiel classification of non-Hodgkin's lymphomas and the Working Formulation for clinical usage. In 1994 after the immunologic revolution (creation the hybridoma technology that led to the development of monoclonal antibodies) and dramatic progress in understanding the genetics of lymphoid malignancies), the International Lymphoma Study Group (ILSG) of experienced hematologists formulated new proposals for a modern lymphoma classification, the so-called Revised European American Lymphoma (REAL) classification. With some additions and corrections, it has been developed into World Health Organization (2001) classification (E.S. Jaffe, N.L. Harris, H. Stein, J.W. Vardiman, eds. *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon: IARC Press, 2001. 351 p.).

The 4<sup>th</sup> edition of the WHO classification of Tumours of Haematopoietic and Lymphoid Tissues (2008) incorporates new information that has emerged from basic and clinical investigations and includes new defining criteria for some diseases as well as number of new entities defined by a combination of immunophenotype, genetic criteria and clinical features.

The recent WHO classification (2008) has been updated as a joint effort of more than 130 hemopathologists from 22 countries. WHO classification of the B cell, T cell and NK cell neoplasms that in many respects recapitulate normal stage of lymphoid cell differentiation subdividing tumors into those with an immature or blastic appearance versus more mature stage of lymphoid development. This classification system represents a significant advance in our ability to understand, identify and treat different lymphoma entities. It is based on the concept of clinicopathologic entities in which histology, immunophenotype, molecular genetic data as well as clinical features are integrated. The putative cell origin and stage of differentiation of different types of lymphoid malignancies is also taken into account.

According to our experience, the application of immunocytochemical and molecular genetic studies has led to the detection of small number of pathologic cells

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 choose 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  $5 \times 10^9/L$  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<sup>+</sup>, CD23<sup>+</sup>, CD43<sup>+/−</sup>, CD10<sup>−</sup>, CD19<sup>+</sup>, CD20 dim, sIgdim<sup>+</sup> and cyclin D1<sup>−</sup> [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 ~16% 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*, *NOTCH1*), inflammatory pathways (*MYD88*, *DDX3X*, *MAPK1*), and RNA splicing/processing (*SF3B1*, *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  $\geq 5$  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<sup>+</sup>, CD20<sup>+</sup>, CD10<sup>+</sup>, CD22<sup>+</sup> and BCL2<sup>+</sup>, 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<sup>+</sup> 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.

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## VIRUSES AND LYMPHOMA

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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- $\alpha$  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  $\gamma$ -herpesvirus lymphomas occurring in severely immunocompromised patients and ATLL continues to be poor. Lytic-inducing therapies targeting NF- $\kappa$ B, 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.

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## LEUKEMIC PHASE OF B-LINEAGE NHL

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B-cell non-Hodgkin lymphomas (NHL) mostly present as disseminated diseases involving lymph nodes, spleen and liver and often the bone marrow (BM). Tumor cells can also be found in the blood (leukemic disease), especially in the indolent lymphomas. High white blood cell counts and a differential demonstrating a lymphocytosis in the blood require immunophenotyping for characterization of the leukemic cells. Molecular/cytogenetic analyses may also have a role in the diagnostic classification of the disease. Besides the specific diagnosis, the clinical evaluation of the patient and prognostic markers are of most importance for selecting the best type of therapy.

**Follicular lymphoma (FL)** is the indolent lymphoma with the highest incidence. Most patients present with advanced stage disease with BM involvement in 40–70% of the cases, and few are leukemic at the time of diagnosis. By high-resolution analysis circulating FL cells may be detected in more patients. Leukemic patients mostly have concomitant lymph node involvement and high tumor burden. A pure FL-cell leukemia with CD20<sup>+</sup>CD10<sup>+</sup>CD5<sup>-</sup> clonal cells has also been described, mostly associated with an indolent clinical outcome. FL carry a t(14;18)(q32;q21) translocation in more than 90% of cases, juxtaposing the immunoglobulin heavy chain (IGH) 3' Regulatory Regions (IgH-3'RR) to the *BCL2* gene, resulting in overexpression of the Bcl2 anti-apoptotic protein. FL cells are also dependent on signals from the microenvironment to survive and proliferate.

Several groups, including ours, have reported that immune cells in the lymphoma microenvironment and in blood influence prognosis. In patients treated before the introduction of rituximab, we have found that a high number of PD-1<sup>+</sup>, FOXP3<sup>+</sup> and CD8<sup>+</sup> T-cell subsets in the tumor microenvironment predict superior outcome, while CD4<sup>+</sup> follicular helper T cells and CD68<sup>+</sup> macrophages are associated with an inferior outcome. The introduction of the anti-CD20 antibody rituximab has improved the prognosis for FL patients. The efficacy of this drug is excellent also as monotherapy especially in patients with high numbers of CD8<sup>+</sup> T-cells in the lymph nodes as well as in the blood.

**Mantle cell lymphoma (MCL):** MCL cells carries the t(11;14) translocation resulting in enhanced cyclin D1 expression and cyclin D1-dependent kinase activity, promoting cell cycle progression. Immunological markers show a typical phenotype (CD20<sup>+</sup>CD5<sup>+</sup>CD23<sup>-</sup>), but also atypical phenotypes (CD20<sup>+</sup>CD5<sup>-</sup>CD23<sup>-</sup> or CD20<sup>+</sup>CD5<sup>+</sup>CD23<sup>+</sup>) in some cases. Most MCL patients have an unfavorable prognosis and intensive treatment strategies are required. However, in around 10% of the patients the disease shows an indolent clinical course with often a non-nodal, leukemic disease. In one study the clinicopathologic features, gene expression and genomic profiles were compared in patients with indolent (iMCL) and in those with conventional disease (cMCL). iMCL and cMCL shared a common gene expression profile that differed from other leukemic lymphoid neoplasms and a signature of 13 genes was underexpressed in iMCL, among these *SOX11*. The *SOX11*-negative tumors exhibited more frequent non-nodal presentation and better survival compared with *SOX11*-positive MCL. Recently, our group found that *SOX11*-negative MCL had a higher frequency of lymphocytosis, but also elevated LDH and p53 positivity. Moreover, *SOX11*-negative cases had a shorter overall survival than *SOX11*-positive cases. Due to the conflicting results, the conclusion is that *SOX11* cannot be used for predicting an indolent disease course. In another study, deletions at 17p13 (*TP53*) and 13q14 were frequent in leukemic MCL and involved the majority of the leukemic clone. Cases with *TP53* deletion were more likely to have splenomegaly and marked leucocytosis (> 30 × 10<sup>9</sup>/L), and were less likely to have lymphadenopathy than those without the deletion. Other distinctive biological features in non-nodal leukemic MCL are mutated IGHV and a transcriptional profile lacking tumor invasion properties, which might contribute to the absence of nodal involvement. In conclusion, MCL patients with leukemic disease but without clinical symptoms might be managed conservatively with a “wait and watch” policy, while blastoid morphology, high proliferation and *TP53* aberrations are markers of aggressive disease, which will require intensive immunochemotherapy.

**Marginal zone lymphoma (MZL):** There are three clinicopathological entities of MZL, including extranodal, mucosa-associated lymphoid tissue (MALT) lymphoma, nodal (NMZL), and splenic (SMZL) type. Leukemic presentation is more common in SMZL. The leukemic



lymphocytes are usually small or morphologically “villous”, and the leukemic manifestation of SMZL is named splenic lymphoma with villous lymphocytes (SLVL). The typical immunophenotype is CD19<sup>+</sup>CD20<sup>+</sup>CD22<sup>+</sup>CD45<sup>+</sup> and the clone is often also CD 103<sup>+</sup> and CD38<sup>+</sup>. Moreover, CD11c is highly associated with SMZL. The genetics and pathogenesis of SMZL are poorly understood and specific prognostic features are lacking. Aberrant karyotypes are seen as gains of 3/3q and 12q, deletions of 7q and 6q and translocations involving 8q/1q/14q. Trisomy 3 and deletions of chromosome 7q22–34 are most common and found in approximately 25 and 45% of cases, respectively. A strong association has been described between usage of the IGVH1-2 and deletion 7q and 14q alterations. Clinical and epidemiological data suggest that chronic hepatitis C virus (HCV) infection may have an etiological role in a subset of cases. MicroRNA (miR)-26b, a miRNA known to have tumor suppressive properties, has been shown to be down-regulated in HCV positive cases. Recent data suggest that certain SMZL subtypes could derive from progenitor populations adapted to particular antigenic challenges through selection of VH domain specificities, in particular the IGHV 1-2(\*)04 allele.

The anti-CD20 antibody rituximab is mostly effective in MZL patients as monotherapy, but for many patients with symptomatic splenomegaly, splenectomy is still a therapeutic option.

In summary, the presence of lymphocytosis in the blood in patients with a suspicion of lymphoma requires careful evaluation for the presence of neoplastic lymphocytes, especially in the absence of easily accessible enlarged lymph nodes. The differential diagnosis between the WHO defined mature B-cell malignancies has improved by using multiple-color flow cytometry of phenotypic data of the lymphoma cells. This method is also of value for characterization of the immune cells in the microenvironment and blood. Molecular/cytogenetic analyses have a role in classification of the disease and for understanding of pathogenesis. Therapeutic decisions are always dependant on the specific diagnosis, prognostic factors and a careful clinical evaluation of the patient.

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## PRIMARY GASTROINTESTINAL LYMPHOMAS

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### Epidemiology and classification

Primary gastrointestinal lymphomas comprise less than 5% of all lymphomas diagnosed in the western world, with a variable geographical and ethnic incidence. The biology and management vary with the main diagnostic (WHO) subtypes which include Gastric MALT lymphomas, Enteropathy associated T-cell lymphoma (EATCL). Other lymphomas which frequently involve the gastrointestinal tract include diffuse large B-cell lymphoma, mantle cell lymphoma and Burkitts lymphoma; but are not considered primary gut lymphomas and will not be covered in this lecture. The pathogenesis of MALT and EATCL lymphomas is linked to abnormal antigen drive (gluten/*Helicobacter* infection) resulting in chronic inflammation and lymphoma development. The lymphomas are otherwise radically different; MALT lymphomas are indolent B-NHL, which respond to antigen-drive withdrawal and minimal therapy with an overall survival (OS) > 80% at 5 years, whereas EATCL is an aggressive T-cell lymphomas associated with a poor outcome.

### Gastric MALT lymphoma

**Clinical features:** Gastric MALT lymphomas incidence in the Western World is approximately 6 per

million, with a median onset at 60 years, slight female predominance and almost invariable association with *Helicobacter pylori* infection. Patients typically present with non-specific dyspeptic type symptoms and the diagnosis is made gastroscopically. 80% of patients have Stage I/II disease.

**Pathology:** The pathological appearance is of small- to medium-sized round or minimally irregular cells, with clumped nuclear chromatin, abundant pale cytoplasm and lymphoepithelial lesions. The cells express pan-B markers but are CD5, 10 and 23 negative. The t(11;18)(q21;q21) detectable by FISH is present in up to 50% of cases with PCR-detectable immunoglobulin gene rearrangements in 90% of cases.

**Management:** *H. pylori* eradication is standard treatment for all patients and in those with disease confined to the mucosa and submucosa results in a durable CR in 70% of cases. For persistent or progressive disease chemotherapy with Chlorambucil +/- Rituximab or loco-regional radiotherapy with 20 Gy are standard approaches. There is no evidence that more intensive therapy results in a better outcome. Life long follow-up should include regular endoscopy.

#### Enteropathy-associated T cell lymphoma

**Clinical features:** Coeliac disease (CD) is caused by gluten intolerance resulting in small intestinal sub-villous atrophy and malabsorption of variable severity which is managed with a gluten free diet (GFD). Coeliacs have a 20 fold increased rate of developing lymphoma with 60–75% of them sub-typed as EATCL. Clinical presentation follows 3 patterns (1) development of refractory coeliac disease (RCDII) despite adherence to a GFD (2) acute presentation with gut perforation/acute severe malabsorption despite adherence to a GFD and (3) acute presentation as in (2) with no previous diagnosis of CD. EATCL diagnosis can be challenging as it is usually confined to the small intestine and tissue is usually obtained surgically or by endoscopy (gastroscopy/double balloon enteroscopy).

**Pathology:** EATCL is characterised by a monomorphic population of medium to large cells with round or angulated vesicular nuclei, prominent nucleoli and moderate to abundant, pale-staining cytoplasm with expression of CD3<sup>+</sup>, CD5<sup>+</sup>, CD7<sup>+</sup>, CD8<sup>+/−</sup>, CD4- and CD103<sup>+</sup>.

**Management:** The 5 year OS is 20% with conventional chemotherapy and this poor outcome is thought to be related to poor patient performance status secondary to nutritional deficiency/gastrointestinal surgery and the chemo-refractoriness inherent to T-cell lymphomas. Outcome can be improved using intensive nutritional support and primary chemotherapy followed by an autologous transplantation for patients under the age of 65 resulting in a 5 year OS of between 50–60%.

**Refractory coeliac disease:** Patients who are diagnosed with an RCDII prodrome are interesting both for insights into EATCL lymphomagenesis and also because they may respond to less intensive therapy, thus reducing the risk of EATCL transformation. RCD II is characterised by sub-villous atrophy, loss of CD8 intra-epithelial lymphocytes and clonal

T-lymphocytes with 70% progression to EATCL within 5 years. A small study of patients with RCDII who responded to Cladribine therapy had a 5 year OS of 83% which may be improved further by autologous SCT.

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#### MATURE T- AND NK- CELL NEOPLASMS

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The mature or peripheral T-cell neoplasms are a biologically and clinically heterogeneous group of rare disorders that result from clonal proliferation of mature post-thymic lymphocytes. Natural killer (NK) cells are closely related to T cells and neoplasms derived from these are therefore considered within the same group. The World Health Organization (WHO) classification of haemopoietic malignancies has divided this group of disorders into those with predominantly leukaemic (disseminated), nodal, extra-nodal or cutaneous presentation. Within the WHO classification these malignancies are differentiated on the basis not only of clinical features but also of morphology, immunophenotype and genetics.

The mature T-cell and NK-cell neoplasms account for approximately 10–12% of all lymphoid malignancies, usually affect adults and most of the entities described are more commonly reported in males than in females. The median age at diagnosis for the group as a whole is 61 years with a range of 17–90 years. There is geographical variation in the frequency of the different subtypes and in Europe peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS), anaplastic large cell lymphoma (ALCL) and angio-immunoblastic T-cell lymphoma (AITL) account for

about three quarters of all cases. NK-cell lymphomas (NKTCL) are more common in Asia and are associated with Epstein-Barr virus (EBV). The human T-cell leukaemia virus (HTLV-I) is aetiologically linked to adult T-cell leukaemia/lymphoma (ATLL).

Although some may follow a relatively benign protracted course, most have an aggressive clinical behaviour and poor prognosis. Excluding anaplastic lymphoma kinase (ALK)-positive anaplastic large cell lymphoma (ALCL) and indolent mycosis fungoides (MF), which have a good outcome, 5 year survival for other nodal and extranodal T-cell lymphomas is about 30%. The similarity between progression free survival (PFS) and overall survival (OS) is an indication of the poor response to second line therapies. The rarity of these diseases and the lack of randomized trials mean that there is no consensus about optimal therapy for T- and NK-cell neoplasms.

**Presentation, diagnosis, staging and prognosis.** Extranodal presentation is common in PTCL and this often contributes to a delay in diagnosis. When compared to aggressive B-cell lymphomas, patients tend to present with unfavourable international prognostic index (IPI) scores (> 3), more advanced disease, a poorer performance status and an increased incidence of B symptoms. Paraneoplastic features are well described including eosinophilia, haemophagocytic syndrome and autoimmune phenomena. The latter are particularly seen in AITL.

**Diagnosis** is based on examination of peripheral blood or tissue biopsy for histological features supplemented by detailed immunohistochemistry, flow cytometry, cytogenetics and molecular genetics. Expert haematopathology review is essential for the correct classification of the different subtypes. Unlike B-cell lymphomas, there is no simple test for clonality and this should be established by polymerase chain reaction (PCR) for rearrangement of T-cell receptor genes.

**Staging** is as for all lymphomas, including tests to assess the extent of disease (e.g. imaging and bone marrow biopsy) and to identify the features needed to assign a prognostic score. Investigations include full blood count and differential, tests of renal and hepatic function, lactate dehydrogenase (LDH), beta2 microglobulin, albumin, serum calcium, uric acid, bone marrow core biopsy, chest X-ray and computerized tomography (CT) scan of chest, abdomen and pelvis. The role of <sup>18</sup>F-fluorodeoxyglucose positron emission tomography (FDG-PET)/CT scanning in PTCL is under investigation and has only been reported in the clinical evaluation of patients in a limited number of clinical studies so far. The data suggest that most T-cell lymphomas are FDG-avid although with variable intensity but that in CTCL PET is not sufficiently sensitive or specific. However, in PTCL stage was altered in less than 10% and did not change treatment recommendations. It cannot be recommended yet for routine use and must be prospectively validated in trials.

Lumbar puncture and magnetic resonance imaging (MRI) of the brain are only required if there is any clinical

suspicion of central nervous system (CNS) involvement. The International Prognostic Index (IPI) gives useful prognostic information in PTCL but it clusters many cases in the higher risk groups. Newer T-cell specific prognostic scores (e.g. PIT) appear to be more discriminatory and may be valuable in prospective trials.

**Treatment.** Treatment of all T and NK neoplasms should be within the context of a clinical trial if possible as standard therapy gives disappointing results. Outside trials, CHOP remains the standard first-line therapy for most nodal and extra-nodal subtypes with no clear evidence that alternative or more intensive regimens are more effective. Consideration should be given to consolidation with autologous haemopoietic stem cell transplantation (HSCT), especially in high-risk chemo-sensitive disease and AITL where results appear to be superior. Relapsed or refractory disease should be treated with relapse-schedule chemotherapy with consideration of allogeneic-HSCT (with reduced intensity conditioning) or autologous stem cell transplantation if the disease is chemosensitive. A number of newer agents show promise, particularly gemcitabine (alone and in combination), pralatrexate and romidepsin. Lenalidomide, bendamustine and bortezomib have also shown efficacy.

CNS prophylaxis should be considered using the same criteria as for diffuse large B-cell lymphoma.

**Specific subtypes.** For most subtypes their rarity has meant that there is little evidence to determine the best therapy. For some, however there is emerging data that specific tailored sub-type approaches are beneficial, and some examples are given below.

**T-PLL** should be treated with intravenous alemtuzumab followed by autologous or allogeneic stem cell transplant in first remission. Purine analogues may be helpful in resistant cases.

**T-LGL Leukaemia** is an indolent condition which does not always require treatment. Therapy is indicated for severe or symptomatic cytopenias and relies on immune-modulatory therapies such as oral cyclosporine, weekly oral low-dose methotrexate or low-dose cyclophosphamide. Second line treatments include purine analogues and alemtuzumab. Chronic lymphoproliferative disease of NK cells should be managed as for T-LGL. Rare aggressive NK-cell leukaemias occurring in younger adults require a different therapeutic approach (ALL-type chemotherapy) and consideration of stem cell transplantation.

**ATLL** is mainly seen in far eastern (Japanese) and Afro-Caribbean patients. Several subtypes exist and the smouldering/chronic subtypes may not initially require treatment although they may benefit from anti-retroviral therapy. The acute and lymphoma subtypes, have very poor prognosis and are usually managed with multi-agent chemotherapy regimens followed by allogeneic HSCT where possible. Recent evidence suggests that these patients benefit from anti-viral drugs given concomitantly, sequentially or instead of chemotherapy. Novel therapies e.g. anti-CCR4 are being evaluated.



**ALCL** (particularly ALK+) has the best outcome with conventional CHOP. Relapsed patients have achieved very high response rates with a CD30-targeted immune-conjugate, brentuximab vedotin. This may be effective in other CD30-positive PTCL and is currently being evaluated in combination in the front-line setting.

**Extranodal Nasal NK/T cell lymphoma** occurs most commonly in Asian populations and is EBV positive. The distinction at diagnosis between localized and disseminated disease is important as the latter has a dismal prognosis. Outcome is unsatisfactory with CHOP-like therapy and asparaginase-containing regimens are preferred. High dose radiotherapy (50–55 Gy) is very important in the control of localized disease and contributes significantly to cure of patients with limited stage at presentation.

**EATL** patients often present acutely and with poor PS. It is important to liaise with an experienced gastroenterologist to assist with biopsy, staging and follow up and to manage nutritional problems. CHOP-like or intensified therapy, with an up-front autograft remains a common approach and does appear to be superior to **CHOP** alone in retrospective series.

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