New developments in the pathology of malignant lymphoma: a review of the literature published from November 2010–January 2011

J. Han van Krieken

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Introduction

The present review, like the previous ones, gives data, insight, and practical tips for the practicing hematopathologist. Increasingly molecular pathways that are altered in cancer form the basis for targeted therapy, and several examples can be found in this review.

Biology of lymphoma

Hodgkin's lymphoma

A proportion of Hodgkin's lymphoma (HL) cases carries the Epstein Barr virus (EBV). Normally cells carrying a virus accumulate viral proteins in the endoplasmatic reticulum (ER), which leads to a stress response and, ultimately, to either apoptosis or survival, dependent on the induced proteins. Chang et al. [1] investigated whether HL cases express survival proteins of the ER stress response and found these indeed, but both in EBV-positive and EBV-negative cases. Transfection of EBV-negative HL cell lines with EBV resulted also in a shift towards expression of survival genes. HL cells thus express ER stress response

survival genes, but this is at most only partially due to EBV infection.

Targets for therapy can be found in various ways and increasingly an in silico approach is taken, namely using advanced bioinformatics tools unraveling large data sets from gene expression arrays. Raia et al. [2] used such expression data from cell lines of HL and primary mediastinal diffuse large B cell lymphoma (PMDLBL), tumor types with quite some similarities, including the constitutive activation of the JAK/STAT signaling pathway. They compare the data with those from normal B cells and find an increased level of STAT5 and 6. Furthermore, they show that IL-13 leads in these cells to a high level of STAT5 but not STAT6. For other genes, there was more divergence between the cell line of HL and PMDLBL. Based on the results and those of other genes a model was developed for each entity, resulting in six potential targets for therapy. One of these, blocking of STAT5 phosphorylation, was experimentally tested and indeed resulted in downregulating of the relevant downstream genes.

B cell lymphomas

Copy number aberrations of (parts of) chromosomes indicate the prognosis in chronic lymphocytic leukemia (CLL) but the mechanism is not clear. Fabbri et al. [3] investigated whether microRNAs (miRNAs) and proteins coded for by genes located on the relevant chromosome regions are up- or downregulated in relation to the gain or loss of the chromosome area. Blood samples of 206 patients with CLL underwent cytogenetic evaluation and qPCR for the relevant RNAs.

In CLLs with 13q deletions, the miR-15a/miR-16-1 cluster is directly targeted and its downstream effectors. In

J. H. van Krieken (✉)
Department of Pathology,
Radboud University Nijmegen Medical Centre,
P.O. Box 9101, 6500 HB Nijmegen, The Netherlands
e-mail: j.vankrieken@pathol.umcn.nl

leukemic cell lines and primary CLL cells, TP53 stimulated the transcription of miR-15/miR-16-1 as well as miR-34b/miR-34c clusters, and the miR-34b/miR-34c cluster directly targeted the ZAP70 kinase. The authors conclude that a microRNA/TP53 feedback circuitry is associated with CLL pathogenesis and outcome.

Extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue (MALT) are associated with various infectious pathogens. Aigelsreiter et al. [4] detected in 13/47 nongastrointestinal MALT lymphomas (28%) *Chlamydia psittaci* DNA compared with 4/37 nonmalignant control samples (11%). *C. psittaci* was detected at variable frequencies in MALT lymphomas of different sites: lung, 100% (5/5); thyroid gland, 30% (3/10); salivary gland, 13% (2/15); ocular adnexa, 15% (2/13); and skin, 25% (1/4). Of 27 autoimmune precursor lesions (11 Hashimoto thyroiditis and 16 Sjögren syndrome), 11 (41%) contained *C. psittaci* DNA. Only one (7%) of 14 gastrointestinal MALT lymphomas was positive for *C. psittaci*. All specimens were negative for *Chlamydia trachomatis* and *Chlamydia pneumoniae*. Besides ocular adnexal lymphomas, *C. psittaci* infection is associated with nongastrointestinal MALT lymphomas and autoimmune precursor lesions, suggesting possible involvement of *C. psittaci*-induced antigenic-driven MALT lymphomagenesis.

Follicular lymphoma (FL) and diffuse large B cell lymphoma (DLBCL) show frequently somatic, heterozygous Y641 mutations in the histone methyltransferase EZH2, which leads to high expression of the enzyme. Yap et al. [5] demonstrate using in vitro enzyme assays of reconstituted PRC2 complexes, that Y641 mutations result in a decrease in mono-methylating and an increase in tri-methylation activity of the enzyme relative to the wild-type enzyme. This represents the first example of a disease-associated gain-of-function mutation in a histone methyltransferase, whereby somatic EZH2 Y641 mutations in lymphoma act dominantly to increase, rather than decrease histone methylation.

The SRY (sex determining region Y)-box 11 (SOX11) gene, located on chromosome 2p25, encodes for a transcription factor that is involved in tissue remodeling during embryogenesis and is crucial for neurogenesis but was also described as marker for mantle cell lymphoma (MCL) and Burkitt lymphoma (BL), as referred to in previous reviews in this journal. Normal, mature lymphocytes lack SOX11 but express SOX4, another member of the same group of SOX transcription factors. Currently, no specific functional effects have been linked to SOX11 expression in MCL, and it is not known which genes are under influence of SOX11 in lymphoma. Wang et al. [6] found variable expression of SOX11, SOX4, and SOX12 mRNA in MCL cell lines. Downregulation of SOX11 expression by siRNA verified that SOX11 controlled the

expression of the gene TUBB3 in the MCL cell line Granta 519. Furthermore, 26 new target genes were discovered that were influenced by siRNA SOX11 downmodulation. Among these genes, DBN1, SETMAR, and HIG2 were found to be significantly correlated to SOX11 expression in two cohorts of MCL. Chromatin immunoprecipitation analysis showed that these genes are direct targets of the SOX11 protein. In spite of almost complete downregulation of the SOX11 protein no significant effects on cell proliferation or survival in short-term in vitro experiments was found, so it remains unclear what the role of SOX11 is in the pathogenesis of MCL.

The introduction of the proteasome inhibitor bortezomib in the treatment of MCL patients resulted in response in a subset of the patients but also often resistance after initial treatment. Roue et al. [7] studying biopsies and cell lines of MCL found that there is a high correlation between loss of sensitivity to the proteasome inhibitor and up-regulation of the prosurvival chaperone BiP/Grp78 by an increase in the chaperoning activity of heat-shock protein of 90 kDa (Hsp90). In bortezomib-resistant cells, pretreatment with an Hsp90 inhibitor led to synergistic induction of apoptotic cell death when combined with bortezomib. This combination may therefore form a rational approach to treatment of MCL patients.

The role of EBV in the development of several lymphoma types is well known. The form of infection, i.e., lytic or one of the various latent forms, is variable and related to the lymphoma type. Little is known about especially the lytic infection in EBV-induced lymphoproliferations. Ma et al. [8] developed a mouse model, in which both human fetal CD34-positive hematopoietic stem cells and thymus/liver tissue are transplanted, to compare EBV pathogenesis and lymphoma formation following infection with a lytic replication-defective BZLF1-deleted (Z-KO) virus or a lytically active BZLF1(+) control. Both the control and Z-KO viruses established long-term viral latency in all infected animals. The infection appeared well controlled in some animals, but others eventually developed DLBCL. Animals infected with the control virus developed tumors more frequently than Z-KO virus-infected animals. Specific immune responses against EBV-infected B cells were generated in mice infected with either the control virus or the Z-KO virus. In both cases, forms of viral latency (type I and type IIB) were observed that are less immunogenic than the highly transforming form (type III) commonly found in tumors of immunocompromised hosts, suggesting that immune pressure contributed to the outcome of the infection. These results point to an important role for lytic EBV infection in the development of B cell lymphomas in the context of an active host immune response.

Imig et al. [9] report on a comprehensive miRNA-profiling study in EBV-induced lymphomas. They show

that hsa-miR-424, hsa-miR-223, hsa-miR-199a-3p, hsa-miR-199a-5p, hsa-miR-27b, hsa-miR-378, hsa-miR-26b, hsa-miR-23a, and hsa-miR-23b were upregulated and hsa-miR-155, hsa-miR-20b, hsa-miR-221, hsa-miR-151-3p, hsa-miR-222, hsa-miR-29b/c, and hsa-miR-106a were downregulated more than twofold due to EBV infection of DLBCL. All known EBV miRNAs with the exception of the BHRF1 cluster as well as EBV-miR-BART15 and -20 were present. A computational analysis indicated potential targets such as c-MYB, LATS2, c-SKI, and SIAH1. Downregulation of SIAH1 protein in DLBCL was demonstrated by immunohistochemistry. The inhibition of SIAH1 is in line with the notion that EBV impedes various pro-apoptotic pathways during tumorigenesis. The downmodulation of the oncogenic c-MYB protein, although counter-intuitive, might be explained by its tight regulation in developmental processes.

Also, BL commonly exhibits EBV positivity associated with latent chronic infection. Models of acute EBV infection have been associated with cellular resistance to apoptosis. However, the effect of latent long-term EBV infection on apoptosis induced by drugs is not well defined. To determine this, Lima et al. [10] have studied the response of the Akata EBV-positive cell line (type I latency) to etoposide, before and after downregulating EBV gene expression. Downregulating EBV nuclear antigen-1 (EBNA-1) expression with siRNAs reverted cellular sensitivity to etoposide. In accordance with this finding, Akata EBV-positive cells showed increased sensitivity to etoposide, when compared with the Akata EBV-negative cells. Furthermore, Akata EBV-positive cells showed increased apoptosis levels and decreased Bcl-xL mRNA and protein levels, when compared with the Akata EBV-negative cells. In addition, Akata EBV-positive cells contained less endoplasmic reticulum ER than EBV-negative cells. Finally, downregulation of EBV with EBNA-1 siRNAs caused an increase in the expression of Bcl-xL.

An example of the above-mentioned increased role of bioinformatics for huge data sets is the study from Sung et al. [11] in a relative small series of central nervous system lymphoma (CNSL). They analyzed 12 CNSL samples with high-resolution array-based comparative genomic hybridization and performed expression profiling in seven of the 12 samples. The most frequent deletion found in eight patients (66.7%) occurred in 9p21.3 containing CDKN2A. They compiled the top 96 genes showing the greatest differential expression between CNSL and normal lymph node tissues. From these, eight candidate genes (NPFFR2, C4orf7, OSMR, EMCN, TPO, FNDC1, COL12A1, and MSC) were selected in which expression changes were associated with copy number aberrations. All eight genes showed both downregulation in expression microarray and deletion in array-based comparative genomic hybridization

analyses. These genes participate in cell signaling or cell adhesion. In addition, low mRNA expression of C4orf7 was significantly associated with poor survival. The authors conclude that their study identified novel tumor suppressor genes that may serve as therapeutic targets of CNSL. Obviously, such claims need rigorous testing in larger number of samples and preclinical models.

T cell lymphoma

It is well known that the TLX1 oncogene (encoding the transcription factor T cell leukemia homeobox protein-1) is altered in a substantial number of T cell acute lymphoblastic leukemia (T-ALL). However, the specific mechanisms of T cell transformation downstream of TLX1 remain to be elucidated. De Keersmaecker et al. [12] describe a very interesting new mechanism by which aneuploidy arises. Transgenic mice that express human TLX1 develop T-ALL with frequent deletions and mutations in Bcl11b (encoding B cell leukemia/lymphoma-11B) and recurrent mutations and deletions in BCL11B occur in 16% of human T-ALLs. The mouse TLX1 tumors were typically aneuploid and showed a marked defect in the activation of the mitotic checkpoint. TLX1 directly downregulates the expression of CHEK1 (encoding CHK1 checkpoint homolog) and additional mitotic control genes and induces loss of the mitotic checkpoint in nontransformed preleukemic thymocytes. This results in chromosomal missegregation and aneuploidy at the earliest stages of tumor development a feature that is common in many forms of cancer.

Another mouse model for T-ALL is reported by Chevillard et al. [13]. They exposed mice deficient for Nrf3 (NF-E2-related factor) to benzo(a)pyrene, a carcinogen found in cigarette smoke. Although smoking is related to several forms of cancer, T-ALL is not known to be one of these. Nevertheless, the mice often developed T-ALL resulting in early death.

Ng et al. [14] used genomewide expression profiling on formalin-fixed, paraffin-embedded tissue of nine cases of nasal-type NK/T cell lymphoma (NKTL) and cell lines thereof in comparison with normal natural killer cells. They found an increased proliferative and anti-apoptotic profile in NKTL with activation of Myc and nuclear factor kappa B and also high levels of survivin. The results were confirmed with immunohistochemistry on 33 cases. Since the survivin inhibitor Terameprocol resulted in reduced cell viability and increased apoptosis this may become a new drug for NKTL which is until now untreatable.

Anand et al. [15] investigated the role of beta-catenin in ALK-positive anaplastic large cell lymphoma (ALCL) using tissue samples and cell lines. β -catenin was transcriptionally active in three of three ALK-positive ALCL lymphoma cell lines, which correlates with the nuclear

localization of β -catenin in these cells and the neoplastic cells in most of the patients' tumor samples. β -catenin is biologically significant in ALK-positive anaplastic large cell lymphoma, since downregulation of β -catenin resulted in a significant reduction in cell growth. Downregulation of β -catenin led to a marked reduction in both the total protein level and the activated/phosphorylated form of STAT3, another signaling protein previously shown to be important in the pathogenesis of ALK-positive anaplastic large cell lymphoma. In contrast to some of the oncogenic tyrosine kinases, modulation of nucleophosmin-anaplastic lymphoma kinase expression did not result in any detectable change in the protein level, nuclear localization or tyrosine phosphorylation of β -catenin; however, inhibition of nucleophosmin-anaplastic lymphoma kinase expression significantly downregulated the transcriptional activity of β -catenin. These results suggest that the interaction between oncogenic tyrosine kinases and various cell signaling proteins are more complex than previously believed.

In previous reviews, I have described the emerging new entity breast implant-associated ALK-negative ALCL. Lechner et al. [16] were able to create a cell line for this disease from a biopsy using a human stromal feeder layer and IL-2, referred to as TLBR-1. TLBR-1 is IL-2-dependent, has a relatively long doubling time (55 h), and displays different cellular shapes in culture. Cytogenetic analysis showed 47 chromosomes lacking t(2;5). The cells are positive for CD4/8, CD30, CD71, and CD26, and antigen presentation (HLA-DR-, CD80-, CD86-positive), IL-2 signaling (CD25-, CD122-positive), and NK (CD56-positive) markers, and Western blots demonstrated strong Notch1 expression. Severe combined immunodeficiency mouse TLBR-1 heterotransplants recapitulated the histology and marker characteristics of the original tumor. This cell line is therefore a valuable tool for investigating the pathogenesis of this entity.

Epidemiology of lymphoma

The role of EBV in various lymphoma types is well recognized. There is however still much to be learned. Several reports deal with the incidence in different entities. The HIV epidemic was associated with a sharp increase in the manifestation of EBV-positive lymphoproliferations, which was effectively counteracted by the introduction of HAART-therapy. Wiggill et al. [17] investigated the prevalence of lymphomas and HIV in a tertiary academic center in South Africa. From January 2007 to December 2009, there were 2,225 new diagnoses of lymphoproliferative disorders, as compared with 1,897 cases diagnosed during 2004–2006. A significant increase in both high-

grade B cell lymphomas and HL was documented during 2007–2009. This was associated with a statistically significant increase in HIV prevalence in those tested (from 44.3% in 2004–2006 to 62.0% in 2007–2009). HIV-positive patients presented at a statistically significantly younger median age and showed a relative over-representation of females when compared with HIV negative patients. These data show that untreated HIV prevalence is directly reflected by the pattern of lymphoproliferations.

Another entity that has arisen the last few years is the EBV-positive DLBL of the elderly. This has sparked an interest in the determination of the incidence of EBV in DLBL in various regions. In Europe and the USA, only about 3% of cases is positive, but in East-Asia 9%. Wada et al. [18] found EBER positivity in more than 20% of tumor cells in 3.3% of cases (19/484 cases), which is similar to the incidence in Western countries. The cut-off of 20% is rather high, but with a cut-off of 10% it is still only 4.3%.

A group from 22 institutions [19] collected in total 1,314 cases of peripheral T cell lymphoma and describe the 340 cases of PTCL-not otherwise specified (PTCL-NOS). The median age of the patients was 60 years and the majority (69%) presented with advanced stage disease. Most patients (87%) presented with nodal disease but extranodal disease was present in 62%. The 5-year overall survival (OS) was 32% and the 5-year failure-free survival (FFS) was only 20%. The majority of patients (80%) were treated with combination chemotherapy that included an anthracycline, but there was no survival advantage. The International Prognostic Index (IPI) was predictive of both OS and FFS. Multivariate analysis of clinical and pathological prognostic factors, respectively, when controlling for the IPI, identified bulky disease, thrombocytopenia, and a high number of transformed tumor cells as adverse predictors of survival, but only the latter was significant in final analysis. Thus, the IPI and a single pathological feature could be used to stratify patients with PTCL-NOS for novel and risk-adapted therapies.

Changing immunosuppression regimens results in improved transplant survival but also differences in the development of post-transplant lymphoproliferative disease (PTLD). Wuhdikarn et al. [20] describe their 20-year experience with lung transplantation.

A total of 32 cases (5%) of PTLD were identified in 639 patients. The median interval after transplantation to the diagnosis was 40 (3–242) months. Eight patients (25%) were diagnosed within 1 year of transplantation and had PTLD predominantly within the thorax and allograft. Twenty-four patients (75%) were diagnosed more than 1 year after transplantation, and their tumors mainly affected the gastrointestinal tract. Monomorphic PTLD, DLBL, was diagnosed in 91% and 24 patients (75%) have died. The median overall survival was 10 (0–108) months.

PTLD after lung transplantation remains a challenge as a result of its frequency, complexity, and disappointing outcome.

Defining entities

B cell lymphomas

Since an anti-CD200 agent is used in clinical trials for acute myeloid leukemia Dorfman et al. [21] investigated whether other hematological malignancies express CD200 (Ox-2 membrane glycoprotein). They found that CLL, hairy cell leukemia, and HL are positive and also B-acute lymphoblastic leukemia and thus, these tumor types might be candidates for anti-CD200 directed therapy.

An entity that seems to arise is IgG4-related disease. Zen and Nakanuma describe their experience with 114 cases [22]. On the basis of the location of the lesions, the patients were classified into five groups: head and neck ($n=23$), thoracic ($n=16$), hepatic and pancreatobiliary ($n=27$), retroperitoneal ($n=13$), and systemic ($n=35$). All groups had similar clinicopathologic features in various aspects. However, there were some organ-specific features: for example, the proportion of the female patients was significantly higher in the head and neck group, serum IgG4 concentrations were significantly higher in the head/neck and systemic groups, and all kidney lesions were associated with extrarenal disease. Unique pathologic features were dense fibrosis in dacryoadenitis, numerous lymph follicles in sialadenitis and dacryoadenitis, and obliterative arteritis in lung lesions. In addition, an epithelioid granuloma and rheumatoid nodule were noted within IgG4-related lesions in two patients, one each with a history of tuberculosis and rheumatoid arthritis, respectively. Malignant tumors (two lung cancers and one malignant lymphoma) were identified after the diagnosis of IgG4-related disease in three patients, all in the systemic group. The authors conclude that further study is needed to conclude whether these features reflect different manifestations of a single disease entity or suggest different underlying etiologic factors.

Venkataraman et al. [23] deal with the same entity but from a different angle. They analyzed 32 dura-based marginal zone lymphomas identified in 27 females and five males ranging in age from 33–82 years (median, 50). Most cases presented radiologically as dura-based masses, mimicking meningioma. Histologically, the majority exhibited plasmacytoid differentiation and were clonal either by PCR or immunohistochemical light-chain analysis (28 out of 32). In the subset tested for IgG4, six of 18 primary dural marginal zone lymphoma (including one epidural tumor) showed numerous IgG4-positive plasma

cells; all six were light-chain restricted and clonal by PCR in five of six tested cases. Three IgG4-positive marginal zone lymphomas tested for cytogenetics did not show any cytogenetic aberrations. Across all cases, FISH and RT-PCR identified abnormalities in three out of nine cases (trisomies 3 and 18; trisomies 3 and 1; trisomy 18) without any extranodal marginal zone lymphoma specific translocations. Regardless of the treatment modality, 16 of 17 patients with follow-up are alive without evidence of disease over a period of 4–124 months (median, 19.5). The expression of IgG4 in light-chain-restricted clonal plasma cells of a significant subset of dural marginal zone lymphomas, including one in an epidural location, is a novel finding and points to distinctive biology.

Both these studies show that the birth of a new entity is not easy. Presently, we have too little data to make definite conclusions.

FL is typically a CD10+/CD5-/FMC-7+ B cell lymphoma with variable CD23 expression. Olteanu et al. [24] studied the expression of CD23 by flow cytometry in 69 lymph nodes and correlated it with pathologic and clinical parameters. Of 69 FL, 48 (70%) were CD23-positive. Grade 3 FL were CD23- more often (12/16 [75%]) than grade 1 and 2 cases (9/53 [17%]). CD23 expression was more common in FL in inguinal lymph nodes than in other sites: 20 of 23 (87%) vs 28 of 46. OS and event-free survival were longer in the CD23-positive group. Bear in mind though that CD23 expression as detected by flow cytometry does is often not found by immunohistochemistry due to the lower sensitivity.

Grading of FL is one of the tasks that is unpopular with hematopathologists. The exception is maybe the distinction between FL grades 3A and 3B. There is a general feeling that actually FL grade 3B is a different entity, in which bcl-2 is not involved. Shustic et al. [25] analyze the clinical outcome of the two groups in a retrospective population-based analysis of 161 patients with FL grade 3 composed of 139 with FL3A and 22 with FL3B. Patients with FL3B had a higher overall IPI score than FL3A patients, though no significant difference in individual IPI risk factor frequencies was noted. More patients with FL3B received front-line anthracycline-containing chemotherapy (82% versus 36%). With median follow-up of 45 months, no difference in disease-specific survival or OS was found between FL3A and FL3B, and no survival curve plateau was observed. Although this is relevant information, it is important to realize that survival is not relevant anymore in the definition or acceptance of a disease entity.

The normal counterparts of MCL are naive, quiescent B cells that have not been processed through the germinal center (GC). For this reason, since lymphomas arising from GC or post-GC B cells often exhibit plasmacytic differentiation, MCL rarely presents with plasmacytic features.

Visco et al. [26] describe seven cases of MCL with a monotypic plasma cell (PC) population collected from six centers, indicating the rarity of this feature. The clinical presentation was rather unusual compared with typical MCL, with two cases arising from the extranodal soft tissues of the head. All MCL cases were morphologically and immunohistochemically typical, bearing the t(11;14) (q13;q32). In all cases, the PC population was clonal. In five of the seven cases, the MCL and PC clones showed identical restriction fragments, indicating a common clonal origin of the neoplastic population. The two cases with clonal diversity denoted the coexistence of two different tumors in a composite lymphoma/PC neoplasm. These findings show that MCL can present with a PC component that is often clonally related to the lymphoma, representing a rare but unique biological variant of this tumor.

B cell activating factor receptor (BAFF-R) is one of three known receptors for BAFF. BAFF-R is required for B cell maturation and survival. Takahata et al. [27] determined the expression pattern in normal and neoplastic lymphocytes using immunohistochemistry. BAFF-R expression was detected on B cells of the mantle zones, some cells within germinal centers and scattered cells in the interfollicular areas of reactive lymph nodes. BAFF-R expression was only found in B cell lymphoma (60/120, positive samples/examined samples) but not in T/NK-cell lymphoma (0/10) or Hodgkin's lymphoma (0/10). The proportions were as follows: FL (14/16), DLBL (27/61), MCL (4/4), and BL (0/4). According to Hans' criteria [28], DLBCLs were subclassified into germinal center B cell-like (GCB) and non-germinal center B cell-like (non-GCB) types. Interestingly, in nodal lymphomas, in the GCB subgroup ($n=12$), nine of 12 (75%) were positive for BAFF-R, while six of 20 (30%) were positive in the non-GCB subgroup ($n=20$; $p<0.05$). In addition, expression of BAFF-R related to lower MIB-1 index was associated with GCB-type DLBCL. In conclusion, BAFF-R was only found in some B cell lymphomas, which was closely associated with the expression pattern in normal counterparts, although BAFF-R expression on follicular lymphoma is different from that on germinal center cells, which is similar to bcl-2. BAFF-R was rather specifically related to low growth activity of GCB-type DLBCL of nodal origin.

Another marker that has received attention is FOXP1 (not to be mixed up with the regulator T cell marker FOXP3). Yu et al. [29] investigated its expression in DLBL. Expression of FOXP1 was detected in 67.4% (95/141) of DLBCLs. FOXP1 expression in non-GCB (67/90, 74.4%) was significantly higher than that in GCB (28/51, 54.9%). FOXP1 expression in MUM1-positive cases (62/81, 76.5%) was significantly higher than that in MUM1-negative cases (33/60, 55%). FOXP1 expression was positively correlated with Bcl2 in non-GCB among nodal DLBCL cases. Among

the extranodal group, patients with FOXP1 expression had a significantly inferior OS compared with those with negative FOXP1 expression, which was not seen in nodal group. In conclusion, FOXP1 expression might be involved in the tumorigenesis of both nodal and extranodal DLBCL. The most striking finding of this study was that FOXP1 expression had an adverse effect on survival of patients with extranodal DLBCL, which indicated that FOXP1 function might be mediated by different mechanisms in nodal and extranodal DLBCLs. FOXP1 might play a role in the pathogenesis of nodal non-GCB DLBCL through the pathways in which Bcl2 was involved, and it might be a second important biomarker for non-GCB.

Both these studies therefore indicate that it is probably as important to separate DLBL in nodal and extranodal forms as it is to separate them into GCB and non-GCB.

Plasmablastic lymphoma (PBL) is another entity that keeps interest. Initially characterized as an aggressive lymphoma arising in the jaw and oral mucosa in HIV-infected patients, there are several reports on cases with extraoral manifestations, heterogeneous histologic findings, and variable association with immunodeficiency states. Hansra et al. [30] compared 13 oral and extraoral cases. The oral PBLs were strongly associated with HIV infection and commonly demonstrated plasmablastic morphologic features without plasmacytic differentiation. Extraoral PBLs tended to occur in patients with underlying non-HIV-related immunosuppression and universally demonstrated plasmacytic differentiation. The patients with oral PBL demonstrated better overall survival compared with patients with extraoral PBL. These findings suggest that PBL with oral and extraoral manifestation represent two distinct clinicopathologic entities. Valera et al. [31] investigated the genetic background of PB using FISH for MYC, BCL2, BCL6, MALT1, PAX5, and IGH loci in 42 PBL and three extracavitary primary effusion lymphomas. MYC rearrangements were identified in 20 of 41 (49%) PBL and the immunoglobulin (IG) genes were the partners in most tumors. MYC rearrangements were more common in EBV-positive (14 of 19, 74%) than EBV-negative (nine of 21, 43%) tumors. No rearrangements of BCL2, BCL6, MALT1, or PAX5 were detected in any PBL, but gains of these loci were observed in 31% to 41% of the cases examined. Twelve of the 40 PBL in which three or more loci could be investigated had multiple simultaneous gains in three or more loci. No differences in the survival of the patients according to MYC were observed, but the four patients with the longest survival (>50 mo) had no or low number of gains (<3). No rearrangements of any of these loci were seen in the primary effusion lymphomas. The authors conclude that PBL are genetically characterized by frequent IG/MYC translocations and gains in multiple chromosomal loci. The oncogenic activation of MYC in

these lymphomas may be an important pathogenetic element associated with EBV infection.

T cell lymphomas

New techniques result in new discoveries. The technology of sequencing DNA advances fast and several papers give data on sequencing of the whole genome of tumors, using technology often referred to as next generation sequencing. Feldman et al. [32] use one of the techniques under this heading, namely massively parallel genomic sequencing on ALK-negative anaplastic large cell lymphoma and discover a new recurrent translocation: t(6;7)(p25.3;q32.3). This is a balanced translocation disrupting the DUSP22 phosphatase gene on 6p25.3 and adjoining the FRA7H fragile site on 7q32.3. They also developed FISH probes to detect this translocation. I have recently described the history of the development of a marker, cyclinD1, in MCL [33], starting with the finding of a translocation: this took many years, indicating the enormous advance there has been in techniques that can be used.

Natural killer (NK) cell lymphomas/leukemias are rare neoplasms with an aggressive clinical behavior. The majority of the cases belong to extranodal NK/T cell lymphoma, nasal-type (ENKTL) in the current WHO classification scheme. Iqbal et al. [34] performed gene expression profiling of 21 ENKTL and NK-cell lymphoma/leukemia patients, 17 NK-cell and T cell lines and five indolent NK-cell large granular-lymphocytic proliferation. The results were compared with 125 PTCL patients previously studied. In the groups of ENKTL and PTCL-not otherwise specified, they identified a category of patients who had a $\gamma\delta$ -pattern of transcripts. These transcripts associate with the T cell receptor (TCR)/CD3 complex, suggesting T cell rather than NK-cell lineage. They were very similar to NK-cell tumors by GEP, but were distinct from cytotoxic ($\alpha\beta$)-PTCL and hepatosplenic T cell lymphoma, indicating derivation from an ontogenically and functionally distinct subset of $\gamma\delta$ T cells. They showed distinct expression of V γ 9, V δ 2 transcripts and were positive for TCR γ , but negative for TCR β by immunohistochemistry. These results indicate that these cases may represent a relevant subgroup.

Adult T cell leukemia/lymphoma (ATLL) is a lymphoproliferative disease caused by human T cell lymphotropic virus type 1 (HTLV-1) infection. HTLV-1 is spread by cell-to-cell transmission via the gp46-197 region in the envelope protein gp46. A correlation exists between the prevalence and titer of the antibody recognizing the gp46-197 region (anti-gp46-197 antibody) and the severity of ATLL. Kiyasu et al. [35] performed immunohistochemistry on FFPE samples of three different histological types of ATLL (anaplastic large cell type, $n=10$; pleomorphic type,

$n=10$; and Hodgkin's-like type, $n=10$) and ten cases of HTLV-associated lymphadenitis. Of the three ATLL subtypes, gp46 expression was highest in the anaplastic large cell type, followed by the pleomorphic type and Hodgkin's-like type (mean, 53.4%, 34.9%, and 16.0%). In HTLV-1 associated lymphadenitis cases, gp46-positive cells were rarely seen (4.0%). These results suggest that gp46-197 immunohistochemical staining can be a useful histological indicator for prediction of the aggressiveness of ATLL and prognosis for ATLL patients.

Cutaneous lymphomas

The classification of cutaneous B cell lymphoma is a continuous process resulting in quite small groups due to the rarity of the disease. Edinger et al. [36] collected a relatively large series of 29 cutaneous (C) MZL to investigate whether it has similarities with other MZL of MALT type. The monotypic plasma cells had class-switched heavy-chain expression in 22 cases, technically inadequate staining in one case (included with class-switched cases for analysis) and six were IgM. The 22 class-switched cases had a predominance of T cells in 22 out of 23 cases with a CD4/CD8 >1 in 15 out of 16 cases, usually showed nodules and scattered small B cells often with IgD, lacked CXCR3 B cell expression, never showed a totally diffuse growth pattern, often had prominent mast cells, and lacked known extracutaneous involvement. The six IgM cases showed a predominance of B cells (5/6), a diffuse proliferation of CD20 B cells (6/6), and extracutaneous disease (3/6). CD21 usually disrupted follicular dendritic meshworks were seen in nine out of 12 class-switched and five out of five IgM cases. These findings support the presence of two types of CMZL with the class-switched cases being the most distinctive but still sharing significant features with MALT lymphomas from other sites. The class-switched cases, however, represent a really small disease category.

Der-Petrossian et al. [37] investigate the presence of langerin-positive dendritic cells (LC) in cutaneous T cell lymphomas based on the hypothesis that cutaneous T cell lymphomas (CTCL) are diseases of chronic T cell stimulation by LC-mediated antigen presentation. Fifty-five specimens of CTCL and ten of CBCL were double-stained with monoclonal antibodies against langerin and CD3 or CD20, respectively, and evaluated by confocal laser scan microscopy. Dermal infiltrates in mycosis fungoides ($n=38$), primary cutaneous CD4+ small/medium-sized pleomorphic T cell lymphoma ($n=3$) and primary cutaneous peripheral T cell lymphoma, unspecified ($n=3$) were characterized by a high frequency of dermal langerin-positive DCs. These cells were exclusively present in the malignant infiltrates. Dermal langerin-positive cells were

detected only in one of six primary cutaneous anaplastic large cell lymphomas (C-ALCL), characterized by epidermotropism. In other C-ALCL cases (five of six), in lymphomatoid papulosis ($n=3$), subcutaneous panniculitis-like T cell lymphoma ($n=2$), and all variants of CBCL no dermal langerin-positive DCs could be found. The authors conclude that LCs are abundant in the dermal infiltrates of T cell lymphomas with specific involvement of the epidermis. This might indicate that immature LC and neoplastic T cells interact in the pathogenesis of CTCL.

It is well known that the incidence of EBV infection and EBV-associated lymphomas is highly variable in different regions of the world. Rodriguez-Pinilla et al. [38] analyzed a series of 14 cases of EBV-associated NK/T cell lymphoma from Peru. Most (11/14) of these cases fit well into the category of *Hydroa vacciniforme*-like lymphoma (HVLL), but three have a different clinical presentation, without facial involvement. In all 14 cases, skin lesions present in both the sun-exposed and nonexposed areas exhibited a slowly progressive relapsing course, changing from edema, to blistering, ulceration, and final scarring. The immunophenotype had a cytotoxic T or NK-cell lineage. The mean time of disease before admission to hospital was 69 months (range, 6 months to 31 years). Only two patients had fever, hepatosplenomegaly, systemic lymphadenopathy, and a high lactate dehydrogenase (LDH) level at the time of diagnosis, whereas ten had facial swelling. After treatment, only four patients remain alive, although with persistent disease. Ten patients died after a mean follow-up of 11.6 months after the initial diagnosis (range, 1 to 32 mo), because of concurrent infections (four cases), disease progression (four patients) or both (two patients). Endemic EBV-positive cutaneous NK/T cell lymphoproliferative disorders in childhood and early adulthood are characterized by a protracted clinical course, eventually leading to an aggressive phase characterized by concurrent infections and disease progression.

There are presently no pathologic criteria that reliably distinguish C-ALCL from other CD30-positive T cell lymphoproliferative disorders (lymphomatoid papulosis, systemic anaplastic large cell lymphoma with skin involvement, and transformed mycosis fungoides) and clinicopathologic interaction therefore is crucial. Wada et al. [39] investigated the presence of IRF4 (interferon regulatory factor-4) translocations in 204 biopsies involved by T cell lymphoproliferative disorders from 182 patients. Nine of 45 (20%) C-ALCL and one of 32 (3%) cases of lymphomatoid papulosis with informative results demonstrated an IRF4 translocation. Remaining informative cases were negative for a translocation (seven systemic ALCL; 44 cases of mycosis fungoides/Sézary syndrome (13 transformed); 24 PTCL-NOS; 12 CD4-positive small/medium-sized pleomorphic T cell lymphomas; five extranodal NK/T cell

lymphomas, nasal type; four gamma-delta T cell lymphomas; and five other uncommon T cell lymphoproliferative disorders). This FISH approach is therefore helpful in the differential diagnosis, but not very sensitive.

Pitfalls in lymphoma diagnosis

Intestinal T cell and NK-cell lymphomas are clinically aggressive and can be challenging to diagnose in small endoscopic biopsies. Mansoor et al. [40] collected eight patients in whom atypical NK cell lymphoproliferative lesions mimicked NK- or T cell lymphoma. The patients (two men; six women; ages 27–68 years) presented with vague gastrointestinal symptoms with lesions involving stomach, duodenum, small intestine, and colon. At endoscopy, the lesions exhibited superficial ulceration, edema, and hemorrhage. Biopsies revealed a mucosal infiltrate of atypical cells with an NK-cell phenotype (CD56+/TIA-1+/granzyme B +/cCD3+), which displaced but did not invade the glandular epithelium. EBER in situ hybridization was negative, and TCR gamma gene rearrangement showed no evidence of a clonal process. Based on an original diagnosis of lymphoma, three patients received aggressive chemotherapy followed by autologous bone marrow transplantation in two. Five patients were followed without treatment. However, no patient developed progressive disease or died of lymphoma (median follow-up, 30 M). Repeat endoscopies in 6/8 patients showed persistence or recurrence of superficial gastrointestinal lesions. This unique entity mimics intestinal and NK/T cell lymphomas on endoscopic biopsies and can result in erroneous diagnosis leading to aggressive chemotherapy. The authors propose the term "NK-cell enteropathy" for this syndrome of as yet unknown etiology. It is a good example of carefully evaluating unusual lesions and collecting them for better understanding.

Prognostic factors in lymphoma

In the previous review, I drew attention to the renewed interest for the prognostic relevance of CD68-positive macrophages in classical HL. Now, Tzankov et al. [41] confirm that increased numbers of macrophages indicate a poorer survival and expand on this. They applied a cut-off score of >0.82% tumor macrophages by which cases with increased numbers showed worse OS (mean, 185 months; median, 192) compared with cases with lower numbers (mean, 285 months; median, not reached). The number of macrophages correlated with a low FOXP3-/high granzyme B-/high PD-1-positive micromilieu and patient age but did not have independent prognostic significance. This study

shows some of the difficulties that we have with developing good prognostic markers that are clinically relevant: a cut-off point that is difficult to use and not biologically determined, and multiple interrelated relevant factors.

A new genomic analysis of MCL by Halldorsdottir [42] gives an illustration of yet another problem: this study uses 31 cases and finds prognostic relevance of several genomic alterations from a huge amount tested by a array approach: large/complex 13q losses were associated with improved survival as were losses/copy number neutral LOH at 19p13. In this study, also identified were novel aberrations. It has become clear that such an approach (many factors, few cases) is highly subject to coincidental findings, even though good *p* values are reached.

Camacho et al. [43] use a more well-known approach: immunohistochemistry on FL. In a series of 186 FL, they identified several markers associated with differences in OS in univariate analyses, such as Cyclin E, Mdm2, CD10, p21, IgD, Bcl-xL, CD30, and E2F6. Cases with a higher level of expression of Cyclin E, Mdm2, p21, IgD, Bcl-xL, CD30, and E2F6 were associated with a significantly shorter OS. On the other hand, strong CD10 expression was linked to a significantly better outcome. A Cox model was then constructed, integrating the follicular lymphoma international prognostic index (FLIPI) score and a restricted selection of three immunohistochemical markers: Cyclin E, Mdm2, and CD10 expression. A potentially useful finding is that the integrated FLIPI plus immunohistochemical model can be used to identify a subset of 26 patients (almost 20% of the total series), with a survival probability of 100% at 5 years. This not only confirms that a group of FL cases may have a very good clinical course, but also indicates that this group can be identified using this integrated clinical and immunohistochemical approach. The issue in this study is of course that it needs independent confirmation, which will likely not happen since the methodology is not easily reproducible (cut-off points!), the number of cases is quite low, and there is not a real biological substrate for the algorithm. This is a bit exemplified by the study of Richendollar et al. [44] who looked at the prognostic value of PD-1 in FL (see above, studied in HL by Tsankow, but not in the FL study by Camacho). In their series of 91 newly diagnosed FL, they quantified the mean number of follicular PD-1-positive cells per nine (!, HVK) high-power fields ($\times 1,000$, three follicles with three fields per follicle). PD-1-positive cells correlated with the number of FOXP3-positive regulatory T cells. On multivariate analysis, independent poor prognostic factors were age 55 years or greater, bulky disease, CD68-positive extrafollicular lymphoma-associated macrophages greater than 16.8 cells/high-power field (HVK), and PD-1-positive cells greater than 35.6 cells/high-power field (HVK). These factors allowed construction of a risk score

defining three distinct prognostic groups with 10-year overall survival of 85%, 60%, and 15%. PD-1-positive follicular helper T cells and CD68-positive extrafollicular lymphoma-associated macrophages appear to predict overall survival in follicular lymphoma. The authors indicate indeed that their data need confirmation in an independent study.

There is no doubt that the division of DLBL in GCB and activated (or non-GCB) type is prognostically relevant. These subgroups can be distinguished by expression array, but several immunohistochemical algorithms have also been proposed. There are also other prognostic algorithms, like the ones mentioned above and one described by Muris et al. [45]. Jablonsky et al. [46] are to be applauded, since they performed indeed a study that aims to confirm previous studies and compared two such algorithms in 66 de novo DLBCL cases. Cases classified by the Hans algorithm [28] as GCB had indeed a significantly lower risk of death compared with the non-GCB group. The 5-year OS rate was 85% for the GCB group and only 30% for the non-GCB group. Although the 5-year OS of favorable group 1 from Muris algorithm was slightly better than in group 2, the difference was not significant. These results indicate that the algorithm of Hans et al. has a significantly better prognostic value.

The attentional reader will have noticed that many prognostic markers described above are related to the tumor stroma. This was the topic of the workshop of the European Association of Hematopathology in Bordeaux in 2008 and maybe that meeting has sparked the interest. Meyer et al. [47] performed immunostains for two stromal cell markers, CD68 and SPARC (secreted protein, acidic, and rich in cysteine), in 262 patients with DLBCL treated with rituximab and cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) or CHOP-like therapies. CD68 expression by cells in the microenvironment did not predict survival. Patients with any SPARC-positive cells in the microenvironment had a significantly longer OS, and patients with high SPARC positivity in the microenvironment also had a significantly longer event-free survival. Survival differences were mainly due to the prognostic effect of SPARC-positive cells in non-GCB-type DLBCL, with no effect found in the GCCB–DLBCL. Of clinical features examined, only the number of extranodal sites was significantly associated with SPARC expression. Multivariate analysis revealed that SPARC expression predicted patient survival independent of the IPI or tumor cell of origin. SPARC expression in the microenvironment of DLBCL can be used for prognostic purposes, determining a subgroup of patients with non-GCB-DLBCL who have significantly longer survival. More aggressive chemotherapy protocols should therefore be considered for patients with non-GCB-DLBCL without SPARC-positive stromal cells.

However, a clinical trial confirming this will be very difficult to perform.

Dong et al. [48] selected gastrointestinal DLBL for their study into prognostic markers. They investigated the genetic abnormalities of CARD11, A20, and ABIN-1/2/3 (the A20 binding inhibitor of NF- κ B) and their clinicopathological correlation in 71 gastrointestinal DLBCL. Recurrent somatic mutations were found in CARD11 (10%), A20 (17%), ABIN-1 (4%), and ABIN-2 (3%), but not in ABIN-3. Among these genetic abnormalities, A20 somatic mutation was significantly associated with both poor OS and event-free survival.

Staging

Staging of tumors remains a cornerstone in the diagnostic work-up of patients. Yi et al. [49] looked at the relevance of bone marrow involvement in 25 nodal PTCL in comparison with 42 DLBL. Most of the PTCL patients with BMI had lymphadenopathy (88%), B symptoms (76%), an elevated LDH level (68%), anemia (64%), splenomegaly (60%), and a poor performance status (52%). Except for the differences of lymphadenopathy and thrombocytopenia between PTCL with BMI and DLBCL with BMI, similarities in gender, age, hepatomegaly, splenomegaly, a bulky mass, B symptoms, elevated LDH, ≥ 2 extranodal sites, ECOG scores ≥ 2 , anemia, IPI, and age-adjusted IPI scores were observed between the two groups. The two groups also had similar 3-year OS (25.8% vs. 30.0%) and progressive-free survival (21.3% vs. 25.2%) rates. In contrast to what is often assumed, this study indicated that PTCL with bone marrow involvement have a similar aggressive course and poor survival compared with DLBCL with bone marrow involvement.

Ancillary techniques

Several papers deal with the use of clonality testing in lymphoproliferations. Berget et al. [50] evaluate the now commonly accepted The BIOMED-2 multiplex PCR protocol performance in detection of B cell clonality in 118 FL using formalin-fixed, paraffin-embedded (FFPE) tissue. The highest clonal detection rates were 67.8% with the IGH VH-FR2-JH assay and 66.1% with the IGK V κ -J κ assay. Clonality was detected in 94.9% of all FFPE FL samples when all assays were combined. FFPE samples stored for 1–5 years did not perform significantly differently from those stored for 6–11 years. In line with the advice of the Biomed group [51], they conclude that the improved PCR protocol for detection of clonality in FFPE samples using BIOMED-2 IG primers is a combination of IGH and IGK analyses.

Clonality testing is relevant in several situations, but in skin lesions of limited value due to the fact that pseudolymphomas may have dominant clones and since there is often a skewed T cell repertoire. Felcht et al. [52] investigated a series of cutaneous B cell lymphomas and benign B cell proliferations using the Biomed approach. Monoclonality was detected in all of five primary cutaneous (PC) FCL; five of six PCMZL; all of six PCDLBL, leg type (LT); and two of nine benign lymphocytic infiltrates. In five of six patients with PCLBCL, LT, a clone corresponding to the clone detected in the skin, was detected in three of five bone marrow, four of five blood, and one of three lymph node specimens. DNA amplification using tubes A and B of IgH was not possible in PCFCL/PCMZL, benign lymphocytic infiltrates, and extracutaneous specimens of PCLBCL, LT, even after repeated analysis up to 11 times. Pseudomonoclonality was identified by repeated analyses in one case of PCMZL and in one case of benign lymphocytic infiltrate. Molecular diagnosis supports the clinical and (immuno)-histologic diagnosis in PCBCL. In PCLBCL, LT, molecular staging may be useful. Tubes C through E of IgH and Ig κ analyses seem to be superior to tubes A and B of IgH. Each sample should be analyzed at least twice to assess the possibility of pseudomonoclonality. This latter finding, especially, is quite relevant and is actually the case for all clonality testing.

Proteomics only slowly enters the diagnostic arena. It is a more difficult to standardize compared with DNA and even RNA approaches. Jansen et al. [53] determined the potential value of protein profiling of tissue samples by assessing how precise this approach enables discrimination of B cell lymphoma from reactive lymph nodes and how well the profiles can be used for lymphoma classification. Protein lysates from lymph nodes ($n=239$) from patients with the diagnosis of reactive hyperplasia ($n=44$), FL ($n=63$), DLBL ($n=43$), MCL ($n=47$), and CLL ($n=42$) were analyzed by SELDI-TOF MS. Both the classification and regression tree and logistic regression classification correctly identified the majority of the malignant (87% and 96%, respectively) and benign cases (73% and 75%, respectively). Classification was less successful since approximately one third of the cases of each group were misclassified according to the histological classification. However, an additional MCL case that was misclassified as CLL initially was identified based on the protein profile. These data are promising but much further data need to be gathered.

Monitoring disease by taken blood samples is an attractive approach, since it is relatively simple and not expensive. Spacek et al. [54] monitored in a cohort of 165 adult HL patients EBV-DNA viral load. Diagnostic tissue samples of all patients were histologically reviewed; in

72%, nodular sclerosis was detected, 24% presented with mixed cellularity (MC), and 5% had other type of HL. Tissues from 150 patients were also analyzed for the presence of latent EBV infection using in situ hybridization for EBV-encoded RNA (EBER) and immunohistochemistry for latent membrane protein (LMP1). Using these methods, 29 (19%) patients were classified as EBV-positive. Using real-time quantitative PCR, 22 (76%) of EBV-positive HL patients had detectable EBV-DNA in the plasma and 19 (66%) patients in whole-blood prior to therapy. In the group of EBV-negative HL cases, three (2%) patients had detectable plasma EBV-DNA and 30 (25%) patients whole-blood EBV-DNA before treatment. EBV-positive HL was significantly associated with EBV-DNA positivity both in the plasma and whole-blood in pretreatment samples, increasing age, and MC subtype. Serial analysis of plasma EBV-DNA showed that response to therapy was associated with decline in viral load. Moreover, significantly increased plasma EBV-DNA level recurred before disease relapse in one patient. These results suggest that the assessment of plasma EBV-DNA viral load might be of value for estimation of prognosis and follow-up of patients with EBV-positive HL.

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