EUROPEAN HEMATOLOGY ASSOCIATION

New approaches to indolent lymphoma - Section 1

Molecular profiling of indolent lymphoma

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Take-home messages

- Indolent lymphomas are characterized by a clinical course measurable in years or even decades but remain incurable for the vast majority of patients with a high tumor burden.
- Molecular and cytogenetic techniques have significantly expanded the bio-pathological knowledge of these neoplasms by identifying lesions that are provided with diagnostic, prognostic and/or therapeutic relevance.
- The perspective is the development of targeted therapies that can eradicate indolent lymphomas leading to patients' cure and delay the time to first chemotherapy.

Introduction

Molecular techniques have shed new light on the pathobiology of indolent lymphomas and evidenced lesions provided with diagnostic, prognostic and/or therapeutic relevance, especially in the setting of chronic lymphocytic leukemia (CLL), lymphoplasmacytic lymphoma (LPL), hairy cell leukemia (HCL), marginal zone lymphomas [(MZL), splenic (S), nodal (N) and extranodal (EN)], and follicular lymphoma (FL) (Table 1).[1]

CLL:[1-4] Patients with mutated IGHV have a better prognosis than those with unmutated genes. The commonest alterations in CLL are deletions in 13q14.3 (miR-16-1 and miR-15a) (~50%), trisomy 12 (~20%) and, less commonly, deletion in 11q22-q23 (*ATM* and *BIRC3*), deletion in 17p13 (*TP53*), and deletion in 6q21. The most commonly mutated genes, detected in 3-15% of cases, are *NOTCH1*, *SF3B1*, *TP53*, *ATM*, *BIRC3*, *POT1*, and *MYD88*. Deletion in 11q and, particularly, deletion in 17p confer a worse clinical outcome whereas isolated deletion in 13q14 is associated with a more favorable clinical course. *TP53* abnormalities (i.e., deletion in 17p13 and *TP53* mutations) are predictive of lack of response to fludarabine-containing regimes. Mutations in *TP53*, *ATM*, *NOTCH1*, *SF3B1*, *BIRC3,* among others are associated with a poor outcome.

LPL:[1,5-8] IGHV turn hypermutated. No specific chromosomal abnormalities are recognized in LPL; however, >90% and ~30% of cases have *MYD88* L265P and *CXCR4* mutations, respectively. *ARID1A* mutations are recorded in 17% of patients, and less commonly, mutations of *TP53, CD79B, KMT2D/MLL2* and *MYBBP1A*. Documenting a *MYD88* L265P mutation may be helpful in diagnosing LPL, although it can be recorded also in other B-cell lymphomas. Similarly, *CXCR4* mutations are not exclusive of LPL. These mutations are important in the pathogenesis of LPL by causing NF-kB activation. Notably, cases lacking a *MYD88* L265P mutation

Table 1. Summary of the main cytogenetic and molecular aberrations in the setting of indolent lymphomas.

Lymphoma category	Main cytogenetic findings	Main somatic gene mutations
Chronic lymphocytic leukemia	del13q14.3, +12, del11q22-q23, del17p13, del6q21.	*NOTCH1, SF3B1, TP53, ATM, BIRC3, POT1, MYD88*
Lymphoplasmacytic lymphoma	del6p, +4	*MYD88, CXCR4, ARID1A, TP53, CD79B, MLL2, MYBBP1A*
Hairy cell leukemia	-	*BRAF V600E*
Splenic marginal-zone lymphoma/leukemia	t(2;7)(p12;q21), del7q, +3q	*MLL2, NOTCH2, KLF2, MYD88*
Nodal marginal-zone lymphoma	+3, +18, del6q23	*MLL2, NOTCH2, KLF2, MYD88, PTPRD*
Extranodal marginal-zone lymphoma	t(11;18)(q21;q21), t(1;14)(p22;q32), t(14;18)(q32;q21), t(3;14)(p14.1;q32), +3, +18, del6q23	*TNFAIP3, MYD88, PIM1, TP53, MYC*
Follicular lymphoma	t(14;18)(q32;q21), del1p36	*EZH2, KMT2D/MLL2, CREBBP, RRAGC, TP53, CDKN2A, MYC*

are reported to have an adverse prognosis and a lower response to ibrutinib. Gene expression profiling (GEP) studies suggest upregulation of IL6 and its downstream MAPK signaling pathway.

HCL:[9] Most HCL cases show hypermutated IGHV. A unique feature of HCL is the common co-expression of multiple clonally related Ig-isotypes, suggesting arrest at some point during isotype switching. No cytogenetic abnormality is specific for HCL. The high frequency of *BRAF* V600E mutation suggests a key role in the pathogenesis of the disease and has prompted to the usage of BRAF-inhibitors in patients with HCL resistant to previous lines of therapy. Whether cases that lack *BRAF* V600E mutation, use the IGHV4-34 family and have *MAP2K1* mutations are most closely related to classic HCL or HCL-variant remains to be established.

SMZL:[1,10] SMZL lacks recurrent chromosome translocations observed in other lymphomas. A small number of cases carries a t(2;7)(p12;q21) activating the *CDK6* gene. Approximately 30% of SMZL show a heterozygous deletion in 7q. In addition, gain of 3q is present in a considerable subset of cases. *NOTCH2* and *KLF2* are mutated in 10-25% and 10-40% SMZLs, respectively. Both mutations, however, are also found in other small B-cell neoplasms and have been associated with SMZLs carrying deletion in 7q. *MYD88* mutations are rare in SMZL, and therefore may contribute to the differentiation from LPL. The observation that the most frequently mutated genes in SMZL (*NOTCH2*, *KLF2, KMT2D/MLL2*) are physiologically involved in proliferation and commitment of mature B-cells to the MZ, points to homing to the spleen compartment and MZ differentiation as the major programs deregulated in this lymphoma. Consistently, SMZL has an expression signature characterized by the upregulation of genes belonging to the MZ differentiation program.

NMZL:[1,11,12] IGH are clonally rearranged with a predominance of mutated VH3 and VH4 families, particularly VH4-34. Cases associated with HCV preferentially use VH1-69. NMZL shares gains of chromosome 3 and 18 and loss of 6q23-q24 with ENMZL and SMZL. GEP analysis has demonstrated an increased expression of NFkB-related genes. *MYD88* L256P mutation has been detected in occasional cases. A recent publication has shown that, although NMZL shares with SMZL a common mutation profile, NMZL harbors *PTPRD* lesions that are otherwise absent in SMZL.

ENMZL:[1,13,15] IGHV are rearranged and hypermutated. There is biased usage of certain IGVH families at different anatomic sites, suggesting antigen-induced clonal expansion. Chromosomal translocations associated with ENMZLs include t(11;18)(q21;q21), t(1;14)(p22;q32), t(14;18)(q32;q21), and t(3;14)(p14.1;q32), resulting in the production of a chimeric protein (*BIRC3/MALT1*) or in transcriptional deregulation (*BCL10, MALT1, FOXP1*) respectively. Trisomies 3, 18 or less commonly of other chromosomes are not infrequent in ENMZL, although unspecific. The prevalence at which the translocations or trisomies occur, varies with the primary anatomic site and geographic area. Abnormalities of *TNFAIP3* on chromosome 6q23 occur in 15-30% of cases but are not specific for ENMZL. *MYD88, PIM1, TP53,* and *MYC* mutations have been reported in 6-9% ENMZL.

FL:[1,16-20] IGH and IGL are rearranged; variable region genes show ongoing somatic hypermutation. The BIOMED-2 approach is recommended to detect clonality. FL is associated with the development of multiple sub-clones: transformation develops in an earlier common progenitor, rather than one of the later sub-clones. FL is genetically characterized by the translocation t(14;18)(q32;q21) between the IGH and *BCL2* genes: it is present in up to 90% of grade 1-2 FL. Given the variation in breakpoint regions, FISH is more sensitive than PCR to detect the translocation. Using GEP, FL lacking *BCL2* rearrangement usually has a late germinal center profile and is more frequent graded 3B. In addition to the t(14;18), other genetic alterations are found in 90% of FL. One of the most commonly affected regions is 1p36, which contains *TNFRSF14*. The number of additional alterations increases with histological grade and transformation. Mutations of *EZH2* are relatively common in FL, and appear to be an early event. Additionally, driver mutations in the chromatin regulator genes *CREBBP,* and *KMT2D/ MLL2* play a key role, and *EZH2*, *KMT2D/MLL2*, and *CREBBP* have all been proposed as possible therapeutic targets. More recently, activating somatic mutations in *RRAGC* were found in approximately 17% FL. GEP studies have shown the importance of the microenvironment in the pathogenesis, evolution and prognosis of FL. Transformation to DLBCL may follow different pathways including inactivation of *TP53*, *CDKN2A*, and activation of *MYC*.

In the present era of druggable genome, molecular studies give for the first time the chance to effectively cure indolent lymphomas.

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