Article is available online at http://www.webio.hu/por/2005/11/4/0205

REVIEW

Chromosome Abnormalities with Prognostic Impact in B-cell Chronic Lymphocytic Leukemia

Gábor MÉHES

Department of Pathology, University of Pécs, Medical School

The detailed analysis of the biologic features led to a rapid increase in clinically relevant information in CLL. The recognition of the prognostic role of IgV_H hypermutation status and related phenotypic changes (CD38, ZAP-70 expression) as well as of chromosome abnormalities defined by cytogenetic analysis enabled a refined classification of the disease. Improvements in karyotyping and the introduction of fluorescence in situ hybridization (FISH) in routine hematological diagnostics raised the detection rate of chromosomal aberrations to approx. 60-80% in CLL. Among them, deletions of 17p and 11q have been associated with unfavorable prognosis. The deletion of the p53 locus (17p13) was described as the strongest independent predictor for aggressive behavior, resistance to chemotherapy and early death. On the contrary, an isolated deletion at 13q14 or a normal karyotype was related with a long survival. Classical and molecular cytogenetic analysis became an important tool for individual risk estimation. Unlike any other approaches, cytogenetic monitoring reflects the genetic heterogeneity and clonal growth dynamics during the course of the disease. (Pathology Oncology Research Vol 11, No 4, 205–210)

Key words: CLL, chromsomal deletions, IgV_H, prognosis

Introduction

Chronic lymphocytic leukemia (CLL) was traditionally considered as a disease of elderly patients with a generally long survival lasting up to 30 years from diagnosis. The duration and outcome of the disease is, however, highly variable. The first period of the follow-up is usually free of any symptoms. Progression may appear years later in the form of lymphadenopathy or organ involvement, but the rapid transformation to prolymphocytic leukemia or aggressive lymphoma (Richter's syndrome) is also a well-known complication.[3,17,30] In contrast to the majority of the cases, early progression and fatal outcome can be also seen within the first months/years in a small fraction of the patients.

The number of treatment alternatives is fortunately growing. Beside combined chemotherapy regimens, promising treatment options including immunotherapy and stem cell transplantation approaches are now also under detailed clinical investigation. In the past the clinical stage (Rai, Binet) was the basis for classification and therapeutic decisions. The novel treatment options would, however, necessitate the precise identification of patients with unfavorable prognosis, who are at the highest risk for early progression and who would gain the most benefit from a more aggressive, targeted treatment. For this reason, the value of the biological features characterizing the leukemic transformation process has increased.

CLL arises from a clonal growth of B lymphocytes, which initially may be triggered by yet unknown antigens according to the present hypothesis.[5,20] During the process of normal B-cell maturation, somatic hypermutation of the variable region of the immunoglobulin heavy chain gene (IgV_H) in the lymphatic follicle is an important station. By now it is widely accepted that the hypermutation status of leukemic cells in B-CLL has a major impact on the long-term survival of the patients. Leukemias of unmutated B-cell nature frequently showed early progression or transformation.[29] In reverse, patients with leukemias of rearranged IgV_H status had a significantly longer survival. The determination of the IgV_H mutation status is unfortunately laborious and expensive for the daily diagnostic use.[19] Therefore, accessible biomarkers related to the mutation status (e.g. CD38 or ZAP-70 expression demonstrated by flow cytometry) are increasingly applied for the prognostic classification.[18,21]

Received: Oct 3, 2005; *accepted:* Nov 1, 2005

Correspondence: Gábor MÉHES, MD, PhD, Department of Pathology, University of Pécs, Medical School, Szigeti út 12., Pécs, H-7602, Hungary. Fax: 36 72 536281, e-mail: gabor.mehes@freemail.hu

Table 1. **Occurrence of the most frequent cytogenetic aberrations in B-CLL based on several recent larger studies**

Reference	*Cases (n)*	*Aberrations total*	*Del(13q)*	*Del(11q)*	*Del(17p)*
		(%)			
Döhner et al.[10]	325	82.0	55.0	18.0	7.0
Bacher et al.[1]	153	74.5	66.2	12.6	10.7
Dewald et al.[9]	113	77.0	64.0	15.0	8.0
Glassman et al.[11]	98	65.3	40.8	23.4	12.2
Pécs, Hungary (unpublished)	61	59.0	40.9	16.3	13.1

In CLL, like in many other hematological disorders, a huge amount of knowledge on the genetic changes accumulated in the last ten years. Chromosomal aberrations could be demonstrated at diagnosis or later in the course of the disease in the majority of the cases. Some of them were found to be tightly related to the outcome. In the present overview we would like to highlight the clinically relevant chromosomal aberrations in CLL and point one more time to the clinical role of cytogenetic examinations in this disease.

Chromosomal aberrations in CLL

Cytogenetic changes in general

Chromosomal changes in CLL proved to be far more frequent than they were detected by G-banding in the early days of cytogenetics. Aberrations were more recently reported in 50-80% of the CLL cases thanks to the introduction of the molecular cytogenetic technology.[1,10,14] Interestingly, the general picture is dominated by unbalanced changes, mainly with deletions of well-definable chromosomal regions.[2,14] Translocations usually accompany the progressive phase of the disease and seem to be more sporadic. t(11;14) formerly reported in CLL is characteristic for mantle cell lymphoma (MCL) according to the current lymphoma classification. The rare peripheral manifestation of this entity shares many features with CLL (lymphocytosis, benign appearance, etc.). MCL indicated by t(11;14) has, however, a clinically different, aggressive behavior and a much shorter overall survival.

The most frequent and prognostically relevant chromosomal aberrations in CLL are presented in *Table 1*. In the followings, the biological background and significance of the most frequent chromosomal findings are summarized.

Del(17p)

According to recent observations, the loss of the short arm of chromosome 17 (more precisely chromosome locus 17p13) is associated with the worst outcome ever, predicting a disease progression within 1-2 years and a relative resistance to chemotherapy.[9,15,16] The deletion of 17p has been found to be the strongest independent marker for disease-related death.[3,10] This change can be demonstrated both by karyotyping and FISH (*Figure 1*). The aberration can be found already at diagnosis in 5% of the CLL cases, the overall frequency was 7-12%. Later occurrance of del(17p) was usually reported together with accompanying additional aberrations.

The 17p13 locus hosts several genes with important regulatory function, including the p53 tumor suppressor gene which becomes deleted as a consequence of the aberration. This alone may serve sufficient explanation for the unfavorable effect of this change. p53 is a key molecule in the regulation of apoptosis, also named programmed cell death, a general mechanism for a rigorously controlled tissue turnover.[28] Due to the failure of the p53-dependent major apoptotic pathway, cells may survive and accumulate further genetic defects (*Figure 2*). Deletion of the p53 suppressor gene is frequently associated with the mutation of the homologue allele (30-40% of all deleted cases), completely disabling the apoptotic effector function of the molecule.[31]

p53 deficiency may have a direct impact on the therapeutic success, too. The standard cytotoxic regimens recommended for CLL consist of combinations of purine analogues, the effect of which relies on the induction of the intact p53 dependent pro-apoptotic mechanisms. The exact correlation of the deficient p53 function with therapy failure is subject of intensive discussions.[8,25,32]

Del(11q22-23)

Deletion of the q22-23 locus on the long arm of chromosome 11 is another aberration with unfavorable prognosis. This change was reported in 12-21% of all CLL patients.[9,10,14,15] Within the given locus ATM (ataxia telangiectasia mutated) is deleted. This gene is known to be responsible for *ataxia telangiectasia,* a hereditary syndrome with neurological disorders and increased risk for tumorigenesis.[3,14] ATM is known as an important synergist in the apoptotic pathway, the failure of which has been demonstrated in many malignancies including lymphoproliferative disorders other than CLL. The normal ATM acts

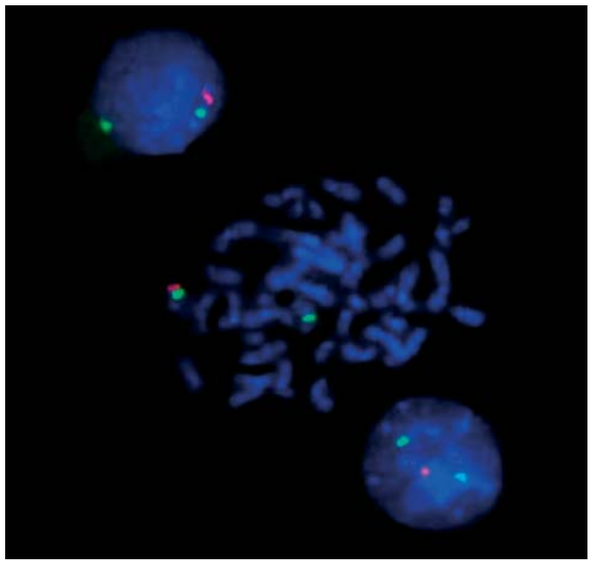

Figure 1. *Deletion of 17p13 as demonstrated by fluorescence in situ hybridization (FISH). A metaphase (center) and two interphase nuclei all show two green centromeric spots representing two copies of chromosome 17. The DNA probe specific for 17p13 (one red signal/cell) indicate the loss of this region in one of the chromosomes. DAPI (blue staining) was used to highlight nuclear chromatin in the preparation.*

predominantly through the activation of the p53-dependent apoptotic pathway,[27,28] and its loss results in reduced cell death similar to p53 deficiency (*Figure 2*).

Trisomy 12

Early cytogenetic studies described trisomy 12 as the most frequent chromosomal aberration in CLL,[23] as it was easy to recognize even in karyograms of poor quality. Due to extended karyotype and FISH studies its frequency was reported to be approx. 15-18%. Long-term clinical studies point to an unfavorable nature of this aberration, although not all data could confirm a difference in the survival from patients with normal karyotype.[14,15] Matrix CGH and expression studies indicated to a group of activated genes at 12q, which may be involved in disease progression.[24] Trisomy 12 is also frequently combined with other karyotype abnormalities.

Del(13q)

Deletion of 13q may be limited to the single locus (q14), or be accompanied with the loss of a larger interstitial region of the long arm of chromosome 13. In the former case the change is almost undetectable for karyotyping. Del(13)(q14) is the most frequent genetic change in CLL, as it was reported in 40-65% of the disease. In approx. 1/3 of the cases the deletion is biallelic. Interestingly, either form of the deletion was associated with a favorable prognosis, provided that this was the only aberration.[9,15,16] Following the acquisition of further chromosomal aberrations (most frequently del(11q) in our collection), the favorable effect of del13q14 on the outcome disappears *(Figure 3)*.

Although several candidates has been suggested,[12] a classical tumor suppressor gene within the deleted region could not be uncovered so far. On the contrary, according to a present exciting hypothesis, the deleted 13q14.3 region would harbor a set of non-protein-coding microRNA genes (4). These genes express short (approx. 20-30 base pairs) functional RNA molecules which would be capable to regulate distant sites of the genome by RNA interference. miRNAs originating from the 13q14 locus could play an important role in the regulation of the cellular activation and, therefore, the altered balance of this control mechanism might turn down the progression of the disease.

Less frequent recurrent aberrations

Due to the high frequency of the above mentioned aberrations, their prognostic impact could be statistically clarified in large series. This is not the case for low frequency aberrations which occur sporadically. Del(6q) (mostly 6q21-23), a recurrent change also occurring in other lymphatic malignancies,[6] is reported in 2-6% of the CLL cases. For cases with del(6q), even a distinct CLL subtype with unclear prognosis was suggested.[6] As a rare aberration, the IgH locus at 14q32 may be affected in translocations and deletions. Involvement of regions at 3q and 8q has also been described.

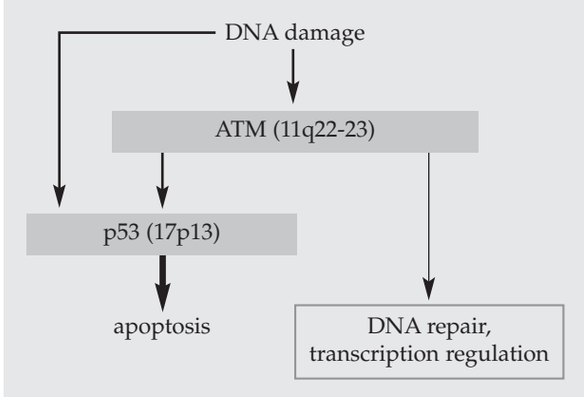

Figure 2. *The role of p53 (17p13) and ATM (11q23) in the elimination of damaged cells. ATM acts basically by the induction of the p53-dependent apoptotic pathway. Deficiency in the function of p53 or ATM may both lead to leukemia cell survival despite of the continuous occurrence of genetic errors and regulatory defects.*

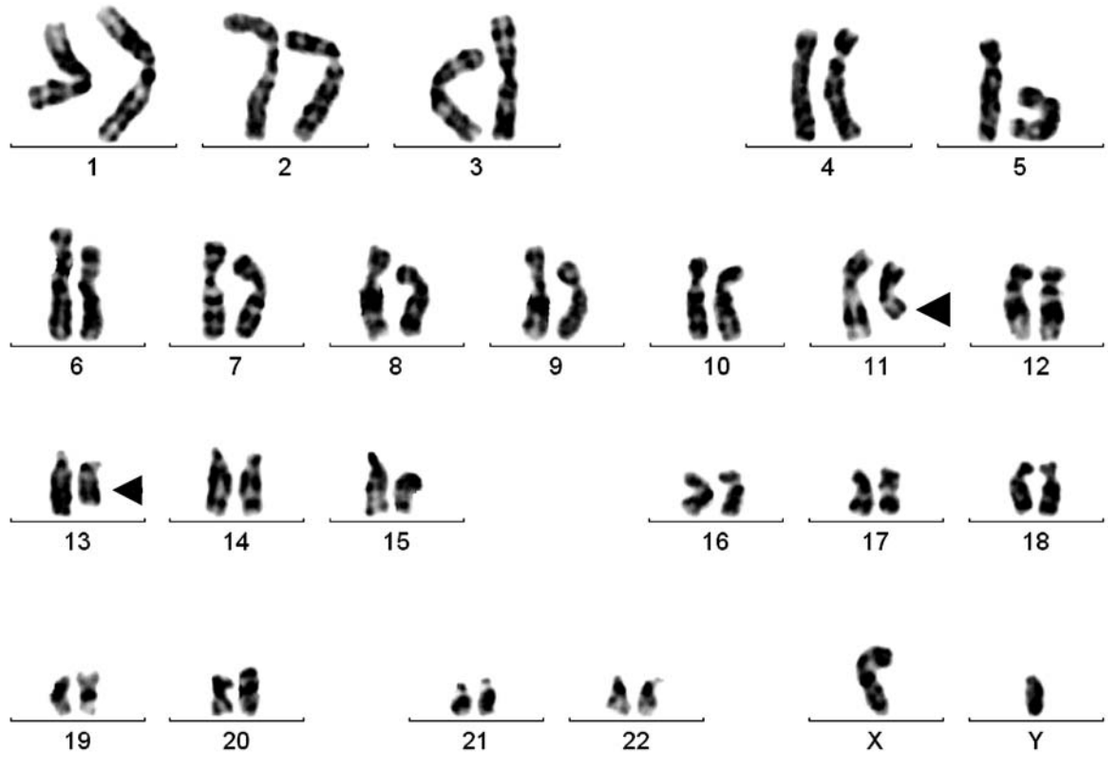

Figure 3. *Karyotype from the peripheral blood of a CLL patient cultivated in the presence of the B-cell mitogen PMA. Beside an interstitial deletion at the long arm of chromosome 13 (arrow), a deletion involving the ATM locus (q22-23) on 11q is also obvious (arrowhead). Due to this complexity, the favorable nature of del(13q) is neutralized.*

Normal karyotype in CLL

In 20-40% of CLL cases no genetic disorders can be demonstrated despite the use of refined cytogenetic technology. This fact is a strong argument for the secondary nature of all previously mentioned chromosomal changes. The negative cytogenetic finding – by exclusion of all presently known unfavorable changes – equals to a favorable clinical prognosis. From the practical point of view we would, however, note that negativity not necessarily will represent the malignant cell population. Exact information on the composition of the material examined is crucial for the proper interpretation of the results.

Cytogenetic examination of CLL

Routine demonstration of cytogenetic aberrations in CLL

Karyotyping or classical cytogenetic analysis is still the standard method for demonstrating chromosomal aberrations. As CLL lymphocytes show only very limited spontaneous growth, special B-cell mitogenic stimulation is necessary to drive the cells in the mitotic phase. For this purpose short-term cultivation of leukemic cells in the presence of B-cell mitogens (phorbol 12-myristate 13-acetate: PMA, Pokeweed mitogen, etc.) from the peripheral blood can be recommended. Cultivation of bone marrow samples for this purpose promise only limited success, moreover, the many different cell types with spontaneous mitotic activity may significantly influence the picture. Karyotyping offers an overview of all existing aberrations within the same cells and also a clear comparison of the frequency of the changes, if the number of metaphases is satisfactory. With good practice a success rate of more than 80% can be achieved, which also depends on the skill and enthusiasm of the laboratory personal.

Fluorescence in situ hybridization (FISH) enables the identification of well-known chromosomal regions in both metaphase and interphase cells.[11] This method is inevitable for the presentation of smaller deletions, where chromosome morphology frequently does not reflect any aberration, like in the case of del(13)(q14). Due to the relatively high number of evaluated cells, the technique of FISH supports the quantification of different cell clones enabling the follow-up at the cytogenetic level. Ready-to-use fluorochrome-labeled DNA probes are commercially available for all interesting regions. Beside chromosomal preparations, these can be applied to any kind of intact cell prepa-

rations including blood and bone marrow films, and also to isolated nuclear suspensions prepared from formalin-fixed and paraffin-embedded tissue blocks.

Research tools for the genetic analysis of CLL

A number of promising novel methods enable recently the extended genotyping of leukemias, including CLL. The general aim of these applications is to acquire complex information on the whole genome and to eliminate the drawbacks of the cell based technologies.

Comparative genomic hybridization (CGH) focuses on the demonstration of genomic gains and losses at a resolution of 1 Mb following whole genome in situ hybridization. Equivalent amounts of differentially labeled DNA from normal and diseased tissue are allowed to hybridize to normal chromosomes. Comparison and relative quantification can be done by the evaluation of the labeling ratios at specific chromosome loci. CGH provided evidence that the most frequent aberrations in CLL were unbalanced losses (deletions).[22] CGH has been applied for the monitoring of progression-dependent genetic changes.

Matrix-CGH. covers the hybridization of the mixture of differentially labeled normal and diseased DNAs to isolated locus-specific probes spotted to a slide in a matrix (array) format. The method enables numerous high resolution in situ hybridizations within a single experiment. In a disease-specific edition of a matrix-CGH hundreds of relevant chromosome regions together with control regions may be addressed. A computational evaluation of the individual fluorochrome ratios at each hybridization spot representing single subchromosomal loci determines the relative quantity for the DNA of the diseased sample. Among many disorders CLL was the first where matrix-CGH was applied in a clinical study. By the use of a CLL-chip, further, yet unknown submicroscopic deletions could be identified (e.g. the involvement of chromosome 19). The diagnostic application of this effective method is presently limited by the high cost of the chip production and by the need of special interpretation expertise.[26]

cDNA microarray (expression profiling). This key molecular method has been applied to demonstrate gene expression, and enables the identification of deregulated genes by the relative quantification of cDNA transcribed in vitro from the total mRNA extracted from leukemic cells. Expression profiles in CLL basically strengthened previous knowledge on chromosomal changes. A clear drop in the expression of p53 or ATM mRNA could be measured in the cases with deletion.[28] The discovery of the highly differential and aberrant expression of the ZAP-70 kinase protein in IgV_H unmutated, high-risk B-cell CLL using expression profiling clearly presented the power of this method. In a very short time ZAP-70 became a reliable prognostic marker for the daily routine analysis of CLL,[13] while other biomarkers showing differential expression are still under intensive evaluation.

Conclusions

Although the survival time is variable, chronic lymphocytic leukemia is basically a long-lasting disease. Treatment decisions in the past relied on the clinical symptoms at diagnosis or progression. For prognostics only indirect and relatively soft parameters (e.g. cell morphology, serum findings) could be considered. The easy and continuous access to the leukemic cells from the peripheral blood, together with the improvements in the molecular methodology led to completely novel understanding of the biologic background of this leukemia. It turned out that beside to the IgV_H mutation status, cytogenetic aberrations may fundamentally influence the outcome of the disease. These enable a clear prognostic classification within the same disease stage, especially demarcating high-risk patients at early stages. In addition, genomic heterogeneity and cell population dynamics during treatment can also be monitored. By the ability to determine a highly unfavorable patient group, CLL became a real prototype for individual risk estimation in cancer.[7] For this reason, both karyotyping and FISH analysis of selected regions gained a significant place in the CLL diagnostic panel.

References

1. *Bacher U, Kern W, Schoch C, et al:* Discrimination of chronic lymphocytic leukemia (CLL) and CLL/PL by cytomorphology can clearly be correlated to specific genetic markers as investigated by interphase fluorescence in situ hybridization (FISH). Ann Hematol 83: 349-355, 2004.
2. *Bea S, Lopez-Guillermo A, Ribas M, et al:* Genetic imbalances in chronic lymphocytic leukemia and transformed large-cell lymphoma (Richter's syndrome). Am J Pathol 161: 957-968, 2002.
3. *Byrd JC, Stilgenbauer S, Flinn IW:* Chronic lymphocytic leukemia. Hematology 163-183, 2004.
4. *Calin GA, Liu CG, Sevignani C, et al.* MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. Proc Natl Acad Sci USA 101: 11755-11760, 2004.
5. *Capello D, Guarini A, Berra E, et al:* Evidence of biased immunoglobulin variable gene usage in highly-stable B-cell chronic leukemia. Leukemia 18: 1941-1947, 2004.
6. *Cuneo A, Rigolin GM, Bigoni R, et al:* Chronic lymphocytic leukemia with 6q- shows distinct hematological features and intermediate prognosis. Leukemia 18: 476-483, 2004.
7. *De Jong D, van't Veer LJ:* Tools for molecular risk-stratification for clinical purposes: CLL as a prototype. Eur J Hum Genet 12: 423, 2004.
8. *De la Fuente MT, Casanova B, Cantero E, et al:* Involvement of p53 in alpha4beta1 integrin-mediated resistance of B-CLL cells to fludarabine. Biochem Biophys Res Commun 311: 708-712, 2003.

9. *Dewald GW, Brockman SR, Paternoster SF, et al:* Chromosome anomalies detected by fluorescence in situ hybridisation: correlation with significant biological features in B-cell chronic lymphocytic leukemia. Br J Haematol 121: 287-295, 2003.
10. *Döhner H, Stilgenbauer S, Döhner K, et al:* Chromosome aberrations in B-cell chronic lymphocytic leukemia: reassessment based on molecular cytogenetic analysis. J Mol Med 77: 266-281, 1999.
11. *Glassman AB, Hayes KJ:* The value of fluorescence in situ hybridization in the diagnosis and prognosis of chronic lymphocytic leukemia. Cancer Genet Cytogenet 158: 88-91, 2005.
12. *Hammarsund M, Corcoran MM, Wilson W, et al:* Characterization of a novel B-CLL candidate gene – DLEU – in the 13q14 tumor suppressor locus. FEBS Lett 556: 75-80, 2004.
13. *Haslinger C, Schweifer N, Stilgenbauer S, et al*: Microarray gene expression profiling of B-cell chronic lymphoid leukemia subgroups defined by genomic aberrations and VH mutation status. J Clin Oncol 22: 3937-3949, 2004.
14. *Hernandez JM, Mecucci C, Criel A, et al:* Cytogenetic analysis of B-cell chronic lymphocytic leukemias classified according to morphologic and immunophenotypic criteria. Leukemia 9: 2140-2146, 1995.
15. *Karhu R, Tobin G, Thunberg U, et al:* More extensive genetic alterations in unmutated than in hypermutated cases of chronic lymphocytic leukemia. Genes Chromosomes Cancer 37: 417-420, 2003.
16. *Kröber A, Seiler T, Benner A, Bullinger L, et al*: VH mutation status, CD38 expression level, genomic aberrations and survival in chronic lymphocytic leukemia. Blood 100: 1410-1416, 2002.
17. *Matolcsy A:* High-grade transformation of low-grade non-Hodgkin's lymphomas: mechanisms of tumor progression. Leuk Lymphoma 34: 251-259, 1999.
18. *Matrai Z:* CD38 as a prognostic marker in CLL. Hematology 10: 39-46, 2005.
19. *Matthews C, Catherwood M, Morris TC, Alexander HD*: Routine analysis of IgVH mutational status in CLL patients using BIOMED-2 standardized primers and protocols. Leuk Lymphoma 45: 1899-1904, 2004.
20. *Messmer BT, Albesiano E, Efremov DG, et al:* Multiple distinct sets of stereotyped antigen receptors indicate a role for antigen in promoting chronic lymphocytic leukemia. J Exp Med 200: 519-525, 2004.
21. *Orchard JA, Ibbotson RE, Davis Z, et al:* ZAP-70 expression and prognosis in chronic lymphocytic leukemia. Lancet 363: 105-111, 2004.
22. *Ottaggio L, Viaggi S, Zunino A, et al:* Chromosome aberrations evaluated by comparative genomic hybridization in B-cell chronic lymphocytic leukemia: correlation with CD38 expression. Haematologica 88: 769-777, 2003.
23. *Que TH, Marco JG, Ellis J, et al:* Trisomy 12 in chronic lymphocytic leukemia detected by fluorescence in situ hybridization: analysis by stage, immunophenotype and morphology. Blood 82: 571-575, 1993.
24. *Rosenwald A, Alizadeh AA, Widhopf G, et al:* Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J Exp Med 194: 1625-1638, 2001.
25. *Rosenwald A, Chuang EY, Davis RE, et al:* Fludarabine treatment of patients with chronic lymphocytic leukemia induces p53 dependent gene expression response. Blood 104: 1428-1434, 2004.
26. *Schwaenen C, Nessling M, Wessendorf S, et al:* Automated array-based genomic profiling in chronic lymphocytic leukemia: development of a clinical tool and discovery of recurrent genomic alterations. Proc Natl Acad Sci USA 101: 1039-1044, 2004.
27. *Stankovic T, Stewart GS, Byrd P, et al:* ATM mutations in sporadic lymphoid tumors. Leuk Lymphoma 42: 1563-1571, 2002.
28. *Stankovic T, Hubank M, Cronin D, et al:* Microarray analysis reveals that TP-53 and ATM-mutant B-CLLs share a defect in activating proapoptotic responses after DNA damage but are distinguished by major differences in activating prosurvival responses. Blood 103: 291-300, 2004.
29. *Timar B, Fülöp Z, Csernus B, et al:* Relationship between the mutational status of VH genes and the pathogenesis of diffuse large B-cell lymphoma in Richter's syndrome. Leukemia 18: 326-30, 2004.
30. *Tsimberiodu AM, Keating MJ:* Richter syndrome. Biology, incidence and therapeutic strategies. Cancer 103: 216-228, 2004.
31. *Thornton PD, Gruszka-Westwood AM, Hamoudi RA, et al:* Characterization of TP53 abnormalities in chronic lymphocytic leukemia. Hematol J 5: 47-54, 2004.
32. *Valganon M, Giraldo P, Agirre X, et al:* P53 aberrations do not predict individual response to fludarabine in patients with B-cell chronic lymphocytic leukaemia in advanced stages Rai III/IV. Br J Haematol 129: 53-9, 2005.
33. *Wiestner A, Rosenwald A, Barry TS, et al:* ZAP-70 expression identifies a chronic lymphocytic leukemia subtype with unmutated immunoglobulin genes, inferior clinical outcome and distinct gene expression profile. Blood 101: 4944-4951, 2003.