

# 2

---

## Genetics of B-Cell Chronic Lymphocytic Leukemia

---

*Stephan Stilgenbauer, MD, Peter Lichter, PhD,  
and Hartmut Döhner, MD*

### 1. INTRODUCTION

B-cell chronic lymphocytic leukemia (B-CLL) is the most frequent type of leukemia among adults in the Western world, with an incidence of about 5 cases per 100,000 residents annually (1,2). The disease affects mainly people of advanced age, but about 20% of patients are younger than 55 (3). B-CLL is characterized by the accumulation of lymphocytes that appear morphologically mature but are functionally incompetent in bone marrow, blood, lymph nodes, and other organs, primarily of the lymphatic system (Fig. 1). During the course of the disease, there is increasing suppression of normal hematopoiesis and impairment of organ functions, resulting in B-symptoms, susceptibility to infection, and hemorrhage (Fig. 1). Currently available conventional therapeutic procedures are aimed at palliation. In younger patients, potentially curative approaches like autologous or allogeneic stem cell transplantation and antibody therapies are currently being investigated. The prognosis is influenced by the degree of dissemination of the disease at the time of diagnosis. This is reflected in the prognostic importance of the clinical staging systems defined by Rai and Binet (4,5). Both systems differentiate among early (Rai 0, Binet A), intermediate (Rai I, II; Binet B) and advanced (Rai III, IV; Binet C) stages, which are characterized by different survival times (Fig. 2) (6). However, the prognostic value of clinical staging is limited, especially in early stages, and there is marked heterogeneity in the speed of disease progression within the individual stages. For this reason, there has been intensive work in recent years on the identification of other clinical and biological factors with potential prognostic relevance. Genetic characteristics of the B-CLL cells have attained considerable importance among these factors (7–10).

### 2. GENOMIC ABERRATIONS IN B-CLL

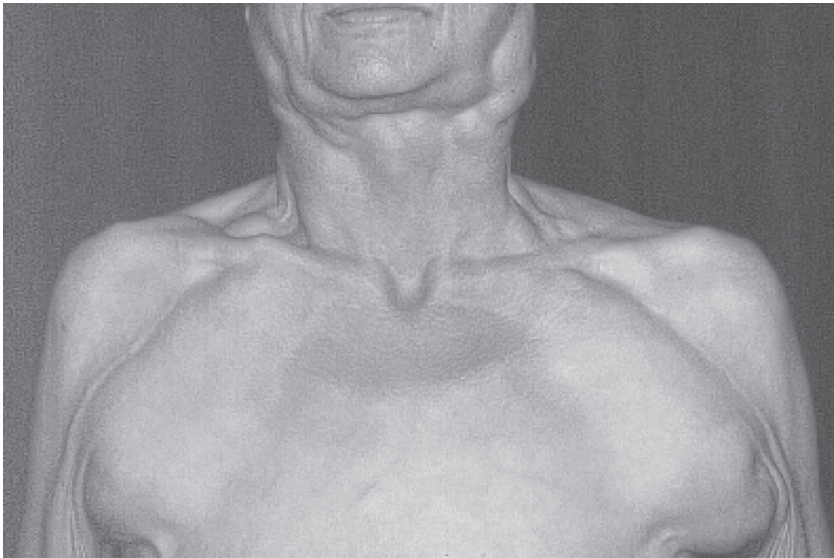
Two major subjects can be differentiated with respect to the genetic analysis of B-CLL: on the one hand, genomic aberrations, which, as acquired changes, may be involved in the initiation and progression of the disease, and, on the other hand, the mutation status of the variable segments of immunoglobulin heavy chain genes ( $V_H$ ), which may reflect the cellular origin of B-CLL.

Since the early 1980s, chromosome banding analyses of malignant B-cells have been performed using B-cell mitogens (11–18). Up to the early 1990s, clonal aberrations could be dem-

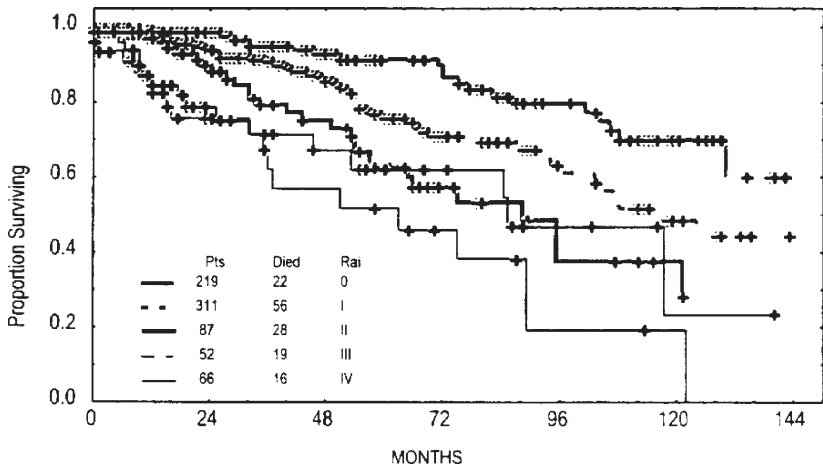
From: *Contemporary Hematology*

*Chronic Lymphocytic Leukemia: Molecular Genetics, Biology, Diagnosis, and Management*

Edited by: G. B. Faguet © Humana Press Inc., Totowa, NJ

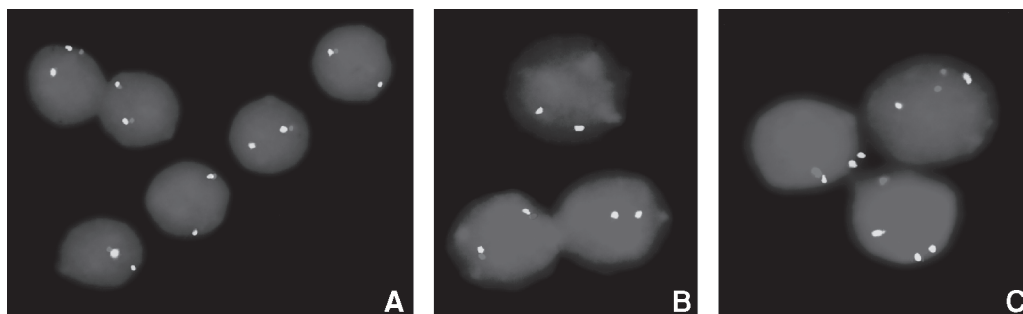


**Fig. 1.** Marked cervical and axillary lymphadenopathy in a B-CLL patient with deletion 11q. (From ref. 9, with permission.)



**Fig. 2.** Estimated survival times of B-CLL subgroups according to Rai stage (from ref. 6, with permission).

onstrated in only 40–50% of B-CLL cases using chromosome banding (19–21). Frequently, and despite the use of B-cell mitogens, nonclonal T-cells without chromosomal aberration were analyzed (22). More recently, the development of molecular cytogenetic techniques like fluorescence *in situ* hybridization (FISH) has led to considerable improvement in the diagnostics of genetic aberrations in tumor cells (23,24). FISH allows sensitive detection of specific sequences in the genome using cloned DNA fragments as probes. Signal number and location reflect numerical and structural changes of the corresponding chromosomal regions. The ability to detect aberrations not only on metaphase chromosomes but also in interphase cell nuclei is of great importance, especially in B-CLL (interphase cytogenetics; Fig. 3) (25). Interphase cytogenetic studies using FISH showed that the incidence of genomic aberrations in B-CLL was markedly



**Fig. 3.** Interphase FISH in B-CLL. **(A)** 11q deletion as demonstrated by the single red signal in five of the six nuclei shown. Two green signals of an internal control probe prove a high hybridization efficiency. The single cell with two red signals probably represents a nonleukemic cell from the specimen. **(B)** Biallelic deletion at 13q. Two of the three nuclei show no red hybridization signal of a probe containing marker D13S272, demonstrating biallelic loss of this region, whereas an adjacent probe containing marker D13S273 is retained in a disomic fashion. The single cell with two red and two green signals probably represents a nonleukemic cell. **(C)** Trisomy 12q (three green hybridization signals) and monoallelic deletion 13q14 (single red signal) in two of three nuclei in a B-CLL specimen. A single cell reflecting the normal disomic status of the two regions is shown for comparison. (From ref. 27, with permission.)

underestimated in banding studies (9). In B-CLL cases with abnormal karyotype by banding analysis but clonal aberrations by interphase FISH, the metaphase cells are derived from nonclonal T-cells and therefore do not reflect the karyotype of the malignant B-CLL cells.

Among the genomic aberrations whose incidence has been underestimated in B-CLL in banding studies are particularly the deletions of bands 13q14 and 11q22-q23, (9); while trisomy 12, which was originally described as the most frequent aberration of B-CLL in studies using chromosome banding, has been rated the third most frequent aberration by interphase FISH (9,26,27). In a study on 325 B-CLL patients, a comprehensive disease-specific probe set was used to detect the most important genomic gains, like partial trisomies 12q13, 3q27, and 8q24 and the most frequent genomic losses in bands 13q14, 11q22-q23, 6q21, 6q27, and 17p13 and translocations in band 14q32 using FISH (9) (Table 1). Overall genomic aberrations were found in more than 80% of all cases. The most frequent aberration by far was deletion of band 13q14, which was found in 55% of the cases. Other frequent aberrations were deletion 11q22-q23 (18%), trisomy 12q13 (16%), deletion 17p13 (7%), deletion 6q21 (6%), trisomy 8q24 (5%), translocation 14q32 (4%), and trisomy 3q27 (3%). Somewhat more than half of the cases showed only a single aberration; one-fifth of the cases showed two and nearly one-tenth showed more than two aberrations.

This precise determination of the incidence of chromosomal aberrations provides the basis for further studies of the role of these changes in the pathogenesis and progression of the disease. Thus, genes assumed to be involved in the pathogenesis of B-CLL could be identified by physical mapping of the minimal affected regions and by the strategy of positional cloning as well as the analysis of candidate genes (for review, see ref. 27).

### ***2.1. Deletions Within Band 13q14 and Identification of Candidate Genes***

The structural chromosome aberration most frequently found in cytogenetic studies of B-CLL is deletion of band 13q14 (18,28–31). Recurrent deletion of a chromosomal region indicates the existence of a tumor suppressor gene, whose inactivation is caused by the loss of an allele and the mutation in the remaining allele (two-hit hypothesis). The retinoblastoma tumor suppressor

Table 1  
Incidence of Genomic Aberrations  
in 325 Patients With B-CLL

<i>Aberration</i>	<i>Patients</i>	
	<i>No.</i>	<i>%</i>
13q deletion	178	55
11q deletion	58	18
12q trisomy	53	16
17p deletion	23	7
6q deletion	21	7
8q trisomy	16	5
t(14q32)	12	4
3q trisomy	9	3
Clonal abnormalities	268	82

From ref. 9, with permission.

gene (*RB1*) is a candidate gene localized in band 13q14 that codes for a nuclear phosphoprotein involved in cell cycle regulation and transcription control. Its inactivation is involved in the pathogenesis of numerous tumors (32). The deletion of an allele of *RB1* was detected using molecular cytogenetic techniques in about one-fourth of all B-CLL cases (33–36). However, the inactivation of both *RB1* gene copies by deletion and/or mutation could only be extremely rarely detected, which raises questions about the pathogenetic role of *RB1* in B-CLL.

Various groups have constructed high-resolution genomic maps of the critical region in 13q14 to identify a new B-CLL-tumor suppressor gene (37–47). By means of positional cloning, several groups identified fragments of several new genes from these subregions in parallel. Based on their localization in the minimal deleted 13q14 region, BCMS (ep272-3-t5, LEU1) and BCMSUN (ep272-3-t4, LEU2) are currently considered the most promising candidate tumor suppressor genes in B-CLL (41–43,45,47). However, in mutation analyses to date, no inactivation of these candidate genes could be demonstrated in B-CLL in the sense of the two-hit hypothesis. BCMS inhibits a complex genomic organization. The gene extends over at least 560 kb genomic DNA and is transcribed in a number of heterogeneous mRNA transcripts (48). Expression analyses are currently being performed to clarify the pathogenetic importance of the candidate genes in the 13q14 region (49,50).

## **2.2. Deletions of Bands 11q22-q23 With *ATM* As the Candidate Gene**

In a study using FISH, a critical region was identified around the neural cell adhesion molecule (NCAM) gene in band 11q23.1 in 15 hematological tumors (51). In another study, the extent of 11q deletions among 40 B-CLL cases was determined using a FISH probe set of overlapping yeast artificial chromosome (YAC) clones spanning bands 11q14-q24 (52,53). All aberrations affected a minimal consensus region of 2–3 Mb in size in bands 11q22.3-q23.1. In the minimal deleted region, the ataxia telangiectasia mutated (*ATM*) gene was localized, which, owing to its role in DNA repair and the frequent observation of lymphomas in *ATM* knockout mice, appeared to be a candidate tumor suppressor gene (54,55). In fact, the changes in both *ATM* alleles by deletion and/or mutation in the sense of the two-hit hypothesis of tumor suppressor gene inactivation could

be demonstrated for the first time in human tumors in T-prolymphocytic leukemia (T-PLL) (56,57). Because of a lack of *ATM* protein expression, the involvement of *ATM* in B-CLL was also postulated, and inactivation of *ATM* by deletion and/or mutation could actually be demonstrated (58–61). It was shown that *ATM* mutant B-CLL cases exhibited a deficient *ATM*-dependent response of p21 to  $\gamma$ -irradiation, failure to upregulate tumor necrosis factor-related apoptosis-inducing ligand receptor 2 (TRAIL-R2), and inability to repair induced chromosomal breaks (62). An association of deletion 11q with a more aggressive clinical course of B-CLL was suggested in a chromosome banding study (63). Interestingly, all *ATM* mutant cases showed absence of somatic V<sub>H</sub> hypermutation (*see* also Subheading 2.8. below), indicating that *ATM* may play a role at the pregerminal center stage of B-cell maturation and may lead to the development of B-CLL derived from pregerminal center cells (10,64). However, mutation of the remaining *ATM* allele was found only in 5 of 22 B-CLL cases with 11q22-q23 deletion of our series, which indicates a possible involvement of additional genes in this region in B-CLL (59). By contrast, in mantle cell lymphoma, in which the 11q22-q23 deletion occurs in nearly half the cases (65,66), mutation of the remaining allele could be demonstrated in all cases with deletion of an *ATM* allele (67). Thus, *ATM* appears to be the tumor suppressor gene inactivated in connection with 11q22-q23 deletions in T-PLL, mantle cell lymphoma, and some cases of B-CLL. Elucidation of the situation in B-CLL cases with 11q deletion without mutation in the remaining *ATM* allele is currently in progress.

### ***2.3. Trisomy 12 As Recurrent Aberration in B-CLL***

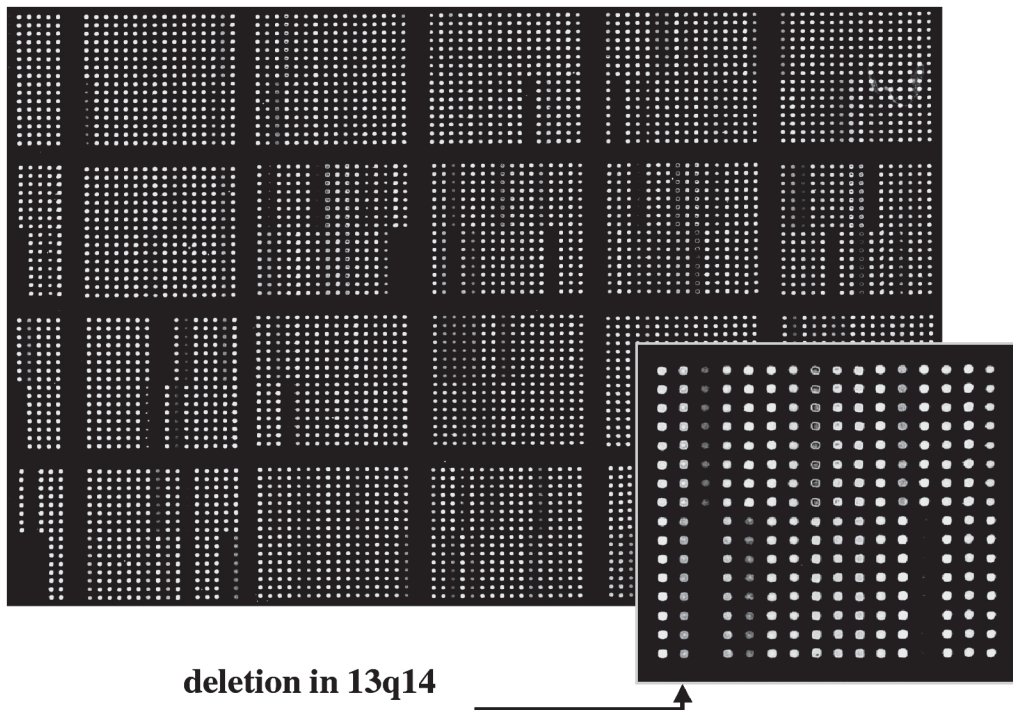
Trisomy 12 was described in the early 1980s as the first recurrent aberration in B-CLL; with a prevalence between 10 and 25%, it was among the most frequent aberrations in nearly all subsequent studies using chromosome banding (14,20,30,68–73). However, the identification of a critical region remained difficult, since usually a complete additional chromosome 12 was present and partial trisomy was observed only in very rare cases (17,68,74).

Molecular cytogenetics by interphase FISH was used in numerous studies to detect trisomy 12 in B-CLL and revealed incidences of 10–20% in European studies and more than 30% in two US studies (17,75–82) (Table 1). The observation of one case of B-CLL with isolated over-representation of bands 12q13-q14 is interesting with respect to identification of a critical segment on chromosome 12 (83). Merup et al. (84) examined a tumor with a complex 12q rearrangement and that found bands 12q13-q15 were most frequently amplified. Dierlamm et al. (85) observed partial trisomy 12 using FISH in 11 of more than 1000 lymphomas. Bands 12q13-q22 were the smallest mutually duplicated segment in four B-CLL cases in this series. Among others, genes of oncogenic potential, like CDK4, GLI, and MDM2, are localized in this genomic region, but no pathogenetic relevance for B-CLL has been shown to date for any of these genes. Currently the innovative approach of DNA microarray chip technology is being used, employing matrix comparative genomic hybridization (CGH) for identification of the smallest replicated genomic regions in bands 12q13-q21 in B-CLL (86–89) (Fig. 4).

### ***2.4. Deletion 6q in Lymphatic Neoplasms***

Among the most frequent aberrations in both acute lymphoblastic leukemia and aggressive as well as indolent lymphoma are deletions involving the long arm of chromosome 6 (90). In B-CLL, 6q deletions were found in 6% of the evaluable cases by means of chromosome banding, whereby bands 6q15 and 6q23 were most often affected (21). In an extensive analysis of various types of lymphoma, at least two independent deletion regions were identified, one in bands 6q21-q23 and





**Fig. 4.** Matrix CGH in B-CLL. Hybridization of DNA derived from a patient with a 13q14 deletion (labeled in green) vs human control DNA (labeled in red). Inset: PAC clone localized in band 13q14 exhibits a dominant red fluorescence signal after hybridization, indicating the deletion of this region (arrow) (89).

another in bands 6q25–q27 (91). Deletion 6q21–q23 was associated with the subgroup of lymphomas with lymphocytic differentiation, which may be considered as nonleukemic correlates to B-CLL (92).

Deletions in band 6q21 in B-CLL were also described in several more recent molecular genetic studies. Merup et al. (93) found 6q deletions in 6% of B-CLL cases, with a minimal deletion region in band 6q21. Gaidano et al. (94) observed 6q deletions in only 4 of 100 B-CLL cases in band 6q27. In another extensive series, 285 B-CLL cases were examined with probes from bands 6q21 and 6q27 (95). The incidence of deletion 6q was 6%, and all deletions affected band 6q21, whereas band 6q27 was deleted in only one-third of the cases, and isolated 6q27 deletion was not observed in any case. In agreement with this, Zhang et al. (96) described a 4–5-Mb large minimal deleted region in band 6q21 in a series of various subtypes of lymphomas and leukemias. Although several candidate genes are located in the critical 6q21 region, it has not yet been possible to demonstrate a pathogenic role for one of these genes.

### ***2.5. Deletion 17p13 and Mutation of the p53 Gene in B-CLL***

Involvement of *p53* in band 17p13 in B-CLL was found in molecular genetic studies. Because of its role in nearly all kinds of tumors, *p53* was examined as a candidate gene in B-CLL. By means of single-strand conformational polymorphism analyses and direct DNA sequencing, *p53* mutations could be proved, with a prevalence between 10 and 15% in B-CLL (94,97–100).

17p13 deletions were found in 4–9% in B-CLL (9,101). To examine the relationship of 17p13 deletion and *p53* inactivation by mutation in the remaining allele, 110 B-CLL cases were analyzed (102). Fifteen showed mutations in the *p53* gene, of which half were biallelic aberrations. Among the cases with deletion, most showed mutations in the remaining *p53* allele, whereas among the cases without 17p13 deletion, *p53* mutation occurred only rarely. The high rate of *p53* mutations in the B-CLL cases with 17p13 deletions suggests that, in the case of 17p13 deletion in B-CLL, *p53* is the tumor suppressor gene affected by the aberrations.

## ***2.6. Rearrangement of the IgH Locus in Band 14q32***

Translocation breakpoints in band 14q32, in which the heavy chain immunoglobulin genes (IgH) are located, were described as the most frequent aberration in B-CLL in early banding studies (13,14,18,20,68,71,72,73,103–105). In the most extensive studies, aberrations of chromosome 14 could be demonstrated in 8% of evaluable cases (21). The aberrations were often the result of translocation t(11;14)(q13;q32), which leads to deregulation of the cyclin-D1 gene (CCND1) in 11q13 by the IgH locus (14q32) (106–110). The t(11;14)(q13;q32) and cyclin-D1 overexpression are now considered characteristic of mantle cell lymphoma and occur rarely in other lymphoproliferative diseases (106,107,110,111). Many of the cases with t(11;14)(q13;q32) in early cytogenetic B-CLL studies were probably leukemic mantle cell lymphoma. Neither the t(11;14)(q13;q32) nor the deregulation of CCND1 was described as a frequent event in B-CLL in recent studies (94,112–115). In our monocentric series of 325 B-CLL cases, there was no case of t(11;14)(q13;q32) (9).

The situation is similar for translocations t(14;18)(q32;q21) and t(14;19)(q32;q13), which are rare but recurrent aberrations (<5%) in B-CLL. In today's view, 14q32 rearrangements are rare events in B-CLL, and the high incidence of these aberrations in early banding analyses was probably caused by the inclusion of other leukemic lymphomas in these series (94,112–122).

## ***2.7. Rare Aberrations in B-CLL: Trisomies 3q27 and 8q24***

Additional genetic aberrations were discovered either by genome-wide screening methods like chromosome banding and CGH, or by analysis of prominent candidate genes. Banding and CGH identified several further aberrations that were rare but recurrent in B-CLL. Often, these were trisomies, like trisomy 3q, which was described in several studies (30,71,73). CGH analyses point to the distal arm of 3q as the minimal duplicated region with possible pathogenetic relevance in B-CLL (83). In addition to trisomy 3, which was also described in banding analyses, CGH analyses identified gains of 8q and 15q as new aberrations in B-CLL (83). With C-MYC in 8q24 and BCL6 in 3q27, for example, candidate genes are known for some of these regions, but their role in the pathogenesis of B-CLL has not yet been confirmed.

## ***2.8. Mutation Status of the V<sub>H</sub> Genes***

A novel genetic parameter of B-CLL is the mutation status of the V<sub>H</sub> genes (123–125). Although in the past, B-CLL was considered to be a lymphoma derived from pregerminal center B-cells, somatically mutated V<sub>H</sub> genes could be demonstrated in about half of the cases in these studies. Accordingly, a separation was made into two different B-CLL groups: one with unmutated V<sub>H</sub> genes, assumed to originate in pregerminal center cells, and another with mutated V<sub>H</sub> genes, thought to stem from postgerminal center cells. Moreover, it could be demonstrated that the V<sub>H</sub> mutation status is clinically relevant. Although B-CLL with unmutated V<sub>H</sub> shows an unfavorable course, with rapid disease progression, B-CLL with mutated V<sub>H</sub> often shows slow progression

(7,8). In addition, there was a correlation between  $V_H$  mutation status and CD38 expression of B-CLL cells as further evidence of the biological difference between the two forms (7). The relationship of the  $V_H$  mutation status to genomic aberrations and the differential influence of these factors in the pathogenesis and progression of B-CLL are currently undergoing intensive examination.

### 3. CLINICAL IMPACT OF GENOMIC ABERRATIONS IN B-CLL

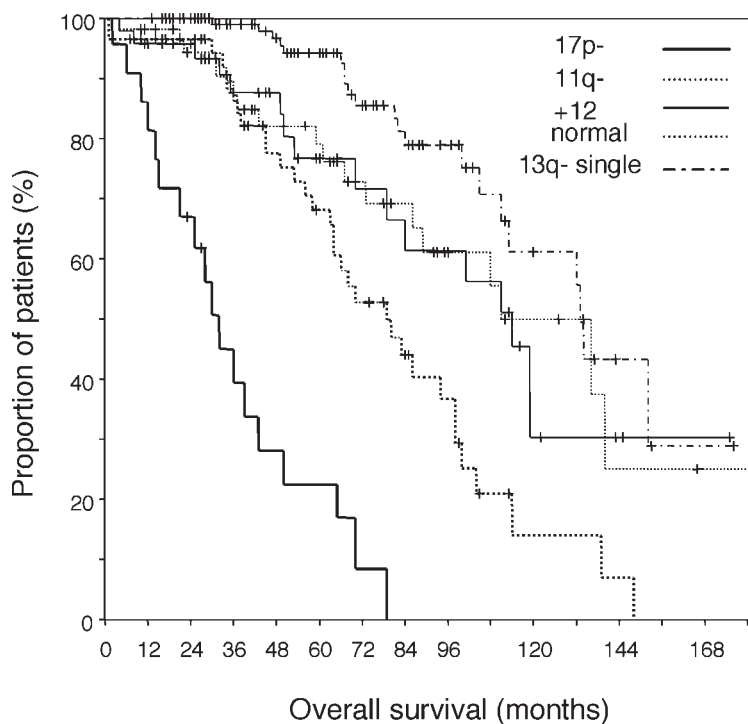
The multicenter International Working Party on Chromosomes in CLL (IWCCLL) studies examined the correlation between clinical data and genomic aberrations based on chromosome banding (19,21). A longer estimated survival time (median 15 yr) was found in the group of patients with normal karyotype compared with the group with clonal aberrations (median 7.7 yr). Moreover, a relation was found between the complexity of the karyotype and unfavorable prognosis. In the subgroup analyses of patients with specific aberrations, a correlation was found between trisomy 12 and shorter survival time; in contrast, aberrations in chromosome 13 were associated with more favorable prognoses. In multivariate analysis, however, neither the presence nor the number of chromosomal aberrations showed independent prognostic relevance.

Precise detection of genomic aberrations using interphase FISH provided a more reliable basis for correlations between genomic aberrations and clinical parameters in B-CLL. In an extensive FISH analysis of 325 B-CLL cases with probes for regions 3q27, 6q21, 8q24, 11q22-q23, 12q13, 13q14, 14q32, and 17p13, multivariate analysis revealed an independent prognostic relevance of genomic aberrations (9) (Fig. 5). It was found that deletion 13q14 as a single aberration was associated with long median survival times (133 mo), whereas deletions 11q22-q23 and 17p13 were associated with poor prognoses (79 and 32 mo, respectively). Intermediate survival times were found for B-CLL cases without aberrations or with trisomy 12 (111 and 114 mo, respectively) (9).

#### 3.1. Prognostic Relevance of Deletion 11q22-q23

B-CLL cases with deletion 11q show more rapid progression of the disease and shorter survival times. In interphase FISH, deletion 11q22-q23 is the second most frequent aberration in B-CLL, with an incidence of approx 20%, and identifies a patient group with a characteristic clinical picture (26). B-CLL patients with 11q deletion present with advanced stages of disease and pronounced lymphadenopathy, reflected by large palpable peripheral, thoracic, and abdominal lymph nodes (Fig. 1). Moreover, patients with 11q deletion have a more rapid progression of disease, as measured by shorter therapy-free intervals (9 mo vs 43 mo;  $p < 0.001$ ). In the survival time analysis, 11q deletion was associated with a poor prognosis, and the effect of this aberration on the course of the disease was age-dependent. In B-CLL patients younger than 55 yr, the survival time was significantly shorter in the group with 11q deletion than in the group without 11q deletion, whereas in patients 55 yr or older, there was only a trend to shorter survival times. Another, likewise age-dependent prognostic relevance was found in an examination of *ATM* protein expression in B-CLL (61). The poor outcome of B-CLL with 11q deletion was confirmed in an independent series (126). In multivariate analysis, 11q deletion was found to be an independent adverse factor (26). Since the 11q deletion appears to be prognostically relevant, especially in younger B-CLL patients, this aberration could serve to identify a patient group that could benefit from modern experimental strategies, such as autologous or allogeneic blood stem cell transplantation.





**Fig. 5.** Survival probability in B-CLL according to risk groups defined by genomic aberrations. The estimated median survival times were as follows: 17p deletion, 32 mo; 11q deletion, 79 mo; normal karyotype, 111 mo; 12q trisomy, 114 mo; and 13q deletion as single abnormality, 133 mo. (From ref.9, with permission.)

### 3.2. Clinical Characteristics of Trisomy 12

In a large comparison of individual chromosomal aberrations in banding analysis, B-CLL with trisomy 12 had the shortest survival time (19,21). However, the negative prognostic relevance of trisomy 12 could not be confirmed in further studies (18,68,69,73).

In interphase FISH studies, trisomy 12 was associated with atypical morphology and immunophenotype in B-CLL (77,79,81). An effect of trisomy 12 on survival time was found in a series of 83 B-CLL patients (78). Patients with trisomy 12 had a mean survival time of 7.9 yr compared with 14.4 yr in the group with normal karyotype. No significant difference was found in a comparison of trisomy 12 vs no trisomy 12 on the basis of the FISH results alone. Patients with trisomy 12 had undergone more intensive prior treatment and were in advanced stages of disease. The response rates to treatment with fludarabine did not differ (78). In a series of 325 B-CLL patients, the prognosis in the group with trisomy 12 (median survival time 114 mo) was intermediate to that of 13q deletion as a single aberration and deletions 11q22-q23 or 17p13 (Fig. 5) (9).

### 3.3. Clinical Relevance of Deletion 6q

In B-CLL patients with deletion 6q, shorter therapy-free intervals could be demonstrated, reflecting more rapid progression of the disease (18). By contrast, however, no association of deletion 6q with shorter survival time was proved in the IWCCLL studies (19,21). An interphase

FISH study on 285 B-CLL patients revealed a correlation between deletion 6q and greater tumor mass, measured by leukocyte count (median  $49.3 \times 10^9/\text{L}$  vs  $31.7 \times 10^9/\text{L}$ ;  $p = 0.036$ ) and lymphadenopathy (95). The sum of the products of the largest cervical, axillary, and inguinal lymph nodes (median  $7.3 \text{ cm}^2$  vs  $3.0 \text{ cm}^2$ ;  $p = 0.029$ ) and the longest lymph node diameter (median 4.0 cm vs 2.0 cm;  $p = 0.008$ ) were greater in the group with 6q deletion. There was, however, no significant difference in survival time or therapy-free intervals between the two groups (95). Thus, 6q deletion does not appear to be of prognostic relevance in B-CLL.

### 3.4. Prognostic Impact of 17p13 (p53) Aberrations

Early mutation analyses showed that *p53* mutations are of significant negative prognostic relevance and are associated with treatment failure in B-CLL (98). In banding studies, the relevance of aberrations of band 17p13, where *p53* is localized, was only recently described in B-CLL. In a study of 480 B-CLL patients with no prior treatment, 17p aberrations were the only chromosomal aberration of prognostic relevance (101). An interphase FISH study also showed that patients whose leukemia cells had a *p53* deletion had significantly shorter survival times than patients without this aberration (100). Moreover, a relationship was found between the deletion and the response to treatment. Whereas 56% of patients without *p53* deletion went into remission on treatment with purine analogs, none of the patients with *p53* deletion responded (100). In a multivariate analysis, *p53* deletion was the strongest prognostic factor, followed by established clinical prognosis factors like stage and age (9). Prediction of the prognosis and therapeutic success in B-CLL thus appears possible with the parameter *p53* aberration/17p deletion. Despite the chemoresistance of B-CLL with 17p deletion, there is evidence that durable therapeutic success can be achieved with the monoclonal antibody campath-1H (127).

### 3.5. Clinical Relevance of 14q32 (IgH) Translocations

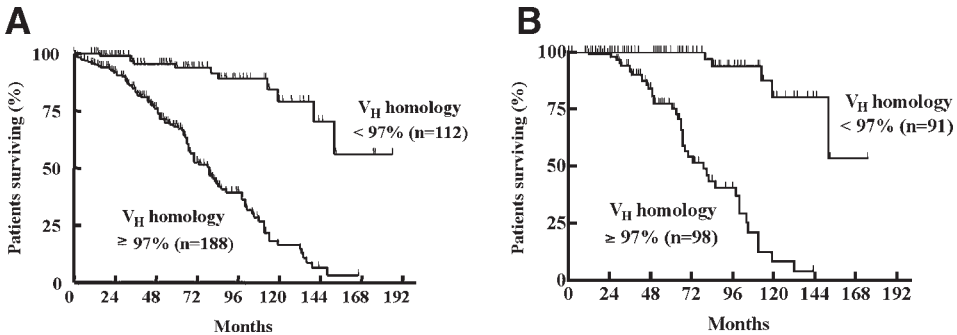
The negative prognostic relevance of translocation breakpoints in band 14q32, often the result of a  $t(11;14)$  (19,21), is probably explicable by the diagnostic ambiguity of these cases, since, for example, differentiation from leukemic mantle cell lymphomas often remains doubtful. Today, in a case with cytogenetic or molecular evidence of the  $t(11;14)(q13;q32)$  or *CCND1* overexpression, a diagnosis of MCL should be considered until another diagnosis is proved otherwise (94,106–115).

## 4. $V_H$ MUTATION, CD38, AND GENOMIC ABERRATIONS IN B-CLL

Several studies over the past few years have demonstrated that there is somatic hypermutation of the rearranged  $V_H$  genes (mutated  $V_H$ ) in about half of the B-CLL cases (123–125). This was surprising since B-CLL had been considered a pregerminal center-derived lymphoma. Pivotal studies on a small number of patients showed an unfavorable prognosis in B-CLL with unmutated  $V_H$  genes (7,8). In some studies, there was a strong correlation between CD38 expression of the B-CLL cells and the  $V_H$  mutation status (7,128). Other authors could not confirm this, so it still remains unclear whether CD38 expression can be applied as a prognostic surrogate marker for the  $V_H$  mutation status (129–131).

### 4.1. Prognostic Impact of the $V_H$ Mutation Status

To examine the  $V_H$  mutation status in a large series ( $n = 300$ ) of B-CLL patients, the VDJ-rearrangement of the immunoglobulin genes was amplified by PCR from genomic DNA, and the



**Fig. 6.** Probability of survival in B-CLL patients with mutated and unmutated  $V_H$  genes according to the 97% cutoff values. **(A)** The estimated median survival time for the  $V_H$  homology  $\geq 97\%$  group was 79 mo. The last observed death in the  $V_H$  homology  $< 97\%$  group was after 152 mo of follow-up time (survival probability 56%). **(B)** When only patients diagnosed at Binet stage A were evaluated the estimated median survival times for the  $V_H$  homology  $\geq 97\%$  and  $V_H$  homology  $< 97\%$  groups were 79 mo vs not reached (last observed death after 152 mo of follow-up time; survival probability 53%). (From ref. 10, with permission.)

mutation status of the  $V_H$  genes was determined by DNA sequencing (10,132). Taking the classical cutoff value of 98% homology to the nearest related germline gene to differentiate between mutated and unmutated  $V_H$  genes, 132 cases (44%) showed mutated and 168 cases (56%) unmutated  $V_H$  genes. The method of maximally selected log rank statistics was applied to test the prognostic relevance of the  $V_H$  mutation status. A corrected  $p$  value ( $p_{\text{cor}}$ ) for the best possible separation of two subgroups with different survival probabilities was found at a  $V_H$  homology to the nearest related germline gene of 97% ( $p_{\text{cor}} < 0.001$ ; 95% confidence interval 96–98%) (10). With a cutoff value of 97% homology to the nearest related germline gene, 112 cases (37%) showed mutated and 188 cases (63%) unmutated  $V_H$ . The Kaplan-Meier estimate of the median survival time in the two  $V_H$  subgroups differed both for the overall group ( $n = 300$ ) and within the subgroup of patients in Binet stage A at the time of diagnosis ( $n = 189$ ) (Fig. 6) (10).

#### 4.2. Structure of the VDJ Rearrangement

In addition to the  $V_H$  mutation status, the study of the structure of the VDJ rearrangement and the character of the mutations with respect to biological factors of disease etiology, like antigen selection, is of interest. Until recently, there were only studies available on small numbers of cases (123,125,133,134, and references therein). In a large B-CLL series, at least one clonal VDJ rearrangement of genomic DNA could be amplified in all 300 cases (132). Cases with mutated  $V_H$  showed a different VDJ rearrangement structure than cases with unmutated  $V_H$ . Genes of the  $V_H3$  and  $V_H4$  families were over-represented in the mutated  $V_H$  subgroup, whereas the  $V_H1$  family was found more frequently in the  $V_H$  unmutated subgroup. Specific  $V_H$  genes were responsible for the differences, and these imbalances were in line with previous studies (123,125,133,134, and references therein). The mean length of the CDR3 region differed significantly between the  $V_H$  mutated and unmutated subgroups. The median mutation rates and ratios of replacement/silent (R/S) mutations were greater within the  $V_H$  subregions in the CDRs than in the FRs. Cases with less than 98% homology to the nearest related germline gene were examined by means of the algorithm of Chang and Casali (135) for evidence of an antigen selection in the mutation pattern of the  $V_H$  gene. In 43 cases, mutation patterns consistent with antigen selection were

found, whereas no such patterns could be recognized in 41 cases (132). The survival probabilities did not differ significantly between the two groups. Taking these data together, there are differences in the biological background of  $V_H$  mutated vs unmutated B-CLL; however, the pathogenetic role of external stimuli still needs to be confirmed.

#### ***4.3. CD38 Expression in Relationship to the $V_H$ Mutation Status***

The prognostic relevance of CD38 expression and particularly the question of whether CD38 might be used as a surrogate marker for the  $V_H$  mutation status in B-CLL is a topic of controversy (7,128–131,136,137). The measurement of CD38 using fluorescence-activated cell sorting (FACS) would be technically less difficult and costly compared with  $V_H$  mutation analysis and thus would be an attractive procedure in estimating prognosis. CD38 expression was tested in 157 B-CLL cases of our series (10). The group with expression of CD38 in more than 30% (56 cases, 36%) or less than 30% (101 cases, 64%) of B-CLL cells were compared, but no significant difference in estimated survival time was found. In this study, a high CD38 expression correlated with unmutated  $V_H$  status, but there was a discrepancy between CD38 expression and  $V_H$  mutation status in about one-third of the cases (10). Thus, CD38 appears to be suitable for predicting the  $V_H$  mutation status only to a limited degree. Moreover, variability in CD38 expression is observed in some studies during the course of the disease (10,130,136).

#### ***4.4. Distribution of Genomic Aberrations in the $V_H$ Subgroups***

Using interphase FISH, genomic aberrations were demonstrable in 246 of 300 (82%) B-CLL cases with known  $V_H$  mutation status in our series (10). The incidences of the individual genomic aberrations in the total group and in dependence on the  $V_H$  mutation status are shown in Table 2. The incidences of genomic aberrations overall and of trisomy 12 in the two  $V_H$  subgroups were comparable; by contrast, prognostically unfavorable aberrations (11q-, 17p-) occurred almost exclusively in the  $V_H$  unmutated, and prognostically favorable aberrations (13q-, 13q- single) more frequently in the  $V_H$  mutated subgroup. This unbalanced distribution of genomic aberrations emphasizes the different biological backgrounds of the B-CLL subgroups with mutated or unmutated  $V_H$  and could in part explain their different clinical course. On the other hand, about two-thirds of the  $V_H$  unmutated B-CLL cases show no unfavorable genomic aberrations, which indicates a differential influence of these factors. Comprehensive studies of gene expression in B-CLL based on DNA chip technology indicate that the global gene expression “signature” of  $V_H$  mutated and unmutated B-CLL is very similar and that only the expression of a small number of genes discriminates between the two groups (138,139).

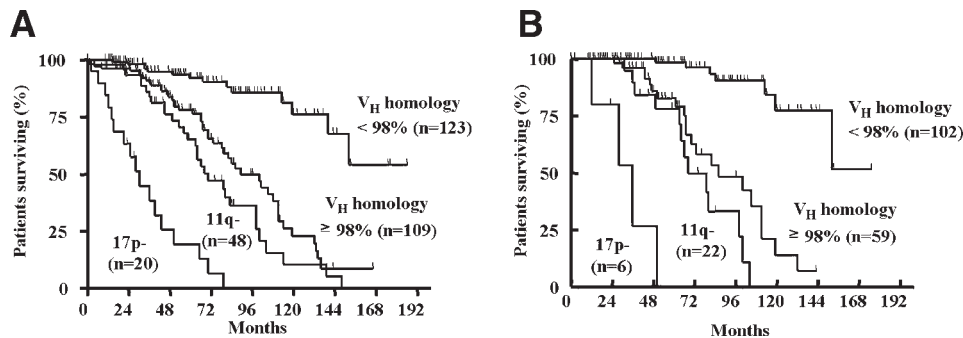
#### ***4.5. Prognostic Relevance of Genomic Aberrations and $V_H$ Mutation Status***

To examine the individual prognostic value of genomic aberrations, the  $V_H$  mutation status, and other clinical and laboratory features, a multivariate analysis was made of the survival time by means of a Cox regression (10). The  $V_H$  mutation status, 17p deletion, 11q deletion, age, leukocyte count and lactate dehydrogenase levels were identified as independent prognostic factors in this analysis. When the  $V_H$  mutation status, and 11q and 17p aberrations were included, the clinical stage of disease according to the systems of Rai or Binet was not identified as an independent prognostic factor. Similar results, demonstrating a very strong prognostic impact of the  $V_H$  mutation status and genomic aberrations, were independently found in two other B-CLL series (140,141). Based on this model, four subgroups with widely differing survival probabilities can be defined by the  $V_H$  mutation status, 11q deletion and 17p deletion (Fig. 7).

Table 2  
Relation of V<sub>H</sub> Mutation Status and Genomic  
Aberrations in 300 B-CLL Cases

Aberration	V <sub>H</sub> (%)		p-value <sup>a</sup>
	Mutated (homology < 98%) [n = 132 (44%)]	Unmutated (homology > 98%) [n = 168 (56%)]	
Clonal aberrations	80	84	0.37
13q deletion	65	48	0.004
13q deletion single	50	26	< 0.001
Trisomy 12	15	19	0.44
11q deletion	4	27	< 0.001
17p deletion	3	10	0.03
17p or 11q deletion	7	35	< 0.001

<sup>a</sup>Fisher's exact test.  
From ref. 10, with permission.



**Fig. 7.** Probability of survival among patients in the following genetic categories: 17p- (17p deletion irrespective of V<sub>H</sub> mutation status), 11q- (11q deletion irrespective of V<sub>H</sub> mutation status), unmutated V<sub>H</sub> (V<sub>H</sub> homology >98% and no 17p or 11q deletion), and mutated V<sub>H</sub> (V<sub>H</sub> homology <98% and no 17p or 11q deletion). **(A)** Among all stages (*n* = 300) the estimated median survival times for the respective genetic subgroups were as follows: 17p deletion, 30 mo; 11q deletion, 70 mo; V<sub>H</sub> unmutated, 89 mo; and V<sub>H</sub> mutated, not reached (54% survival at 152 mo). **(B)** Among Binet stage A patients (*n* = 189) the estimated median survival times for the respective genetic subgroups were as follows: 17p deletion, 36 mo; 11q deletion, 68 mo; V<sub>H</sub> unmutated, 86 mo; and V<sub>H</sub> mutated, not reached (52% survival at 152 mo). (From ref. 10, with permission.)

These studies show that genomic aberrations and V<sub>H</sub> mutation status appear to have complementary relevance in estimating the prognosis in B-CLL. Unmutated V<sub>H</sub> and genomic aberrations were among the strongest prognostic factors and gives us insight into the biological bases of the clinical heterogeneity of B-CLL. For this reason, genomic aberrations and V<sub>H</sub> mutation status are currently being tested in relation to other clinical and laboratory factors in prospective multicenter studies of the German CLL Study Group (GCLLSG). If these factors allow us to predict the course of disease in individual patients at the time of diagnosis, independent of the stage, they could serve as the basis for future risk-adapted treatment strategies.



## 5. PERSPECTIVE: NEW DIAGNOSTIC TOOLS

As in other leukemias, genetic markers correlate with the clinical course, response to therapy, and survival time in B-CLL. To determine the relevance of such genetic markers for the stratification of patient groups to various treatments of different intensity, extensive clinical studies must be performed. As a rapid and robust diagnostic test matrix CGH (comparative genomic hybridization against a matrix of defined genomic DNA fragments) can be utilized (86,89). In this procedure, total genomic tumor DNA is labeled with fluorescent dyes and hybridized on a DNA chip containing microarrays of defined genomic DNA fragments. After cohybridization with differently labeled normal control-DNA, the relative intensity of the two fluorescences is used to determine whether a certain DNA-sequence is over- or under-represented in the genome of the tumor cell population (Fig. 4). Since all relevant genomic aberrations in B-CLL are imbalances, a matrix CGH chip was constructed to test specific aberrations that might occur in B-CLL (89). For this purpose, genomic DNA fragments of the chromosomal areas that are deleted or over represented in B-CLL were selected, isolated, and printed on a matrix CGH chip. This chip allows automated analysis of the genomic imbalances in B-CLL and will be evaluated in clinical studies aiming at risk stratification of individual patients.

## ACKNOWLEDGMENTS

This work was supported by Wilhelm Sander-Stiftung (2001.004.1), Deutsche Krebshilfe (70-2434-DöI), and BMBF (01KW9934, 01KW9938).

## REFERENCES

1. Rozman C, Montserrat E. Chronic lymphocytic leukemia. *N Engl J Med* 1995;1333:1052–1057.
2. Zwiebel JA, Cheson BD. Chronic lymphocytic leukemia: staging and prognostic factors. *Semin Oncol* 1998;25:42–59.
3. Mauro FR, Foa R, Giannarelli D, et al. Clinical characteristics and outcome of young chronic lymphocytic leukemia patients: a single institution study of 204 cases. *Blood* 1999;94:448–454.
4. Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Paternack BS. Clinical staging of chronic lymphocytic leukemia. *Blood* 1975;46:219–234.
5. Binet JL, Auquier A, Dighiero G, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. *Cancer* 1981;48:198–206.
6. Keating MJ Improving the complete remission rate in CLL. In: *Hematology 1999. The American Society of Hematology Education Program Book*, 1999, pp. 262–269.
7. Damle JN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood* 1999;94:1840–1847.
8. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig VH genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood* 1999;94:1848–1854.
9. Döhner H, Stilgenbauer S, Benner A. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med* 2000;343:1910–1918.
10. Kröber A, Seiler T, Benner A, et al. VH mutation status, CD38 expression level, genomic aberrations, and survival in chronic lymphocytic leukemia. *Blood* 2002;100:1410–1416.
11. Robèrt KH, Möller E, Gahrton G, Eriksson H, Nilsson B. B-cell activation of peripheral blood lymphocytes from patients with chronic lymphocytic leukaemia. *Clin Exp Immunol* 1978;33:302–308.
12. Mitelman F, Levan G. Clustering of aberrations to specific chromosomes in human neoplasms. *Hereditas* 1978;89:207–232.
13. Autio K, Turunen O, Penttilä O, Erämaa E, de la Chapelle A, Schröder J. Human chronic lymphocytic leukemia: Karyotypes in different lymphocyte populations. *Cancer Genet Cytogenet* 1979;1:147–155.
14. Gahrton G, Robèrt KH, Friberg K, Zech L, Bird AG. Nonrandom chromosomal aberrations in chronic lymphocytic leukemia revealed by polyclonal B-cell-mitogen stimulation. *Blood* 1980;56:640–647.

15. Hurley JN, Fu SM, Kunkel HG, Chaganti RSK, German J. Chromosome abnormalities of leukaemic B lymphocytes in chronic lymphocytic leukaemia. *Nature* 1980;283:76–78.
16. Crawford DH, Catovsky D. In vitro activation of leukaemia B cells by interleukin-4 and antibodies to CD40. *Immunology* 1993;80:40–44.
17. Döhner H, Pohl S, Bulgay-Mörschel M, Stilgenbauer S, Bentz M, Lichter P. Detection of trisomy 12 in chronic lymphoid leukemias using fluorescence in situ hybridization. *Leukemia* 1993;7:516–520.
18. Oscier DG, Stevens J, Hamblin TJ, Pickering RM, Lambert R, Fitchett M. Correlation of chromosome abnormalities with laboratory features and clinical course in B-cell chronic lymphocytic leukaemia. *Br J Haematol* 1990;76:352–358.
19. Juliusson G, Oscier DG, Fitchett M, et al. Prognostic subgroups in B-cell chronic lymphocytic leukemia defined by specific chromosomal abnormalities. *N Engl J Med* 1990;323:720–724.
20. Juliusson G, Gahrton G. Chromosome aberrations in B-cell chronic lymphocytic leukemia. Pathogenetic and clinical implications. *Cancer Genet Cytogenet* 1990;45:143–160.
21. Juliusson G, Oscier D, Gahrton G, for the International Working Party on Chromosomes in CLL (IWCCLL). Cytogenetic findings and survival in B-cell chronic lymphocytic leukemia. Second IWCCLL compilation of data on 662 patients. *Leuk Lymphoma* 1991;5:21–25.
22. Autio K, Elonen E, Teerenhovi L, Knuutila S. Cytogenetic and immunologic characterization of mitotic cells in chronic lymphocytic leukemia. *Eur J Haematol* 1986;39:289–298.
23. Lichter P, Ward DC. Is non-isotopic in situ hybridization finally coming of age? *Nature* 1990;345:93–95.
24. Lichter P, Bentz M, Joos S. Detection of chromosomal aberrations by means of molecular cytogenetics: Painting of chromosomes and chromosomal subregions and comparative genomic hybridization. *Methods Enzym* 1995;254:334–359.
25. Cremer T, Landegent J, Brückner A, et al. Detection of chromosome aberrations in the human interphase nucleus by visualization of specific target DNAs with radioactive and non-radioactive in situ hybridization techniques: diagnosis of trisomy 18 with probe L1.84. *Hum Genet* 1986;74:346–352.
26. Döhner H, Stilgenbauer S, James MR, et al. 11q deletions identify a new subset of B-cell chronic lymphocytic leukemia characterized by extensive nodal involvement and inferior prognosis. *Blood* 1997;89:2516–2522.
27. Stilgenbauer S, Bullinger L, Lichter P, Döhner H. Genetics of chronic lymphocytic leukemia: genomic aberrations and V(H) gene mutation status in pathogenesis and clinical course. *Leukemia* 2002;16:993–1007.
28. Fitchett M, Griffiths MJ, Oscier DG, Johnson S, Seabright M. Chromosome abnormalities involving band 13q14 in hematologic malignancies. *Cancer Genet Cytogenet* 1987;24:143–150.
29. Peterson LC, Lindquist LL, Church S, Kay NE. Frequent clonal abnormalities of chromosome band 13q14 in B-cell chronic lymphocytic leukemia: Multiple clones, subclones, and nonclonal alterations in 82 Midwestern patients. *Genes Chromosom Cancer* 1992;4:273–280.
30. Ross FM und Stockdill G. Clonal chromosome abnormalities in chronic lymphocytic leukemia patients revealed by TPA stimulation of whole blood cultures. *Cancer Genet Cytogenet* 1987;25:109–121.
31. Zech L, Mellstedt H. Chromosome 13—a new marker for B-cell chronic lymphocytic leukemia. *Hereditas* 1988;108:77–84.
32. Weinberg RA. The retinoblastoma protein and cell cycle control. *Cell* 1995;81:323–330.
33. Döhner H, Pilz T, Fischer K, et al. Molecular cytogenetic analysis of Rb-1 deletions in chronic B-cell leukemias. *Leuk Lymph* 1994;16:97–103.
34. Liu Y, Grandér D, Söderhäll S, Juliusson G, Gahrton G, Einhorn S. Retinoblastoma gene deletions in B-cell chronic lymphocytic leukemia. *Genes Chrom Cancer* 1992;4:250–256.
35. Liu Y, Szekely L, Grandér D, et al. Chronic lymphocytic leukemia cells with allelic deletions at 13q14 commonly have one intact RB1 gene: Evidence for a role of an adjacent locus. *Proc Natl Acad Sci USA* 1993;90:8697–8701.
36. Stilgenbauer S, Döhner H, Bulgay-Mörschel M, Weitz S, Bentz M, Lichter P. High frequency of monoallelic retinoblastoma gene deletion in B-cell chronic lymphoid leukemia shown by interphase cytogenetics. *Blood* 1993;81:2118–2124.
37. Bouyge-Moreau I, Rondeau G, Avet-Loiseau H, et al. Construction of a 780-kb PAC, BAC, and cosmid contig encompassing the minimal critical deletion involved in B cell chronic lymphocytic leukemia at 13q14.3. *Genomics* 1997;46:183–190.
38. Brown AG, Ross FM, Dunne EM, Steel CM, Weir-Thompson EM. Evidence for a new tumour suppressor locus (DBM) in human B-cell neoplasia telomeric to the retinoblastoma gene. *Nat Genet* 1993;3:67–72.
39. Bullrich F, Veronese ML, Kitada S, et al. Minimal region of loss at 13q14 in B-cell chronic lymphocytic leukemia. *Blood* 1996;88:3109–3115.

40. Chapman RM, Corcoran MM, Gardiner A, Hawthorn LA, Cowell JK, Oscier DG. Frequent homozygous deletions of the D13S25 locus in chromosome region 13q14 defines the location of a gene critical in leukaemogenesis in chronic B-cell lymphocytic leukaemia. *Oncogene* 1994;9:1289–1293.
41. Corcoran MM, Rasool O, Liu Y, et al. Detailed molecular delineation of 13q14.3 loss in B-cell chronic lymphocytic leukemia. *Blood* 1998;91:1382–1390.
42. Migliazza A, Bosch F, Komatsu H, et al. Nucleotide sequence, transcription map, and mutation analysis of the 13q14 chromosomal region deleted in B-cell chronic lymphocytic leukemia. *Blood* 2001;97:2098–2104.
43. Kalachikov S, Migliazza A, Cayanis E, et al. Cloning and gene mapping of the chromosome 13q14 region deleted in chronic lymphocytic leukemia. *Genomics* 1997;42:369–377.
44. Liu Y, Hermanson M, Grandér D, et al. 13q deletions in lymphoid malignancies. *Blood* 1995;86:1911–1915.
45. Liu Y, Corcoran M, Rasool O, et al. Cloning of two candidate tumor suppressor genes within a 10 kb region on chromosome 13q14, frequently deleted in chronic lymphocytic leukemia. *Oncogene* 1997;15:2463–2473.
46. Stilgenbauer S, Leupolt E, Ohl S, et al. Heterogeneity of deletions involving RB-1 and the D13S25 locus in B-cell chronic lymphocytic leukemia revealed by FISH. *Cancer Res* 1995;55:3475–3477.
47. Stilgenbauer S, Nickolenko J, Wilhelm J, et al. Expressed sequences as candidates for a novel tumor suppressor gene at band 13q14 in B-cell chronic lymphocytic leukemia and mantle cell lymphoma. *Oncogene* 1998;16:1891–1897.
48. Wolf S, Mertens D, Schaffner C, Korz C, Döhner H, Stilgenbauer S, Lichter P. B-cell neoplasia associated gene with multiple splicing (BCMS): the candidate B-CLL gene on 13q14 comprises more than 560 kb covering all critical regions. *Hum Mol Genet* 2001;10:1275–1285.
49. Korz C, Pscherer A, Benner A, et al. Evidence for distinct pathomechanisms in B-cell chronic lymphocytic leukemia and mantle cell lymphoma by quantitative expression analysis of cell-cycle and apoptosis associated genes. *Blood* 2002;99:4554–4461.
50. Mertens D, Wolf S, Schroeter P, et al. Downregulation of candidate tumor suppressor genes within chromosome band 13q14.3 is independent of the DNA methylation pattern in B-cell chronic lymphocytic leukemia. *Blood* 2002;99:4116–4121.
51. Kobayashi H, Espinosa R III, Fernald AA, et al. Analysis of deletions of the long arm of chromosome 11 in hematologic malignancies with fluorescence in situ hybridization. *Genes Chrom Cancer* 1993;8:246–252.
52. James MR, Richard III CW, Schott JJ, et al. A radiation hybrid map of 506 STS markers spanning human chromosome 11. *Nat Genet* 1994;6:70–76.
53. Stilgenbauer S, Liebisch P, James MR, et al. Molecular cytogenetic delineation of a novel critical genomic region in chromosome bands 11q22.2-q23.1 in lymphoproliferative disorders. *Proc Natl Acad Sci USA* 1996;93:11,837–11,841.
54. Barlow C, Hirotsumi S, Paylor R, et al. Atm-deficient mice: a paradigm of ataxia telangiectasia. *Cell* 1996;86:159–171.
55. Rotman G, Shiloh Y. ATM: from gene to function. *Hum Mol Genet* 1998;7:1555–1563.
56. Stilgenbauer S, Schaffner C, Litterst A, et al. Biallelic mutations in the ATM gene in T-prolymphocytic leukemia. *Nat Med* 1997;3:1155–1159.
57. Vorechovsky I, Luo L, Dyer MJS, et al. Clustering of missense mutations in the ataxia-telangiectasia gene in a sporadic T-cell leukaemia. *Nat Genet* 1997;17:96–99.
58. Bullrich F, Rasio D, Kitada S, et al. ATM mutations in B-cell chronic lymphocytic leukemia. *Cancer Res* 1999;59:24–27.
59. Schaffner C, Stilgenbauer S, Rappold G, Döhner H, Lichter P. Somatic ATM mutations indicate a pathogenic role of ATM in B-cell chronic lymphocytic leukemia. *Blood* 1999;94:748–753.
60. Stankovic T, Weber P, Stewart G, et al. Inactivation of ataxia telangiectasia mutated gene in B-cell chronic lymphocytic leukaemia. *Lancet* 1999;353:26–29.
61. Starostik P, Manshoury T, O'Brien S, et al. Deficiency of the ATM protein defines an aggressive subgroup of B-cell chronic lymphocytic leukemia. *Cancer Res* 1998;58:4552–4557.
62. Pettitt AR, Sherrington PD, Stewart G, Cawley JC, Taylor AM, Stankovic T. p53 dysfunction in B-cell chronic lymphocytic leukemia: inactivation of ATM as an alternative to TP53 mutation. *Blood* 2001;98:814–822.
63. Fegan C, Robinson H, Thompson P, Whittaker JA, White D. Karyotypic evolution in CLL. Identification of a new sub-group of patients with deletions of 11q and advanced or progressive disease. *Leukemia* 1995;9:2003–2008.
64. Stankovic T, Stewart GS, Fegan C, et al. Ataxia telangiectasia mutated-deficient B-cell chronic lymphocytic leukemia occurs in pregerminal center cells and results in defective damage response and unrepaired chromosome damage. *Blood* 2002;99:300–309.

65. Monni O, Zhu Y, Franssila K, et al. Molecular characterisation of deletion at 11q22.1-23.3 in mantle cell lymphoma. *Br J Haematol* 1999;104:665–671.
66. Stilgenbauer S, Winkler D, Ott G, et al. Molecular characterization of 11q deletions points to a pathogenic role of the ATM gene in mantle cell lymphoma. *Blood* 1999;94:3262–3264.
67. Schaffner C, Idler I, Stilgenbauer S, Döhner H, Lichter P. Mantle cell lymphoma is characterized by inactivation of the ATM gene. *Proc Natl Acad Sci USA* 2000;97:2773–2778.
68. Bird ML, Ueshima Y, Rowley JD, Haren JM, Vardiman JW. Chromosome abnormalities in B cell chronic lymphocytic leukemia and their clinical correlations. *Leukemia* 1989;3:182–191.
69. Han T, Ozer H, Sadamori N, et al. Prognostic importance of cytogenetic abnormalities in patients with chronic lymphocytic leukemia. *N Engl J Med* 1984;310:288–292.
70. Juliusson G, Robèrt KH, Öst A, et al. Prognostic information from cytogenetic analysis in chronic B-lymphocytic leukemia and leukemic immunocytoma. *Blood* 1985;65:134–141.
71. Morita M, Minowada J, Sandberg AA. Chromosomes and causation of human cancer and leukemia. XLV. Chromosome patterns in stimulated lymphocytes of chronic lymphocytic leukemia. *Cancer Genet Cytogenet* 1981;3:293–306.
72. Nowell PC, Vonderheid EC, Besa E, Hoxie JA, Moreau L, Finan JB. The most common chromosome change in 86 chronic B cell or T cell tumors: a 14q32 translocation. *Cancer Genet Cytogenet* 1986;19:219–227.
73. Pittman S, Catovsky D. Prognostic significance of chromosome abnormalities in chronic lymphocytic leukaemia. *Br J Haematol* 1984;58:649–660.
74. Gahrton G, Robèrt KH, Friberg K, Juliusson G, Biberfeld P, Zech L. Cytogenetic mapping of the duplicated segment of chromosome 12 in lymphoproliferative disorders. *Nature* 1982;297:513–514.
75. Anastasi J, Le Beau MM, Vardiman JW, Fernald AA, Larson RA, Rowley JD. Detection of trisomy 12 in chronic lymphocytic leukemia by fluorescence in situ hybridization to interphase cells: a simple and sensitive method. *Blood* 1992;79:1796–1801.
76. Arif M, Tanaka K, Asou H, Ohno R, Kamada N. Independent clones of trisomy 12 and retinoblastoma gene deletion in Japanese B cell chronic lymphocytic leukemia, detected by fluorescence in situ hybridization. *Leukemia* 1995;9:1822–1827.
77. Criel A, Wlodarska I, Meeus P, et al. Trisomy 12 is uncommon in typical chronic lymphocytic leukaemias. *Br J Haematol* 1994;87:523–528.
78. Escudier SM, Pereira-Leahy JM, Drach JW, et al. Fluorescence in situ hybridization and cytogenetic studies of trisomy 12 in chronic lymphocytic leukemia. *Blood* 1993;81:2702–2707.
79. Matutes E, Oscier D, Garcia-Marco J, et al. Trisomy 12 defines a group of CLL with atypical morphology: correlation between cytogenetic, clinical and laboratory features in 544 patients. *Br J Haematol* 1996;92:382–388.
80. Perez Losada A, Wessman M, Tiainen M, et al. Trisomy 12 in chronic lymphocytic leukemia: an interphase cytogenetic study. *Blood* 1991;78:775–779.
81. Que TH, Garcia Marco J, Ellis J, et al. Trisomy 12 in chronic lymphocytic leukemia detected by fluorescence in situ hybridization: Analysis by stage, immunophenotype, and morphology. *Blood* 1993;82:571–575.
82. Raghoebier S, Kibbelaar RE, Kleiverda K, et al. Mosaicism of trisomy 12 in chronic lymphocytic leukemia detected by non-radioactive in situ hybridisation. *Leukemia* 1992;6:1220–1226.
83. Bentz M, Huck K, du Manoir S, et al. Comparative genomic hybridization in chronic B-cell leukemias reveals a high incidence of chromosomal gains and losses. *Blood* 1995;85:3610–3618.
84. Merup M, Juliusson G, Wu X, et al. Amplification of multiple regions of chromosome 12, including 12q13-15, in chronic lymphocytic leukaemia. *Eur J Haematol* 1997;58:174–180.
85. Dierlamm J, Wlodarska I, Michaux L, et al. FISH identifies different types of duplications with 12q13-15 as the commonly involved segment in B-cell lymphoproliferative malignancies characterized by partial trisomy 12. *Genes Chrom Cancer* 1997;20:155–166.
86. Solinas-Toldo S, Lampel S, Stilgenbauer S, et al. Matrix-based comparative genomic hybridization: Biochips to screen for genomic imbalances. *Genes Chrom Cancer* 1997;20:399–407.
87. Pinkel D, Segraves R, Sudar D, et al. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet* 1998;20:207–211.
88. Wessendorf S, Schwaenen C, Barth Th, et al. Automated genomic profiling using microarray based hybridization (Matrix-CGH) - a powerful technique for the detection of DNA-amplification in aggressive lymphoma. *Blood* 2001;98(suppl 1):1940.
89. Schwaenen C, Nessling M, Wessendorf S, et al. Automated Genomic Profiling in Chronic Lymphocytic Leukemia using Array Based Comparative Genomic Hybridization (Matrix-CGH). 2003, submitted for publication.

90. Johansson B, Mertens F, Mitelman F. Cytogenetic deletion maps of hematologic neoplasms: circumstantial evidence for tumor suppressor loci. *Genes Chrom Cancer* 1993;8:205–218.
91. Offit K, Parsa NZ, Gaidano G, et al. 6q deletions define distinct clinico-pathologic subsets of non-Hodgkin's lymphoma. *Blood* 1993;82:2157–2162.
92. Offit K, Louie DC, Parsa NZ, et al. Clinical and morphologic features of B-cell small lymphocytic lymphoma with del(6)(q21q23). *Blood* 1994;83:2611–2618.
93. Merup M, Moreno TC, Heyman M, et al. 6q deletions in acute lymphoblastic leukemia and non-Hodgkin's lymphomas. *Blood* 1998;91:3397–4000.
94. Gaidano G, Newcomb EW, Gong JZ, et al. Analysis of alterations of oncogenes and tumor suppressor genes in chronic lymphocytic leukemia. *Am J Pathol* 1994;144:1312–1319.
95. Stilgenbauer S, Bullinger L, Benner A, et al. Incidence and clinical significance of 6q deletions in B-cell chronic lymphocytic leukemia. *Leukemia* 1999;13:1331–1334.
96. Zhang Y, Matthiesen P, Harder S, et al. A 3-cM commonly deleted region in 6q21 in leukemias and lymphomas delineated by fluorescence in situ hybridization. *Genes Chromosomes Cancer* 2000;27:52–58.
97. Gaidano G, Ballerini P, Gong JZ, et al. p53 mutations in human lymphoid malignancies: association with Burkitt lymphoma and chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 1991;88:5413–5417.
98. El Rouby S, Thomas A, Costin D, et al. p53 gene mutation in B-cell chronic lymphocytic leukemia is associated with drug resistance and is independent of MDR1/MDR3 gene expression. *Blood* 1993;82:3452–3459.
99. Fenaux P, Preudhomme C, Laï JL, et al. Mutations of the p53 gene in B-cell chronic lymphocytic leukemia: a report on 39 cases with cytogenetic analysis. *Leukemia* 1992;6:246–250.
100. Döhner H, Fischer K, Bentz M, et al. p53 gene deletion predicts for poor survival and non-response to therapy with purine analogs in chronic B-cell leukemias. *Blood* 1995;85:1580–1589.
101. Geisler CH, Philip P, Egelund Christensen B, et al. In B-cell chronic lymphocytic leukaemia chromosome 17 abnormalities and not trisomy 12 are the single most important cytogenetic abnormalities for the prognosis: a cytogenetic and immunophenotypic study of 480 unselected newly diagnosed patients. *Leuk Res* 1997;21:1011–1023.
102. Kröber A, Scherer K, Leupolt E, Stilgenbauer S, Döhner H. p53 aberrations in B-CLL predict survival and are associated with in vivo resistance to therapy. *Blood* 2000;96(suppl 1):abstract 4463.
103. Bloomfield C, Arthur D, Frizzera G, Levine E, Peterson B, Gajl-Peczalska K. Nonrandom chromosome abnormalities in lymphoma. *Cancer Res* 1983;43:2975–2984.
104. Ueshima Y, Bird ML, Vardiman JW, Rowley JD. A 14;19 translocation in B-cell chronic lymphocytic leukemia: a new recurring chromosome aberration. *Int J Cancer* 1985;36:287–290.
105. Van den Berghe H, Parloir C, David G, Michaux JL, Sokal G. A new characteristic karyotypic anomaly in lymphoproliferative disorders. *Cancer* 1979;44:188–195.
106. Bosch F, Jares P, Campo E, et al. PRAD-1/Cyclin D1 gene overexpression in chronic lymphoproliferative disorders: a highly specific marker of mantle cell lymphoma. *Blood* 1994;84:2726–2732.
107. Rosenberg CL, Wong E, Petty EM, et al. PRAD1, a candidate BCL1 oncogene: Mapping and expression in centrocytic lymphoma. *Proc Natl Acad Sci USA* 1991;88:9638–9642.
108. Tsujimoto Y, Yunis J, Onorato-Showe L, Erikson J, Nowell PC, Croce CM. Molecular cloning of the chromosomal breakpoint of B-cell lymphomas and leukemias with the t(11;14) chromosome translocation. *Science* 1984;224:1403–1406.
109. Levy V, Ugo V, Delmer A, et al. Cyclin D1 overexpression allows identification of an aggressive subset of leukemic lymphoproliferative disorder. *Leukemia* 1999;13:1343–1351.
110. Withers DA, Harvey RC, Faust JB, Melnyk O, Carey K, Meeker TC. Characterization of a candidate bcl-1 gene. *Mol Cell Biol* 1991;11:4846–4853.
111. Raffeld M, Jaffe ES. bcl-1, t(11;14), and mantle cell-derived lymphomas. *Blood* 1991;78:259–263.
112. Medeiros J, van Krieken JH, Jaffe ES, Raffeld M. Association of bcl-1 rearrangements with lymphocytic lymphoma of intermediate differentiation. *Blood* 1990;76:2086–2090.
113. Newman RA, Peterson B, Davey FR, et al. Phenotypic markers and BCL1 rearrangements in B-cell chronic lymphocytic leukemia: a cancer and leukemia group B study. *Blood* 1993;82:1239–1246.
114. Raghoebier S, van Krieken JHJM, Kluin-Nelemans JC, et al. Oncogene rearrangements in chronic B-cell leukemia. *Blood* 1991;77:1560–1564.
115. Rechavi G, Katzir N, Brok-Simoni F, et al. A search for bcl1, bcl2, and c-myc oncogene rearrangements in chronic lymphocytic leukemia. *Leukemia* 1988;3:57–60.
116. Adachi M, Cossmna J, Longo D, Croce CM, Tsujimoto Y. Variant translocation of the bcl-2 gene to Ig in a chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 1989;86:2771–2774.



117. Crossen PE, Morrison MJ. Lack of 5' bcl2 rearrangements in B-cell leukemia. *Cancer Genet Cytogenet* 1993;69:72–73.
118. Dyer MJS, Zani VJ, Lu WZ, et al. BCL2 translocations in leukemias of mature B cells. *Blood* 1994;83:3682–3688.
119. Hanada M, Delia D, Aiello A, Stadtmauer E, Reed JC bcl-2 gene hypomethylation and high-level expression in B-cell chronic lymphocytic leukemia. *Blood* 1993;82:1820–1828.
120. McKeithan TW, Rowley JD, Shows T, Diaz M. Cloning of the chromosome translocation breakpoint junction of the t(14;19) in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 1987;84:9257–9260.
121. McKeithan TW, Takimoto GS, Ohno H, et al. BCL3 rearrangements and t(14;19) in chronic lymphocytic leukemia and other B-cell malignancies: a molecular and cytogenetic study. *Genes Chrom Cancer* 1997;20:64–72.
122. Michaux L, Mecucci C, Stul M, et al. BCL3 rearrangements and t(14;19)(q32;q13) in lymphoproliferative disorders. *Genes Chrom Cancer* 1996;15:38–47.
123. Fais F, Ghiotto F, Hashimoto S, et al. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. *J Clin Invest* 1998;102:1515–1525.
124. Küppers R, Klein U, Hansmann ML, Rajewsky K. Cellular origin of human B-cell lymphomas. *N Engl J Med* 1999;341:1520–1529.
125. Schroeder HW, Dighiero G. The pathogenesis of chronic lymphocytic leukemia: analysis of the antibody repertoire. *Immunol Today* 1994;15:288–294.
126. Neilson JR, Auer R, White D, et al. Deletions at 11q identify a subset of patients with typical CLL who show consistent disease progression and reduced survival. *Leukemia* 1997;11:1929–1932.
127. Stilgenbauer S, Döhner H. Campath-1H induced complete remission of chronic lymphocytic leukemia despite p53 gene mutation and resistance to chemotherapy. *N Engl J Med* 2002;347:452–453 (letter).
128. Damle JN, Wasil T, Allen SL, Schulman P, Rai KR, Chiorazzi N, Ferrarini M. Updated data on V gene mutation status and CD38 expression in CLL. *Blood* 2000;95:2456–2457 (letter).
129. Hamblin TJ, Orchard JA, Gardiner A, Oscier DG, Davis Z, Stevenson FK. Immunoglobulin V genes and CD38 expression in CLL. *Blood* 2000;95:2455–2456 (letter).
130. Hamblin TJ, Orchard JA, Ibbotson RE, et al. CD38 expression and immunoglobulin variable region mutations are independent prognostic variables in chronic lymphocytic leukemia, but CD38 expression may vary during the course of the disease. *Blood* 2002;99:1023–1029.
131. Thunberg U, Johnson A, Roos G, et al. CD38 expression is a poor predictor for VH gene mutational status and prognosis in chronic lymphocytic leukemia. *Blood* 2001;97:1892–1894.
132. Kröber A, Bühler A, Kienle D, Benner A, Döhner H, Stilgenbauer S. Analysis of VDJ rearrangement structure and VH mutation status in chronic lymphocytic leukemia. *Blood* 2001;98:abstract 1509.
133. Kipps TJ, Tomhave E, Pratt LF, Duffy S, Johnson T, Kobayashi R, Carson D. Developmentally restricted VH gene expressed at high frequency in chronic lymphocytic leukaemia. *Proc Natl Acad Sci USA* 1989;86:5913.
134. Küppers R, Gause A, Rajewsky K. B-cells of chronic lymphatic leukaemia express V genes in unmutated form. *Leuk Res* 1991;15:487–496.
135. Chang B, Casali P. The CDR1 sequences of a major proportion of human germline Ig VH genes are inherently susceptible to amino acid replacement. *Immunol Today* 1994;15:367–373.
136. Ibrahim S, Keating M, Do KA, et al. CD38 expression as an important prognostic factor in B-cell chronic lymphocytic leukemia. *Blood* 2001;98:181–186.
137. Matrai Z, Lin K, Dennis M, et al. CD38 expression and Ig VH gene mutation in B-cell chronic lymphocytic leukemia. *Blood* 2001;97:1902–1903.
138. Klein U, Tu Y, Stolovitzky GA, et al. Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. *J Exp Med* 2001;194:1625–1638.
139. 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* 2001;194:1639–1647.
140. Oscier DG, Gardiner AC, Mould SJ, et al. Multivariate analysis of prognostic factors in CLL: clinical stage, IGVH gene mutational status, and loss or mutation of the p53 gene are independent prognostic factors. *Blood* 2002;100:1177–1184.
141. Lin K, Sherrington PD, Dennis M, Matrai Z, Cawley JC, Pettitt AR. Relationship between p53 dysfunction, CD38 expression, and IgV(H) mutation in chronic lymphocytic leukemia. *Blood* 2002;100:1404–1409.





<http://www.springer.com/978-1-58829-099-1>

Chronic Lymphocytic Leukemia  
Molecular Genetics, Biology, Diagnosis, and  
Management

Faguet, G. (Ed.)

2004, XIV, 421 p., Hardcover

ISBN: 978-1-58829-099-1

A product of Humana Press