

REVIEW

Genetics of chronic lymphocytic leukemia: genomic aberrations and V_H gene mutation status in pathogenesis and clinical course

S Stilgenbauer¹, L Bullinger¹, P Lichter² and H Döhner¹ and the German CLL Study Group (GCLLSG)

¹Abteilung Innere Medizin III, University of Ulm, Germany; and ²Abteilung 'Organisation komplexer Genome', Deutsches Krebsforschungszentrum, Heidelberg, Germany

The genetic characterization of chronic lymphocytic leukemia (CLL) has made significant progress over the past few years. While conventional cytogenetic analyses only detected chromosome aberrations in 40–50% of cases, new molecular cytogenetic methods, such as fluorescence *in situ* hybridization (FISH), have greatly enhanced our ability to detect chromosomal abnormalities in CLL. Today, genomic aberrations are detected in over 80% of CLL cases. Genes potentially involved in the pathogenesis were identified with *ATM* in a subset of cases with 11q deletion and *p53* in cases with 17p13 deletion. For the most frequent aberration, the deletion 13q14, candidate genes have been isolated. Genetic subgroups with distinct clinical features have been identified. 11q deletion is associated with marked lymphadenopathy and rapid disease progression. 17p deletion predicts for treatment failure with alkylating agents, as well as fludarabine and short survival times. In multivariate analysis 11q and 17p deletions provided independent prognostic information. Recently, another important issue of genetic risk classification in CLL was identified with the mutation status of the immunoglobulin variable heavy chain genes (V_H). CLL cases with unmutated V_H show more rapid disease progression and shorter survival times. Whether CD38 expression can serve as a surrogate marker for V_H mutation status is currently discussed controversially. V_H mutation status and genomic abnormalities, such as 17p and 11q deletion, have recently been shown to be related to each other, but were of independent prognostic information in multivariate analysis. Moreover, genomic aberrations and V_H mutation status appear to give prognostic information irrespective of the clinical stage and may therefore allow a risk assessment for individual patients early in the course of their disease. *Leukemia* (2002) 16, 993–1007. DOI: 10.1038/sj/leu/2402537

Keywords: CLL; genomic aberrations; *p53*; *ATM*; V_H mutation status

Introduction

In a review by Mitelman and Levan on chromosome aberrations in human neoplasia no specific aberration was associated with chronic lymphocytic leukemia (CLL) in 1978.¹ Since then our knowledge of cytogenetic and molecular cytogenetic findings in this disease has increased tremendously. In the late 1970s, specific chromosomal aberrations in CLL were identified through the use of B cell mitogens. Clonal chromosome abnormalities are detected in 40–50% of cases by conventional cytogenetics.^{2,3} Chromosome banding studies are still hampered by the problem of the low *in vitro* mitotic activity of the CLL cells. In recent years, modern molecular cytogenetic methods, such as fluorescence *in situ* hybridization (FISH), made it possible to identify chromosome

aberrations in approximately 80% of CLL cases using a disease-specific probe set.⁴ Deletion 11q22-q23 and deletion 17p13 are independent prognostic markers in multivariate analysis identifying subgroups of patients with rapid disease progression and short survival times. On the other hand, deletion of chromosome band 13q14 as the sole abnormality is associated with a favorable prognosis.⁴ With the exception of *p53* and *ATM*, most of the affected tumor suppressor genes and oncogenes in CLL are as yet unknown.

Another important genetic marker is related to the stage of differentiation of the CLL cells. The recombination of variable (V), diversity (D) and joining (J) immunoglobulin gene segments and the insertion of nontemplated nucleotides at the V–D and D–J junction occur physiologically in the pre-germinal center phase of B cell differentiation. In the following germinal center phase, the variable region genes (V_H) of the B cells can be modified by somatic hypermutation through introduction of point mutations and occasional deletions and duplications at a very high rate.⁵ Initially, it was thought that the clonal accumulation of CD5⁺ B cells in CLL was comprised of antigen-inexperienced lymphocytes with V_H in germline configuration corresponding to naive B cells. However, recent studies showed the presence of significant somatic mutation of the V_H genes, indicating that approximately in half of all CLL cases the neoplastic cells correspond to postgerminal-center memory B cells.^{6,7} In pivotal studies Damle *et al*⁸ and Hamblin *et al*⁹ have shown that the presence of unmutated V_H genes predicts for an inferior survival in CLL.

Characterization of genomic aberrations and the V_H mutational status may help to understand the pathogenesis of CLL and may give prognostic information independent from conventional clinical markers for a risk-adapted management of CLL patients.

Methodological approaches for the genetic analysis of CLL

Conventional chromosome banding analysis

With the aid of B cell mitogens such as TPA, Epstein–Barr virus, lipopolysaccharide, pokeweed mitogen, cytochalasin B, anti-human IgM, B cell growth factor, and an anti-CD40 antibody recurrent chromosome aberrations were identified in the 1980s.^{10–17} Despite the use of these mitogens, conventional chromosome banding analysis has remained difficult in CLL and even with improved culture techniques clonal genomic abnormalities can be detected in only 40–50% of CLL cases.^{2,3} In cases with normal karyotype a study combining immunophenotyping and karyotype analysis showed that metaphase spreads without clonal aberrations often originate from non-leukemic T-lymphocytes.¹⁸

Correspondence: H Döhner, Department of Internal Medicine III, University of Ulm, Robert-Koch-Str 8, 89081 Ulm, Germany; Fax: 49 731 500 24493

Received 15 February 2002; accepted 22 February 2002

Comparative genomic hybridization

Without knowledge of candidate regions involved in a specific tumor type, a genome-wide screening for chromosome imbalances can also be performed by comparative genomic hybridization (CGH).^{19–21} As this method works with differently labeled total genomic DNA samples (normal tissue DNA vs tumor DNA) that are co-hybridized to normal metaphase spreads, no tumor cell metaphase spreads are needed. In CLL, comparison of CGH data with banding results showed that the incidence of aberrations detected by CGH was higher.²²

Fluorescence *in situ* hybridization (FISH)

The development of fluorescence *in situ* hybridization (FISH) in the late 1980s and early 1990s, provided a very powerful tool for the detection of chromosome aberrations in tumors,²³ especially in CLL. With the aid of specific DNA probes genomic abnormalities can be detected by FISH on the single cell level in interphase nuclei or metaphase spreads. Therefore, this approach is also referred to as ‘interphase cytogenetics’ (see also Figure 1).²⁴ At the molecular level, many critical genomic regions have only very recently been characterized in CLL and in contrast to PCR-based methods no sequence information of the region under investigation is required. Another major advantage of FISH compared to conventional banding analysis or CGH is a higher spatial resolution for the detection of genomic aberrations resulting in a higher sensitivity. Therefore, the different findings regarding the spectrum and frequency of genomic abnormalities seen in various FISH studies compared to banding studies are not surprising.²⁵ Using the molecular cytogenetic FISH approach with a comprehensive probe set, today genomic aberrations are detected in approximately 80% of CLL cases.⁴

Molecular genetic techniques

Deletion screening detecting loss of heterozygosity (LOH) by quantitative Southern blot or microsatellite analyses and mutation analyses of genes by single strand conformational polymorphism (SSCP) or DNA sequence analysis have long been limited in CLL due to the lack of candidate genes. Recently, the elucidation of the pathogenic role of *p53* and *ATM* (in a subset of CLL patients) have made molecular gen-

etic screening possible (see below). The identification and sequencing of clonal VDJ rearrangements will be of growing importance to further evaluate the prognostic impact of the *V_H* gene mutational status as biological risk factor in CLL. Therefore, the diagnostic procedure for the detection of mutated *V_H* genes has to become less labor- and cost-intensive. We currently use a set-up consisting of a multiplex PCR from genomic DNA with a mixture of family specific unlabeled 3′-*J_H* primers and fluorochrome-labeled 5′-*V_H* primers. The PCR product is subsequently subjected to a genescan analysis through which the *V_H* gene family involved in the clonal VDJ rearrangement can be identified. The product of the initial multiplex PCR is then directly sequenced with the unlabeled primer corresponding to the *V_H* family involved in the clonal VDJ rearrangement of the respective case.²⁶

Pathogenic and clinical implications of genomic aberrations

Incidence of genomic aberrations in CLL

In conventional chromosome banding studies, trisomy 12 was the first recurrent chromosome aberration described in the late 1970s and early 1980s.^{10–14} Several investigators confirmed trisomy 12 as frequent aberration in CLL in the following years.^{27–35} Deletions and less frequently translocations involving chromosome band 13q14 were another recurrent aberration identified by several different groups in the late 1980s.^{32,36–38} Further genomic aberrations detected in varying frequencies by conventional cytogenetics included deletions of chromosome bands 11q,^{29,30,32,37,39} 6q^{14,29,30,34,35} and 17p,³⁴ partial or total trisomy 3q,^{27,29,32} and translocations involving band 14q32.^{11,14,27,29,31,34,35,40–42} This breakpoint was most frequently the result of a t(11;14)(q13;q32), today considered a hallmark of mantle cell lymphoma (MCL).⁴³ Therefore, many of these cases most likely represented leukemic MCL variants, rather than bona fide CLL cases.

In 1990 and 1991 the largest CLL banding series were reported by the First and Second International Working Party on Chromosomes in CLL (IWCCLL).^{2,3} Of 662 cases compiled in the Second IWCCLL, 604 were cytogenetically evaluable. Clonal genomic aberrations could be identified in 311 of these CLL cases, with trisomy 12 (19%) being the most frequent abnormality followed by aberrations of chromosome 13 (10%), 14 (8%), 11 (8%), 6 (6%), and 17 (4%).³ However, in 351 cases no clonal abnormality was found.

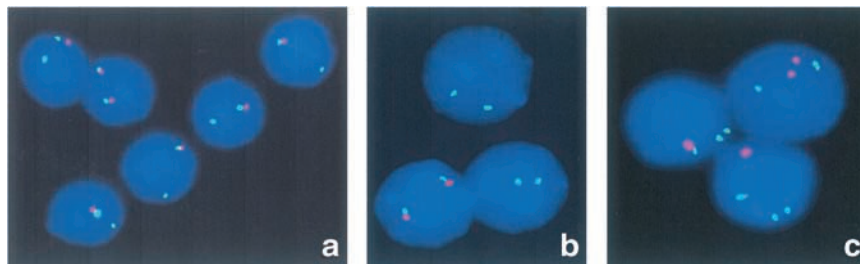


Figure 1 Examples of FISH images demonstrating genomic aberrations in CLL. (a) CLL with monoallelic 17p13 deletion as demonstrated by the single red signal in five of the six nuclei shown. Two green signals of an internal control probe hybridizing to an adjacent disomic genomic region proof a high hybridization efficiency. The single cell with two red signals likely represents a non-leukemic cell from the blood specimen. (b) CLL with biallelic deletion at 13q14. Two of the three nuclei show no red hybridization signal of a probe containing marker *D13S272* demonstrating biallelic loss of this region, while an adjacent probe containing marker *D13S273* is retained in disomic fashion. The single cell with two red and two green signals likely represent a non-leukemic 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.

Due to the methodological problems of conventional chromosome banding studies, it became necessary to reassess the incidence of genomic aberrations in CLL with the aid of novel molecular cytogenetic techniques. Based on conventional cytogenetic analyses and CGH data, a comprehensive DNA probe set was developed allowing the evaluation of the incidence and prognostic significance of the most important CLL-associated genomic aberrations. In our single center study, 325 CLL cases were analyzed by FISH for deletions in the chromosome regions 6q21, 11q22-q23, 13q14, 17p13, for trisomies of bands 3q26, 8q24, 12q13 and for translocations involving the immunoglobulin heavy chain locus on band 14q32. The prevalence of specific genomic aberrations in this large cohort was 82% (268 of 325 cases) and therefore almost twice as high as assumed from chromosome banding studies. The most common aberration was deletion 13q14 (55%), followed by deletion 11q22-q23 (18%), trisomy 12q13 (16%) deletion 17p13 (7%) and deletion 6q21 (7%) (Table 1).⁴ In multivariate analysis, the 17p and 11q deletions gave significant prognostic information showing that genomic aberrations are important independent predictors of disease progression and survival in CLL (Figure 2a and b).⁴ However, the prognostic significance of genomic aberrations in CLL has to be confirmed in prospective multicenter controlled treatment trials.

First results from prospective investigations within the CLL1 (fludarabine vs watch and wait in Binet A patients) and CLL3 (high-dose therapy followed by autologous transplantation in Binet B and C patients <60 years) treatment trials of the German CLL Study Group (GCLLSG) are shown in comparison to our unicentric cohort in Table 1.^{44,45} Regarding the overall incidence of genomic aberrations, these data are consistent with the results from our single center study. The early stage CLL patients entered in the CLL1 trial more frequently show 13q deletions as a single abnormality compatible with a favorable prognosis, whereas in the CLL3 population there is a high incidence of 11q deletions most likely reflecting the preferred inclusion of patients with rapid disease progression. However, among the Binet A patients in the CLL1 trial, 15% show high risk chromosomal aberrations such as deletions 11q or 17p (Table 1).⁴⁵

Clonal evolution of genomic aberrations has been documented in CLL, however, there are little data so far addressing this topic using molecular cytogenetic techniques. In a conventional cytogenetic analysis carried out by Oscier *et al*⁴⁶ karyotypic evolution was seen in 18 out of 112 patients (16%),

but there was no correlation between the incidence of clonal evolution and disease progression. In two other chromosome banding studies a significant association between the presence of ongoing karyotype changes and disease progression was seen.^{47,48} In these investigations 6q and 11q deletions were the most commonly acquired secondary chromosome aberrations associated with a shorter progression-free survival. Using a molecular cytogenetic approach we performed a sequential interphase cytogenetic study applying FISH on 55 CLL patients over a median observation time period of 42 months.⁴⁹ Clonal evolution was seen in nine out of 55 patients (16%) with 17p deletion (four cases), 6q deletion (three cases), 11q deletion (one case) and evolution from mono- to biallelic 13q deletion (three cases) being the acquired aberrations. We found a significant association between the presence of clonal evolution and progressive disease. Only 20% of the patients with a stable karyotype have died compared to two-thirds of those exhibiting clonal evolution.

In consideration of these studies the sensitive detection of genomic aberrations by interphase FISH provides a basis for a more accurate correlation of genomic aberrations with clinical features in CLL. Interphase FISH and molecular genetic techniques represent excellent tools for a better characterization of the critical genomic regions and have allowed the identification of candidate genes involved in pathogenesis or disease progression of CLL. In the following sections the most frequently involved genomic regions are discussed in detail.

13q14 deletions

Structural aberrations involving the long arm of chromosome 13 were initially reported in smaller chromosome banding studies in the late 1980s.^{32,35-38} While in the beginning trisomy 12 was often identified at a higher frequency, in more recent banding series deletions involving 13q turned out to be the most common abnormalities. Most aberrations that involve chromosome band 13q14 are deletions, whereas some appeared as balanced translocations at the resolution power of metaphase chromosome analysis. However, the translocation breakpoints in 13q14 are accompanied by submicroscopic deletions as demonstrated by molecular genetic techniques.^{50,51}

Chromosome band 13q14 harbors the retinoblastoma gene *RB1* and this well-known tumor suppressor gene was initially considered to play a pathogenic role in the development of CLL. However, abnormalities disrupting both alleles of *RB1* are rarely observed in CLL which argues against the involvement of this gene.^{50,52-54} Because reciprocal translocations involving band 13q14 were more frequently associated with deletions of *D13S25* than with *RB1*, a novel tumor suppressor gene was postulated 1.6 cM distal of *RB1* in the genomic region containing the marker *D13S25*.^{50,51} The aim of subsequent molecular genetic studies was to define this critical region more precisely.^{50,51,55-59} Several groups constructed high resolution physical maps spanning several hundred kilobases at the *RB1-D13S25* interval.⁶⁰⁻⁶⁵ A commonly deleted segment of approximately 300 kb around *D13S272* was identified by Kalachikov *et al*.⁶⁰ Liu *et al*⁶¹ described a minimally deleted interval of 10 kb centromeric to *D13S272*. Mutation analysis of two candidate genes in this region, *Leu1* and *Leu2*, today termed *BCMS* and *BCMSUN*, respectively, failed to show inactivation of these genes. We constructed a 1.4 Mb sized contig of DNA fragments at the critical *D13S273-D13S25* interval and used clones from this to screen

Table 1 Incidence of genomic aberrations in one large retrospective unicentric FISH study compared with preliminary results of two prospective multicenter trials of the German CLL Study Group (GCLLSG)

Aberration	Ref. 4 (n = 325)	CLL1 ^a (n = 258)	CLL3 ^b (n = 139)
13q deletion	55%	62%	51%
13q deletion single	36%	44%	31%
11q deletion	18%	11%	25%
Trisomy 12	16%	10%	12%
17p deletion	7%	6%	3%
Normal karyotype	18%	22%	19%

^aCLL1 trial of the German CLL Study Group (GCLLSG) for Binet A patients.⁴⁵

^bCLL3 trial of the GCLLSG evaluating high-dose therapy followed by autologous stem cell transplantation among Binet B and C patients.⁴⁴

322 B-CLL cases by FISH.⁶⁴ 51% of the examined CLL and 70% of MCL cases showed a 13q14 deletion with a commonly deleted segment involving marker *D13S272*. However our experiments also did not reveal any evidence for mutational disruption of *BCMS* or *BCMSUN* according to the two-hit hypothesis of tumor suppressor gene inactivation. An independently expressed *BCMSUN* homolog of unclear pathogenic significance was identified in bands 1p22-p31.⁶⁶ *BCMS* is organized in a complex fashion spanning more than 560 kb of genomic DNA and is processed into a myriad of transcripts through differential splicing.⁶⁷ There is currently no evidence for other frequently deleted regions on chromosome 13q, such as the *BRCA2* gene in band 13q12,⁶⁴ therefore, the putative tumor suppressor gene involved in 13q deletions in CLL still remains to be determined.

The clinical significance of 13q14 aberrations was first shown by the multicenter studies of the First and Second IWCCLL, where patients with structural abnormalities of chromosome 13 seemed to have a more favorable prognosis exhibiting survival probabilities similar to those with a normal karyotype.^{2,3} These findings were supported by the results of our uncentric interphase FISH study.⁴ The patients with a deletion 13q14 as single aberration (no additional aberration detectable with a comprehensive FISH probe set) had the longest estimated median treatment-free interval and survival time (133 months; see Figure 2a and b). In particular, the estimated median survival time of this group was longer as compared to the groups without detectable aberrations (111 months) and the group with trisomy 12 (114 months).

11q22-q23 deletions

In most chromosome banding studies in CLL, the frequency of 11q22-q23 aberrations has been underestimated. The Second IWCCLL reported abnormalities of the long arm of chromosome 11 in 49 of 604 (8%) cytogenetically evaluable cases.³ Most of these resulted from the translocation t(11;14)(q13;q32) and aberrations involving 11q other than translocations at 11q13 were found in less than 5% of CLL cases. In three more recently published large conventional cytogenetic studies 11q deletion did not occur as frequent aberration.⁶⁸⁻⁷⁰ Evidence for the significance of 11q22-q23 aberrations came from smaller chromosome banding studies.^{29,30,32,37,39,71} In one report, deletion 11q was the second most common chromosomal abnormality identifying a subset of patients who showed rapid disease progression and short survival.⁷²

Regarding the molecular characterization of the critical region affected by deletions of 11q21-q25, only few data were available. One FISH study of diverse hematological neoplasms identified a commonly deleted segment at 11q23.1 containing the neural cell adhesion molecule (*NCAM*) gene, whereas *BCL1* at 11q13 and *MLL* at 11q23.3 were located outside this critical region.⁷³ For further delineation of the commonly deleted region we performed a molecular cytogenetic study applying FISH in 40 CLL cases,⁷⁴ using a YAC contig spanning bands 11q14.3-q23.3.⁷⁵ The minimally deleted region could be narrowed down to 2-3 Mb in bands 11q22.3-q23.1. This finding was later confirmed in an independent series.⁷⁶ Among the genes contained in this genomic fragment *RDX* (radixin) and *ATM* (ataxia telangiectasia mutated) appeared to be the most potential candidate tumor suppressor genes because of their function.^{77,78} Murine knock-out models gave evidence for a growth suppressor function of *ATM* as mice deficient for *ATM* developed T-neoplasms.⁷⁹ Furthermore, in

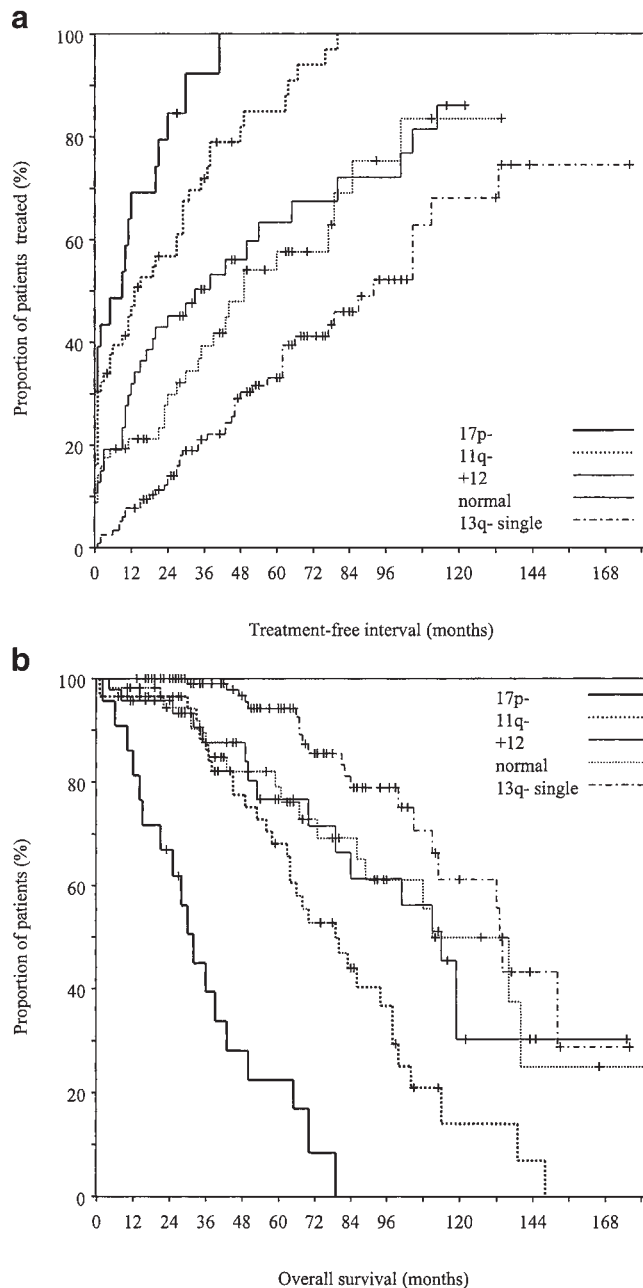


Figure 2 Impact of genomic aberrations on the clinical course of CLL. (a) Rate of disease progression as assessed by the treatment-free interval in CLL according to risk groups defined by genomic aberrations.⁴ The median treatment-free intervals were: 17p deletion, 9 months; 11q deletion, 13 months; 12q trisomy, 33 months; normal karyotype, 49 months; and 13q deletion as single abnormality, 92 months (from Ref. 4 with permission). (b) Survival probability in CLL according to risk groups defined by genomic aberrations.⁴ The estimated median survival times were: 17p deletion, 32 months; 11q deletion, 79 months; normal karyotype, 111 months; 12q trisomy, 114 months; and 13q deletion as single abnormality, 133 months (from Ref. 4 with permission).

human T cell prolymphocytic leukemia (T-PLL), *ATM* deletions and missense mutations leading to disruption of both alleles have been reported.^{80,81} Absent *ATM* protein expression in a subset of CLL cases and the description of *ATM* mutations in a small CLL subgroup made *ATM* a likely candidate tumor suppressor in this disease as well.⁸²⁻⁸⁵ It

was shown that *ATM* mutant CLL cases exhibited a deficient *ATM*-dependent response to gamma irradiation, failure to up-regulate TRAIL/R2 and inability to repair induced chromosomal breaks.⁸⁶ Interestingly, all *ATM* mutants showed absence of somatic V_H hypermutation (see also below) indicating that *ATM* may play a role at the pre-germinal center stage, where loss of *ATM* function during B cell maturation may lead to tumorigenesis in pre-germinal cells by a defect in *p53* damage response and repair of chromosomal breaks.⁸⁷ Bullrich *et al*⁸³ observed *ATM* mutations not only in neoplastic, but also in normal cells of CLL patients, suggesting the predisposition of heterozygous *ATM* mutation carriers to develop CLL. However, we were not able to confirm this finding. Furthermore, in our study *ATM* mutations were only found in five of 22 CLL cases with 11q deletion.⁸⁵ Therefore, and in contrast to MCL where all cases with loss of 11q show disruption of the remaining *ATM* allele,^{88–90} the search for additional candidate genes in 11q22–q23 in CLL is ongoing.

First evidence for a prognostic role of 11q aberrations in CLL came from two banding studies showing a correlation between 11q deletions and progressive disease with reduced survival times.^{71,72} In our large interphase FISH study, we were able to show that CLL patients with 11q abnormalities present with a characteristic clinical picture. Patients with 11q deletion exhibit extensive lymphadenopathy as assessed by the extent of peripheral lymph node involvement and the frequency of mediastinal or abdominal lymphadenopathy and have a more rapid disease progression as shown by a shorter treatment-free interval and reduced overall survival (see Figure 2a and b).⁹¹ In multivariate analysis with survival as a dependent variable the presence of 11q deletion gave significant prognostic information.⁴ Therefore, 11q aberrations identify a new subset of CLL with extensive lymphadenopathy, rapid disease progression and inferior survival. First results from the prospective CLL3 trial of the GCLLSG indicate that deletion 11q23 appears to be associated with an inferior molecular remission rate after high-dose therapy and autografting.⁴⁴ If this will also translate into inferior clinical outcome remains to be determined by a longer follow-up.

Trisomy 12q13

Trisomy 12 was reported as the first recurrent chromosome aberration in CLL and was the most common chromosome aberration in many chromosome banding studies.^{13,27–35} The frequency ranged from 7% to more than 25% according to the different investigations.⁹² On banding analysis, only few CLL cases exhibiting a partial trisomy 12 have been identified.^{16,34,93} Recurrent duplication of chromosome band 12q13–q21.2 was found in all cases, indicating that this region may contain the candidate oncogene(s) playing a pathogenic role in CLL. By restriction length polymorphism (RFLP) analyses, it was shown that trisomy 12 results from duplication of one homolog rather than from loss of one homolog and triplication of the remaining one.⁹⁴

Trisomy 12 was assessed by interphase FISH by numerous groups,^{16,68,95–102} determining the frequency of this aberration between 10% and 20% and in two studies from the US even more than 30%.^{96,98} This variation may be related to patient selection, and may be in some extent due to different geographical distribution of this chromosome aberration as well. However, in all studies directly comparing interphase FISH with conventional cytogenetic chromosome banding techniques, a higher frequency of trisomy 12 was found with the

molecular cytogenetic method. In our extended FISH analysis trisomy 12 was only the third most common chromosome aberration seen in 16% of 325 CLL cases.⁴ Regarding further evaluation of the minimally duplicated segment we were able to identify a CLL case with isolated overrepresentation of a fragment in 12q13–q14.²² Analyzing a complex chromosome 12 rearrangement by FISH, Merup *et al*¹⁰³ described a highly amplified region spanning bands 12q13–q15. Another FISH study identified bands 12q13–q22 as minimal duplication segment in B cell non-Hodgkin's lymphoma (NHL).¹⁰⁴ So far, no gene from this segment has been proven to be involved in CLL pathogenesis. Recently high resolution mapping of copy number changes using DNA-chip technology became available which may be helpful for narrowing the region of interest.^{105,106}

The clinical significance of trisomy 12 was shown in the First and Second IWCCLL, where patients with this aberration had the shortest survival times among patients with single chromosomal abnormalities.^{2,3} Some single center banding studies also showed an association with shorter treatment-free intervals and shorter overall survival.^{30,107} However, this adverse prognostic effect was only shown in univariate analysis and it was not confirmed by other single center chromosome banding analyses.^{28,29,34,35} Interphase cytogenetics showed an association between trisomy 12 and an increased percentage of atypical lymphocytes or prolymphocytes within the leukemic cell population, as well as an atypical immunophenotype.^{68,99,100,102} Evaluating the prognostic impact of trisomy 12 by FISH, no significant difference in survival probabilities between patients with or those without trisomy 12 was seen.⁹⁸ However, when conventional chromosome banding data were included in the analysis of this study the estimated median survival in patients with trisomy 12 was significantly shorter than that in patients with normal karyotype. Furthermore, patients exhibiting an aberration of chromosome 12 were more heavily pretreated and had advanced Binet stages. A sequential FISH analysis of trisomy 12 in CLL showed over a 4-year period similar requirement for treatment and similar overall survival for patients with and without FISH-defined trisomy 12.¹⁰⁸ A long-term follow-up of a FISH study that initially reported increased need of therapy and reduced survival in patients with trisomy 12 found no statistical significant difference in survival between patients with and without chromosome 12 aberration after a median observation time of 87 months.¹⁰⁹ In our interphase cytogenetic study the estimated median survival time for patients with trisomy 12 was 114 months compared to 111 months in patients with normal karyotype and 108 months for the entire group (see Figure 2a and b).⁴ On the other hand, preliminary results of the CLL1 trial of the GCLLSG show a significant correlation between trisomy 12 and the high risk group of the study (as determined by diffuse bone marrow infiltration pattern and/or lymphocyte doubling time below 12 months and elevated serum thymidine kinase level and/or elevated serum β_2 microglobulin level).⁴⁵ Whether this association with risk factors indicating rapid disease progression also results into an inferior outcome will be seen in the future.

17p13 deletions and *p53* mutations

Structural aberrations of chromosome 17, most commonly resulting from loss of the short arm, were only observed in 4% of CLL cases by the multicenter study of the Second IWCCLL.⁴ Recently, another large chromosome banding study confirmed

the incidence of 4% for chromosome 17 abnormalities in CLL as detected by conventional cytogenetics.⁶⁹ Evidence for more frequent disruption of chromosome 17 leading to the loss of the short arm came from smaller banding series.³⁴ However, the pathogenic role of 17p13 deletions, the location of the tumor suppressor gene *p53*, became more evident through molecular genetic studies investigating this candidate gene. Gaidano *et al*¹¹⁰ found *p53* mutations in six of 40 (15%) CLL cases by SSCP analysis and sequencing of the PCR amplified fragments. Subsequent studies also reported *p53* mutations at frequencies ranging from 10% to 15%.^{111–113} Based on these molecular genetic findings we applied FISH using a *p53* containing genomic probe to screen a large series of CLL patients for 17p13 deletions. In our initial series of 100 cases we found *p53* deletions at an incidence of 17%,¹¹⁴ whereas our extended series showed only an incidence of 7%.⁴ This difference is most likely due to patient selection as the initial study also included cases of B-PLL which showed a high frequency of 17p deletions. In line with the two-hit hypothesis we were able to demonstrate a disruption of the remaining *p53* allele by mutation in most cases leading to inactivation of this recessively acting tumor suppressor gene.¹¹⁵ Until recently, *p53* was the only gene shown to be involved in the pathogenesis of CLL, and it is interesting to note that *ATM* mutations which are found in a subset of CLL cases with 11q deletion can also result into dysfunction of the *p53* pathway.⁸⁷

A strong prognostic impact of *p53* abnormalities was demonstrated in the study by El-Rouby *et al*,¹¹² who showed that *p53* abnormalities predicted for treatment failure with alkylating agents. A recent banding analysis of 480 untreated CLL patients within a randomized trial of alkylator therapy found abnormalities involving 17p13 to be the only chromosomal aberration of prognostic significance.⁶⁹ Our initial interphase cytogenetic study on 100 CLL cases identified 17 patients exhibiting a 17p deletion whose clinical course was characterized by significantly shorter survival times compared to patients without this aberration.¹¹⁴ In addition, patients with 17p deletion showed no response to therapy with purine analogs. Whereas 56% of patients without *p53* disruption responded to treatment with fludarabine, none of the patients with a *p53* deletion did. In multivariate analysis *p53* deletion was revealed as the strongest prognostic factor (see Figure 2a and b).⁴ A recent study on 122 CLL patients also showed that *p53* abnormalities are more common in refractory advanced disease and that *p53* aberrations are associated with treatment resistance and shorter survival.¹¹⁶ There is anecdotal evidence that a response may be achieved in CLL with *p53* inactivation with the monoclonal anti-CD52 antibody Campath-1H.¹¹⁷

6q21 deletions

Aberrations of the long arm of chromosome 6 are among the most common chromosomal abnormalities in lymphoid neoplasms.¹¹⁸ The Second IWCCLL found structural aberrations of chromosome 6 in 6% of the evaluable tumors and described chromosome bands 6q15 and 6q23 as most commonly involved.³ Based on molecular genetic analysis of several subtypes of malignant lymphomas, at least two independent regions of commonly deleted segments, one at 6q21–q23 and one at 6q25–q27, have been identified.¹¹⁹ In small lymphocytic lymphoma (SLL), the lymphomatous counterpart of CLL, especially deletions of 6q21–q23 were identified.¹²⁰ Recently, in CLL the proximal location of the minimally deleted region was confirmed by Merup *et al*,¹²¹ who identified a critical

deletion region spanning markers D6S283 to D6S270 on chromosome band 6q21 in 6% of CLL cases. Our investigations with FISH using DNA probes mapping on 6q21 and 6q27 confirmed these findings showing that all deletions can be detected with the 6q21 probe, whereas only one-third of the patients also showed 6q27 deletions.¹²² Zhang *et al*¹²³ determined a 4–5 Mb minimal deletion region in band 6q21 in a variety of lymphomas and lymphoid malignancies. Several genes in this region have been identified, but none of these has so far been shown to play a role in the pathogenesis of CLL.

Patients with deletion of the long arm of chromosome 6 were shown to have shorter treatment-free intervals in one single center study.³⁵ In patients with follicular NHL deletion 6q has also been identified as the negative prognostic factor.¹²⁴ On the other hand, the two large multicenter studies of the IWCCLL did not observe any adverse prognostic impact of 6q deletions.^{2,3} In our interphase cytogenetic study 6q deletion was associated with a higher tumor mass as represented by higher white blood cell counts and more extensive lymphadenopathy, but the median treatment-free intervals and overall survival times were not significantly different between the groups with or without 6q deletions.¹²²

14q32 (*IgH*) translocations

The Second IWCCLL reported aberrations of chromosome 14 clustering in band 14q32, the locus of the immunoglobulin heavy chain (*IgH*) gene, in 8% of evaluable cases.³

t(11;14)(q13;q32): The translocation *t(11;14)(q13;q32)* is leading to the fusion of the *BCL1* locus at 11q13 to *IgH* at 14q32 resulting in overexpression of cyclin D1 (*CCND1*).^{125–130} However, this translocation today is considered a hallmark of MCL and may occur at low frequencies in other lymphoproliferative disorders distinct from CLL.^{43,125–127} Although early molecular studies suggested a pathogenic role of *CCND1* in CLL,^{128–130} a re-evaluation classified the examined cases as MCL.⁴³ Recent analyses showed no evidence for a frequent involvement of *CCND1* in CLL.^{4,113,131–134}

t(14;18)(q32;q21): Translocations involving *BCL2* at 18q21 were also suggested to play a pathogenic role in CLL.^{135,136} The *t(14;18)(q32;q21)* is characteristic of follicular NHL, but overexpression of *BCL2* is also observed in other lymphoid malignancies, such as CLL. In follicular lymphoma breakpoints of *t(14;18)* occur in the major breakpoint region (mbr) or in the minor cluster region (mcr) at the 3' end of *BCL2*, whereas in CLL the breakpoints have been found to be at the 5' end juxtaposing *BCL2* to the immunoglobulin light chains. However, large series showed *BCL2* rearrangements to be rare events in CLL.^{4,113,133,137–139}

t(14;19)(q32;q13): From the 19q13 breakpoint of this translocation the *BCL3* gene was cloned.^{140–144} Rearrangement involving the *BCL3* gene locus at 19q13 were found in six out of 4487 cytogenetically analyzed lymphoproliferative disorders with five of the six cases classified as CLL.^{4,140}

In the light of large studies of well classified tumors the previously described '14q+ marker' does not appear to be a fre-

quent aberration of CLL. Rearrangements of the *IgH* locus with oncogenes appear to be rare events in CLL.¹⁴⁵

Infrequent genomic aberrations

Other prominent cancer genes such as *p16* (*CDKN2A*) located in chromosome band 9p21, frequently involved in other types of neoplasms,^{146–148} do not appear to have a role in the pathogenesis of CLL.¹⁴⁹ Chromosome banding analyses and CGH studies also reported other recurrently involved genomic regions in CLL. These abnormalities are commonly gains of chromosomal regions, such as trisomy 3.^{27,29,32} CGH data suggested that the minimally duplicated segment comprised the distal region of the long arm, which could be a locus for an oncogene of possible pathogenic significance in CLL.²² CGH data also revealed another region of interest, gains of the long arm of chromosome 8.²² Automated genomic profiling using microarray based hybridization (matrix-CGH) may be a new powerful technique for detection of genomic aberrations in lymphoid malignancies.¹⁵⁰ Preliminary analyses in CLL identified additional genomic gains in bands 2q22, 7q31, and 11q25.¹⁵¹

Pathogenic and clinical implications of V_H gene mutations

Characterization of V_H gene mutations in CLL

Based on the expression of CD5, CLL cells appeared to correspond phenotypically to mantle-zone-derived, naive B cells. Early studies confirmed the expectation that no somatic mutation would be found in the V_H genes of CLL cells.^{152–154} However, an extensive review of Schroeder and Dighiero⁶ showed that approximately half of 75 reported CLL immunoglobulin V_H gene sequences varied by more than 2% in sequence similarity as compared to the nearest germline gene, consistent with somatic hypermutation. In a subsequent study, Fais *et al*⁷ found that approximately 50% of IgM⁺ CLL cases and approximately 75% of IgG⁺ and IgA⁺ cases showed significant V_H gene mutations. The degree of V_H gene mutation in these CLL cells was considerable, since more than a third of the IgM⁺ CLL cells and more than two-thirds of the isotype-switched CLL tumors showed 5% differences from their most similar germline genes. Based on these data, CLL can be segregated in two subgroups on the basis of the presence or absence of significant numbers of V_H gene mutations.^{7,8} The discrepancy between initial studies of V_H mutations in CLL and the recent results may be due to the observation that V_H gene mutations are distributed nonstochastically and appear to relate to the V_H family gene expressed in the CLL cell.⁷ A V_H family-related hierarchy of mutation ($V_{H3} > V_{H4} > V_{H1}$), which was most obvious when comparing the V_{H3} –07 (90% of cases mutated), V_{H4} 4–34 (73%), and V_{H1} 1–69 (17%) genes, was reported by Fais *et al*.⁷ Therefore, small patient cohorts with nonrandom V_H gene family expression may demonstrate different levels of somatic mutation. The reason for the V_H family-related hierarchy of mutation in CLL is still unclear, but might reflect differences in the types of antigens that have driven the individual B cells before their leukemic transformation and/or differences in the maturation stages at which they were transformed into leukemic cells.^{7–9,155} In addition to the overrepresentation of V_{H3} and V_{H4} family gene members in V_H mutated cases and overrepresentation of V_{H1} family genes in the V_H unmutated group, there is also a difference in J_H

gene usage and in the complementary determining region (*CDR3*) structure reflected by *CDR3* length and D segment sequence conservation rates between the two subgroups of CLL.^{7,156,157} However, to date the biological significance of these findings is still unknown.

B cell tumors have recently been classified in three categories: (1) those with unmutated V_H genes, in which it was postulated that the cell of origin had not entered the germinal center; (2) tumors with ongoing V_H gene mutations, such as follicle center lymphoma, in which it was postulated that the malignant cells remain under the influence of the germinal center reaction; and (3) B cell tumors with mutated stable V_H genes, such as multiple myeloma, which were postulated to have irreversibly traversed the germinal center.¹⁵⁸ Therefore, the finding that CLL can either exhibit V_H gene sequences in germline configuration, or with somatically mutated V_H genes, as independently reported in several studies,^{7–9,159} illustrates a novel scenario with a single tumor entity being composed of different subtypes, with regard to the germinal center reaction of V_H gene hypermutation. In addition and in contrast to FCL, the mutational pattern in CLL appears stable and does not show any intraclonal heterogeneity.^{7–9,158–160}

Correlation of V_H gene mutational status with *BCL6* mutations

Somatic hypermutation can also be observed in non-immunoglobulin genes as evidenced in the 5'-intronic region of the *BCL6* gene.¹⁶¹ *BCL6* is a proto-oncogene mapping to chromosome 3q27,¹⁶² which encodes a transcription protein with a POZ/Zinc finger motif.¹⁶³ In NHL, a high frequency of chromosomal translocations cluster at band 3q27, specifically at the *BCL6* promoter, which might lead to deregulated *BCL6* function with a potential role in lymphomagenesis.^{164–166} However, mutation events at this locus also occur in the absence of translocations.¹⁶¹ In both, the V_H and *BCL6* loci, somatic mutation is only found in memory and not in naive cells in normal B cells.^{167–169} At the single cell level, *BCL6* mutations tended to occur in normal B cells containing V_H gene mutations.¹⁶⁷ In addition, one study reported that the occurrence of *BCL6* mutations in eight out of 34 CLL cases was restricted to V_H mutated cases, supporting the hypothesis that *BCL6* mutations result from the same process that targets immunoglobulin genes.¹⁷⁰ However, in the initial study, 80% to 100% of the cells displayed V_H gene mutations, whereas only approximately 30% of these cells had corresponding mutations in *BCL6*, indicating different targeting of the two loci.¹⁶⁷ In CLL two studies were able to identify somatic mutation not only in the cases with mutated V_H genes, but also in cases with no V_H mutations.^{171,172} One further unexpected feature was intraclonal variation in *BCL6* evident in six out of eight mutated cases.¹⁷² As in CLL intraclonal heterogeneity in V_H is very rare, it was not found in the V_H genes of the cases with heterogeneity in *BCL6*, suggesting that the mutation mechanism may operate differently on the two loci. Recently, ongoing mutations in CLL were also described in the *CD79* genes, regardless of the mutational status of the corresponding V_H gene.¹⁷³ The pathogenic role of mutations in the non-coding region of *BCL6* is so far unknown and in contrast to the V_H status *BCL6* mutations do not apparently correlate with prognosis.¹⁷²

Correlation of V_H gene mutational status with CD38 expression

It still remains unclear whether CLL cells with unmutated V_H genes are naive B cells or represent B cells that have been antigen-stimulated, but have not accumulated mutations. To more accurately define the stage of maturation at which the CLL cells are arrested, Damle *et al*⁸ studied the surface membrane expression of CD38 and IgD on CD5⁺/CD19⁺ B cells in a series of well-characterized cases. Analyses of these surface markers have been especially useful in distinguishing B cells at various stages of differentiation from naive through memory cells.¹⁷⁴ Damle *et al*⁸ found a heterogeneous CD38 expression in CLL with some cases showing an expression of CD38 on almost 100% of the malignant clone, whereas in other cases only few, if any leukemic cells expressed CD38. Based on the percentage of clonal cells expressing CD38, CLL could be subdivided in two categories: one with <30% CD38⁺ CLL cells and another with ≥30% CD38⁺ CLL cells.

The analysis of Damle *et al*⁸ showed that the set with a higher percentages of CD38⁺ cells (≥30% CD38⁺ CLL cells) was comprised of unmutated CLL cases, whereas the set with lower percentages (<30% CD38⁺ CLL cells) contained almost exclusively the mutated cases indicating a strong inverse relationship between V_H gene mutation and CD38 expression. CD38 expression was also reported to be stable over time and it was not influenced by chemotherapy in this study. However, other studies failed to find this strong correlation between unmutated V_H genes and high CD38 expression, which is therefore currently a matter of discussion.^{175–177} In agreement with the above-mentioned studies, we also observed an inverse correlation between V_H mutational status and CD38 expression, but in approximately one-third of cases CD38 expression failed to predict the V_H mutational status.²⁶ The variation observed among different studies might be due to methodological aspects, but with the current knowledge CD38 expression appears not to serve as a valid surrogate marker for the V_H gene mutational status in CLL.

Correlation of V_H gene mutational status with genomic aberrations

With the V_H gene mutation status and genomic aberrations, there are two genetic parameters among the most powerful risk factors in CLL, but the relation of these two has only recently been evaluated (Table 2).^{26,178} The overall incidence of aberrations was not different between the V_H mutated and unmutated subgroups in our study (80% vs 84%). However,

Table 2 Correlation of V_H mutation status with genomic aberrations in 300 CLL cases

Aberration	V_H mutated (homology <98%) n = 132 (44%)	V_H unmutated (homology ≥98%) n = 168 (56%)	P value ^a
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

^aFisher's exact test.

there was a significant difference in the incidence of high risk aberrations deletion 17p (3% vs 10%) and deletion 11q (4% vs 27%), as well as a difference in the incidence of favorable aberrations such as deletion 13q (65% vs 48%) and del(13q14) as single aberration (50% vs 26%).^{26,178} In a smaller interphase cytogenetic study, our finding that genomic aberrations are seen in the V_H mutated, as well as in the unmutated subgroup were confirmed, but no association between specific aberrations and the V_H gene mutational status was observed possibly due to the small patient number.¹⁷⁹ The difference compared with the results of Oscier *et al*¹⁵⁹ and Hamblin *et al*⁸ who observed more cases exhibiting trisomy 12 in the V_H unmutated subgroup is most likely attributable to different technology used. Chromosome banding was performed in the Oscier/Hamblin study, while we applied FISH, a much more sensitive technique to detect genomic aberrations in CLL. This is underlined by the observation that by banding generally a lower incidence of aberrations is detectable in particular in V_H mutated CLL possibly due to the generally lower *in vitro* proliferative potential of the V_H mutated CLL cells.^{9,159}

Clinical impact of V_H gene mutation status, CD38 expression and genomic aberrations

The pioneer studies by Damle *et al*⁸ and Hamblin *et al*⁸ reported a significant correlation of the V_H gene mutational status with disease course and survival in CLL. Collectively, the two studies reported results on 131 CLL patients showing that cases with a V_H gene homology ≥98% (ie unmutated V_H) experienced a more aggressive clinical course with shorter survival times compared to cases with homology <98% (ie mutated V_H). The estimated median survival time in the study by Damle *et al*⁸ was in the unmutated group 108 months and not reached in the mutated group, whereas Hamblin *et al*⁸ reported 117 months in unmutated and 293 months in unmutated patients, respectively. The latter study also showed this correlation in Binet A patients with an estimated median survival time of 95 months for patients without mutation and 293 months for those with mutations. Subsequent studies confirmed the prognostic impact of the V_H gene mutational status on survival.^{176,180}

Analogous to the pivotal studies, our patients with mutated V_H genes had significantly higher survival probabilities compared to the patients with unmutated V_H genes.^{26,178} Interestingly, in our series the estimated V_H homology rate yielding the best separation of two subgroups with different survival probabilities was not the classical cut-off value of 98% but 97% V_H homology to the nearest germline gene (Figure 3a and b). This finding was derived from careful statistical analysis of survival time in relation to V_H homology, instead of applying the classical 98% cut-off value for the definition of mutated V_H derived from germline polymorphism considerations.²⁶ Further studies are needed to determine the biological significance of this finding. Furthermore, first data examining the influence of the V_H mutation status on outcome after specific treatment modalities are becoming available. The group of Dreger *et al* has recently demonstrated that the adverse prognostic influence of unmutated V_H may be retained even after aggressive treatment, such as high-dose therapy and autologous stem cell transplantation.¹⁸¹

Damle *et al*⁸ initially demonstrated the CD38 expression level as significant prognostic marker in CLL. The estimated median survival time for patients with ≥30% CD38⁺ CLL cells

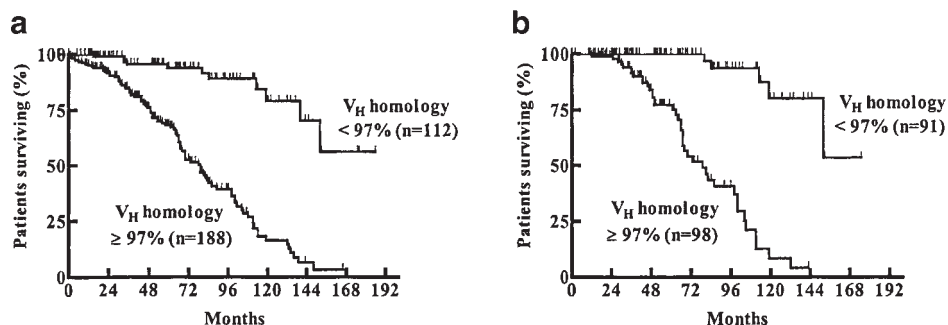


Figure 3 Probability of survival from the date of diagnosis among patients with mutated and unmutated V_H genes according to the 97% and the 98% cut-off values. (a) The estimated median survival time for the V_H homology $\geq 97\%$ group was 79 months. The last observed death in the V_H homology $< 97\%$ group was after 152 months 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 months vs not reached (last observed death after 152 months of follow-up time; survival probability 53%).

was 120 months in this study, as compared to the group of patients with lower CD38 expression level, in which the estimated median survival time was not yet reached. Subsequent studies confirmed the prognostic value of the CD38 expression level.^{177,182,183} However, our investigations in 157 CLL cases and a study by Thunberg *et al*, failed to show a significant difference in survival probability when using the 30% cut-off for CD38 positivity.^{26,176} By maximally selected log rank statistics, the best separation of two subgroups with different survival probability was achieved for a cut-off value of 7% CD38-positive CLL cells in our study. The estimated median survival times for the group with $< 7\%$ CD38⁺ clonal cells was 79 months, and 114 months for the group with $< 7\%$ CD38⁺ clonal cells.^{26,178}

For the evaluation of the relative prognostic impact of the V_H mutation status, CD38 expression level, genomic aberrations, clinical, and laboratory parameters we performed a multivariate analysis.^{26,178} Unmutated V_H , 17p deletion, age, white blood cell count, and serum lactate dehydrogenase were identified as significant prognostic factors at the 97% V_H homology cut-off, while at the 98% cut-off 11q deletion entered the model as additional independent prognostic factor. The hazard ratios together with their 95% confidence intervals are shown in Table 3. Of note is the fact that clinical staging according to Binet provided a significant separation of

subgroups with respect to their survival time distributions, but was not an independent prognostic factor in the knowledge of V_H mutation and 17p deletion status. This observation is illustrated in Figure 4 showing the survival probabilities in the dominant genetic subgroups for all (a), and for Binet-A patients (b). In conclusion, it appears that with the V_H mutation status and genomic aberrations, parameters became available which may allow a risk assessment of CLL patients at the time of diagnosis independently of the stage of their disease.

Perspectives

Over the past decade, rapid progress has been made in the genetic analysis of CLL. With the aid of interphase cytogenetics recurrent genomic aberrations are observed in more than 80% of cases and with *ATM* and *p53* genes involved in disease pathogenesis and progression were identified. However, for the most frequent genomic abnormalities candidate genes still have to be isolated. The discovery of the two CLL subtypes as defined by the mutation status of the V_H genes has further highlighted the molecular heterogeneity of the disease. Novel tools such as DNA microarrays already had strong impact on the further clarification of the molecular background of CLL.^{151,184–186} In particular, these studies have shown that both the V_H mutated and the unmutated type of CLL show gene expression patterns similar to memory B-cells and have therefore shed doubt on the theory that CLL with unmutated V_H genes is derived from naïve pre-germinal center lymphocytes. Furthermore, genetic parameters have shown their prognostic value in the identification of CLL patients who are at risk for rapid disease progression, resistance to therapy and short survival. Particularly, unmutated V_H genes, 11q and 17p deletions were among the strongest independent risk factors and appear to identify patients at risk for poor outcome irrespectively of their clinical stage. Therefore, at the time of diagnosis a “state of the art risk assessment” should today ideally include a comprehensive genomic screening, an evaluation of the V_H mutation status and CD38 expression level, in addition to other well known clinical and biological parameters. Further evaluation of these prognostic markers within prospective treatment trials is needed to give us the opportunity for a more refined disease management in the future, especially with regard to the availability of highly effective treatment approaches such as purine analogs, antibodies and autologous or allogeneic stem cell transplantation.

Table 3 Cox regression analysis of survival time from diagnosis in 300 cases of CLL²⁶

Variable	Hazard ratio for death (95% CI) at V_H homology cut-off value	
	97%	98%
V_H unmutated	5.27 (2.67–10.40)	2.91 (1.71–4.95)
17p deletion	5.53 (2.98–10.26)	8.19 (4.29–15.65)
11q deletion	—	1.78 (1.07–2.96)
Age	1.49 (1.18–1.87)	1.68 (1.33–2.13)
LDH	1.26 (1.10–1.44)	1.2 (1.05–1.38)
WBC	1.11 (1.06–1.17)	1.11 (1.05–1.16)

Hazard ratios and confidence intervals (CIs) are computed for 10-year increments in age; 50 IU per liter increments in lactate dehydrogenase; and for 20 000 per cubic millimeter increments in the white cell count. The estimated shrinkage factor for the effect of unmutated V_H was 0.956 (V_H homology $\geq 97\%$) and 0.935 (V_H homology $\geq 98\%$) yielding a corrected estimated hazard ratio of 4.90 (V_H homology $\geq 97\%$) and 2.71 (V_H homology $\geq 98\%$).

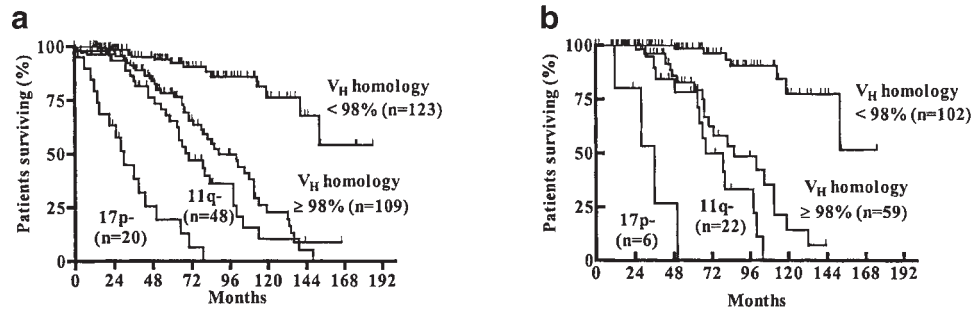


Figure 4 Probability of survival among patients in the following genetic categories: 17p⁻ (17p deletion irrespective of V_H mutation status), 11q⁻ (11q deletion irrespective of V_H mutation status), unmutated V_H (V_H homology $\geq 98\%$ and no 17p or 11q deletion), and mutated V_H (V_H homology $< 98\%$ and no 17p or 11q deletion). (a) Among the entire cohort ($n = 300$), the estimated median survival times for the respective genetic subgroups were: 17p deletion, 30 months; 11q deletion, 70 months; V_H unmutated, 89 months; and V_H mutated; not reached (54% survival at 152 months). (b) Among Binet A patients ($n = 189$) the estimated median survival times for the respective genetic subgroups were: 17p deletion, 36 months; 11q deletion, 68 months; V_H unmutated, 86 months; and V_H mutated, not reached (52% survival at 152 months).

Acknowledgements

This work was supported by the Wilhelm Sander-Stiftung (No. 2001.004.1), University of Ulm (No. P.679), Deutsche Krebshilfe (No. 10-1289-Stl), and BMBF (Nos. 01KW9934, 01KW9938).

Editor's note

We are very indebted to Dr Peter Daniel who recruited and evaluated all the Reviews published in this Spotlight. Authors who are interested in contributing a Review for this Spotlight are invited to contact the Editor-in-Chief, Dr Muller Bérat.

References

- Mitelman F, Levan G. Clustering of aberrations to specific chromosomes in human neoplasms. *Hereditas* 1978; **89**: 207–232.
- Juliussen G, Oscier DG, Fitchett M, Ross FM, Stockdill G, Mackie MJ, Parker AC, Castoldi GL, Cuneo A, Knuutila S, Elonen E, Gahrton G. Prognostic subgroups in B-cell chronic lymphocytic leukemia defined by specific chromosomal abnormalities. *N Engl J Med* 1990; **323**: 720–724.
- Juliussen 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.
- Döhner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, Döhner K, Bentz M, Lichter P. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med* 2000; **343**: 1910–1916.
- Küppers R, Klein U, Hansmann ML, Rajewsky K. Cellular origin of human B-cell lymphomas. *N Engl J Med* 1999; **341**: 1520–1529.
- Schroeder HW, Dighiero G. The pathogenesis of chronic lymphocytic leukemia: analysis of the antibody repertoire. *Immunol Today* 1994; **15**: 288–294.
- Fais F, Ghiotto F, Hashimoto S, Sellars B, Valetto A, Allen SL, Schulman P, Vinciguerra VP, Rai K, Rassenti LZ, Kipps TJ, Dighiero G, Schroeder HW Jr, Ferrarini M, Chiorazzi N. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. *J Clin Invest* 1998; **102**: 1515–1525.
- Damle JN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, Buchbinder A, Budman D, Dittmar K, Kolitz J, Lichtman SM, Schulman P, Vinciguerra VP, Rai KR, Ferrarini M, Chiorazzi N. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood* 1999; **94**: 1840–1847.
- Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V_H genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood* 1999; **94**: 1848–1854.
- 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.
- 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.
- 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.
- Gahrton G, Robèrt KH, Friberg K, Zech L, Bird AG. Extra chromosome 12 in chronic lymphocytic leukaemia. *Lancet* 1980; **1**: 146–147.
- 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.
- Oscier DG. Cytogenetic and molecular abnormalities in chronic lymphocytic leukaemia. *Blood Rev* 1994; **8**: 88–97.
- 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.
- Crawford DH, Catovsky D. *In vitro* activation of leukaemia B cells by interleukin-4 and antibodies to CD40. *Immunology* 1993; **80**: 40–44.
- 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.
- Kallioniemi A, Kallioniemi O-P, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* 1992; **258**: 818–821.
- Du Manoir S, Speicher MR, Joos S, Schröck E, Popp S, Döhner H, Kovacs G, Robert-Nicoud M, Lichter P, Cremer T. Detection of complete and partial chromosome gains and losses by comparative genomic *in situ* hybridization. *Hum Genet* 1993; **90**: 590–610.
- Joos S, Scherthan H, Speicher MR, Schlegel J, Cremer T, Lichter P. Detection of amplified genomic sequences by reverse chromosome painting using genomic tumor DNA as probe. *Hum Genet* 1993; **90**: 584–589.
- Bentz M, Huck K, du Manoir S, Joos S, Werner CA, Fischer K, Döhner H, Lichter P. Comparative genomic hybridization in chronic B-cell leukemias reveals a high incidence of chromosomal gains and losses. *Blood* 1995; **85**: 3610–3618.
- Lichter P, Ward DC. Is non-isotopic *in situ* hybridization finally coming of age? *Nature* 1990; **345**: 93–95.
- Cremer T, Landegent J, Brückner A, Scholl HP, Schardin M,

- Hager HD, Devilee P, Pearson PP, van der Ploeg M. 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.
- 25 Döhner H, Stilgenbauer S, Döhner K, Bentz M, Lichter P. Chromosome aberrations in B-cell chronic lymphocytic leukemia: reassessment based on molecular cytogenetic analysis. *J Mol Med* 1999; **77**: 266–281.
- 26 Kröber A, Seiler T, Benner A, Bullinger L, Brückle E, Lichter P, Döhner H, Stilgenbauer S. V_H mutation status, CD38 expression level, genomic aberrations, and survival in chronic lymphocytic leukemia. *Blood* (in press).
- 27 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.
- 28 Han T, Ozer H, Sadamori N, Emrich L, Gomez GA, Henderson ES, Bloom JL, Sandberg AA. Prognostic importance of cytogenetic abnormalities in patients with chronic lymphocytic leukemia. *N Engl J Med* 1984; **310**: 288–292.
- 29 Pittman S, Catovsky D. Prognostic significance of chromosome abnormalities in chronic lymphocytic leukaemia. *Br J Haematol* 1984; **58**: 649–660.
- 30 Juliusson G, Robèrt KH, Öst A, Friberg K, Biberfeld P, Nilsson B, Zech L, Gahrton G. Prognostic information from cytogenetic analysis in chronic B-lymphocytic leukemia and leukemic immunocytoma. *Blood* 1985; **65**: 134–141.
- 31 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.
- 32 Ross FM, 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.
- 33 Han T, Sadamori N, Block AMW, Xiao H, Henderson ES, Emrich L, Sandberg AA. Cytogenetic studies in chronic lymphocytic leukemia, prolymphocytic leukemia and hairy cell leukemia: a progress report. *Nouv Rev Fr Hematol* 1988; **30**: 393–395.
- 34 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.
- 35 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.
- 36 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.
- 37 Zech L, Mellstedt H. Chromosome 13 – a new marker for B-cell chronic lymphocytic leukemia. *Hereditas* 1988; **108**: 77–84.
- 38 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 Chromos Cancer* 1992; **4**: 273–280.
- 39 Callen DF, Ford JH. Chromosome abnormalities in chronic lymphocytic leukemia revealed by TPA as a mitogen. *Cancer Genet Cytogenet* 1983; **10**: 87–93.
- 40 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.
- 41 Bloomfield C, Arthur D, Frizzera G, Levine E, Peterson B, Gajl-Peczalska K. Nonrandom chromosome abnormalities in lymphoma. *Cancer Res* 1983; **43**: 2975–2984.
- 42 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.
- 43 Raffeld M, Jaffe ES. bcl-1, t(11;14), and mantle cell-derived lymphomas. *Blood* 1991; **78**: 259–263.
- 44 Stilgenbauer S, Ritgen M, Bullinger L, Kröber A, Lichter P, Dreger P, Döhner H. Genomic aberrations in the CLL3 trial of the German CLL Study Group (GCLLSG): deletion 11q23 identifies patients with molecular disease persistence after autologous high dose therapy. *Blood* 2001; **98** (Suppl. 1): Abstr. 3180.
- 45 Bullinger L, Kräutle C, Busch R, Kröber A, Emmerich B, Hallek M, Lichter P, Stilgenbauer S, Döhner H. Incidence and correlation of genomic aberrations with clinical and biological risk factors in B-CLL stage Binet-A within the CLL1 trial of the GCLLSG. *Blood* 2001; **98** (Suppl. 1): Abstr. 1512.
- 46 Oscier D, Fitchett M, Herbert T, Labert R. Karyotypic evolution in B-cell chronic lymphocytic leukemia. *Genes Chromos Cancer* 1991; **3**: 16–20.
- 47 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.
- 48 Finn WG, Kay NE, Kroft SH, Church S, Peterson LC. Secondary abnormalities of chromosome 6q in B-cell chronic lymphocytic leukemia: a sequential study of karyotypic instability in 51 patients. *Am J Hematol* 1998; **59**: 223–229.
- 49 Leupolt E, Stilgenbauer S, Kröber A, Bullinger L, Lichter P, Döhner H. Clonal evolution in B-CLL: acquisition of deletions involving 6q21, 11q22 and 17p13 (*TP53*) associated with disease progression. *Blood* 2001; **98** (Suppl. 1): Abstr. 1968.
- 50 Liu Y, Szekeley L, Grandér D, Söderhäll S, Juliusson G, Gahrton G, Linder S, Einhorn S. 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.
- 51 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.
- 52 Liu Y, Grandér D, Söderhäll S, Juliusson G, Gahrton G, Einhorn S. Retinoblastoma gene deletions in B-cell chronic lymphocytic leukemia. *Genes Chromos Cancer* 1992; **4**: 250–256.
- 53 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.
- 54 Döhner H, Pilz T, Fischer K, Cabot G, Diehl D, Fink T, Stilgenbauer S, Bentz M, Lichter P. Molecular cytogenetic analysis of Rb-1 deletions in chronic B-cell leukemias. *Leuk Lymphoma* 1994; **16**: 97–103.
- 55 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.
- 56 Devilder MC, François S, Boscic C, Moreau A, Mellerin MP, Le Paslier D, Bataille R, Moisan JP. Deletion cartography around the D13S25 Locus in B cell chronic lymphocytic leukemia. *Cancer Res* 1995; **55**: 1355–1357.
- 57 Stilgenbauer S, Leupolt E, Ohl S, Weiß G, Schröder M, Fischer K, Bentz M, Lichter P, Döhner H. 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.
- 58 Liu Y, Hermanson M, Grandér D, Merup M, Wu X, Heyman M, Rasool O, Juliusson G, Gahrton G, Detlofsson R, Nikiforova N, Buys C, Söderhäll S, Yankovsky N, Zabarovsky E, Einhorn S. 13q deletions in lymphoid malignancies. *Blood* 1995; **86**: 1911–1915.
- 59 Bullrich F, Veronese ML, Kitada S, Jurlander J, Caligiuri MA, Reed JC, Croce CM. Minimal region of loss at 13q14 in B-cell chronic lymphocytic leukemia. *Blood* 1996; **88**: 3109–3115.
- 60 Kalachikov S, Migliazza A, Cayanis E, Fracchiolla NS, Bonaldo MF, Lawton L, Jelenc P, Ye X, Qu X, Chien M, Hauptschein R, Gaidano G, Vitolo U, Saglio G, Resegotti L, Brodjansky V, Yankovsky N, Zhang P, Soares MB, Russo J, Edelman IS, Efstratiadis A, Dalla-Favera R, Fischer SG. Cloning and gene mapping of the chromosome 13q14 region deleted in chronic lymphocytic leukemia. *Genomics* 1997; **42**: 369–377.
- 61 Liu Y, Corcoran M, Rasool O, Ivanova G, Ibbotson R, Grandér D, Iyengar A, Baranova A, Kashuba V, Merup M, Wu X, Gardiner A, Mullenbach R, Poltarau A, Hultström AL, Juliusson G, Chapman R, Tiller M, Cotter F, Gahrton G, Yankovsky N, Zabarovsky

- E, Einhorn S, Oscier D. 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.
- 62 Bouyge-Moreau I, Rondeau G, Avet-Loiseau H, André MT, Béziau S, Chérel M, Saleün S, Cadoret E, Shaikh T, De Angelis MM, Arcot S, Batzer M, Moisan JP, Devilder MC. 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.
- 63 Corcoran MM, Rasool O, Liu Y, Iyengar A, Grandt D, Ibbotson RE, Merup M, Wu X, Brodyansky V, Gardiner AC, Juliusson G, Chapman RM, Ivanova G, Tiller M, Gahrton G, Yankovsky N, Zabarovsky E, Oscier DG, Einhorn S. Detailed molecular delineation of 13q14.3 loss in B-cell chronic lymphocytic leukemia. *Blood* 1998; **91**: 1382–1390.
- 64 Stilgenbauer S, Nickolenko J, Wilhelm J, Wolf S, Weitz S, Döhner K, Böhm T, Döhner H, Lichter P. 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.
- 65 Rondeau G, Moreau I, Bezieau S, Cadoret E, Moisan JP, Devilder MC. Exclusion of Leu1 and Leu2 genes as tumor suppressor genes in 13q14.3-deleted B-CLL. *Leukemia* 1999; **10**: 1630–1632.
- 66 Mertens D, Wolf S, Bullinger L, Ohl S, Schaffner C, Döhner H, Stilgenbauer S, Lichter P. The candidate gene for B-cell chronic lymphocytic leukemia localized at 13q14.3, *BCMSUN*, has an independently expressed homolog on 1p22-p31, *BCMSUN-like*. *Int J Cancer* 2000; **88**: 692–697.
- 67 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.
- 68 Matutes E, Oscier D, Garcia-Marco J, Ellis J, Copplestone A, Gillingham R, Hamblin T, Lens D, Swansbury GJ, Catovsky D. 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.
- 69 Geisler CH, Philip P, Egelund Christensen B, Hou-Jensen K, Tinggaard Pedersen N, Myhre Jensen O, Thorling K, Andersen E, Birgens HS, Drivsholm A, Ellegard J, Larsen JK, Plesner T, Brown P, Kragh Andersen P, Mørk Hansen M. 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.
- 70 Hernandez JM, Mecucci C, Criel A, Meeus P, Michaux L, van Hoof A, Verhoef G, Louwagie A, Scheiff JM, Michaux JL, Boogaerts M, van den Berghe H. Cytogenetic analysis of B cell chronic lymphoid leukemias classified according to morphologic and immunophenotypic (FAB) criteria. *Leukemia* 1995; **9**: 2140–2146.
- 71 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.
- 72 Neilson JR, Auer R, White D, Bienz N, Waters JJ, Whittaker JA, Milligan DW, Fegan CD. Deletions at 11q identify a subset of patients with typical CLL who show consistent disease progression and reduced survival. *Leukemia* 1997; **11**: 1929–1932.
- 73 Kobayashi H, Espinosa R III, Fernald AA, Begy C, Diaz MO, Le Beau MM, Rowley JD. Analysis of deletions of the long arm of chromosome 11 in hematologic malignancies with fluorescence *in situ* hybridization. *Genes Chromos Cancer* 1993; **8**: 246–252.
- 74 Stilgenbauer S, Liebisch P, James MR, Schröder M, Schlegelberger B, Fischer K, Bentz M, Lichter P, Döhner H. 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**: 11837–11841.
- 75 James MR, Richard III CW, Schott JJ, Yousry C, Clark K, Bell J, Terwilliger JD, Hazan J, Dubay C, Vignal A, Agrapart M, Imai T, Nakamura Y, Polymeropoulos M, Weissenbach J, Cox DR, Lathrop GM. A radiation hybrid map of 506 STS markers spanning human chromosome 11. *Nat Genet* 1994; **6**: 70–76.
- 76 Zhu Y, Monni O, El-Rifai W, Siitonen SM, Vilpo L, Vilpo J, Knuutila S (1999) Discontinuous deletions at 11q23 in B cell chronic lymphocytic leukemia. *Leukemia* 1999; **13**: 708–712.
- 77 Rotman G, Shiloh Y. ATM: from gene to function. *Hum Mol Genet* 1998; **7**: 1555–1563.
- 78 Trofatter JA, MacCollin MM, Rutter JL, Murrell JR, Duyao MP, Parry DM, Eldridge R, Kley N, Menon AG, Pulaski K. A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. *Cell* 1993; **72**: 791–800.
- 79 Barlow C, Hirotsune S, Paylor R, Liyanage M, Eckhaus M, Collins F, Shiloh Y, Crawley JN, Ried T, Tagle D, Wynshaw-Boris A. Atm-deficient mice: a paradigm of ataxia telangiectasia. *Cell* 1996; **86**: 159–171.
- 80 Stilgenbauer S, Schaffner C, Litterst A, Liebisch P, Gilad S, Bar-Shira A, James MR, Lichter P, Döhner H. Biallelic mutations in the ATM gene in T-prolymphocytic leukemia. *Nat Med* 1997; **3**: 1155–1159.
- 81 Vorechovsky I, Luo L, Dyer MJS, Catovsky D, Amlot PL, Yaxley JC, Foroni L, Hammarström L, Webster ADB, Yuille MAR. Clustering of missense mutations in the ataxia-telangiectasia gene in a sporadic T-cell leukaemia. *Nat Genet* 1997; **17**: 96–99.
- 82 Starostik P, Manshouri T, O'Brien S, Freireich E, Kantarjian H, Haidar M, Lerner S, Keating M, Albitar M. Deficiency of the ATM protein defines an aggressive subgroup of B-cell chronic lymphocytic leukemia. *Cancer Res* 1998; **58**: 4552–4557.
- 83 Bullrich F, Rasio D, Kitada S, Starostik P, Kipps T, Keating M, Albitar M, Reed JC, Croce CM. ATM mutations in B-cell chronic lymphocytic leukemia. *Cancer Res* 1999; **59**: 24–27.
- 84 Stankovic T, Weber P, Stewart G, Bedenham T, Murray J, Byrd PJ, Moss PAH, Taylor AMR. Inactivation of ataxia telangiectasia mutated gene in B-cell chronic lymphocytic leukaemia. *Lancet* 1999; **353**: 26–29.
- 85 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.
- 86 Stankovic T, Stewart GS, Fegan C, Biggs P, Last J, Byrd PJ, Keenan RD, Moss PA, Taylor AM. 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.
- 87 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.
- 88 Monni O, Zhu Y, Fransilla K, Oinonen R, Höglund P, Elonen E, Joensuu H, Knuutila S. Molecular characterisation of deletion at 11q22.1–23.3 in mantle cell lymphoma. *Br J Haematol* 1991; **104**: 665–671.
- 89 Stilgenbauer S, Winkler D, Ott G, Schaffner C, Leupolt E, Bentz M, Möller P, Müller-Hermelink HK, James MR, Lichter P, Döhner H. Molecular characterization of 11q deletions points to a pathogenic role of the ATM gene in mantle cell lymphoma. *Blood* 1999; **94**: 3262–3264.
- 90 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.
- 91 Döhner H, Stilgenbauer S, James MR, Benner A, Weilguni T, Bentz M, Fischer K, Hunstein W, Lichter P. 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.
- 92 Juliusson G, Gahrton G. Chromosome aberrations in B-cell chronic lymphocytic leukemia. Pathogenetic and clinical implications. *Cancer Genet Cytogenet* 1990; **45**: 143–160.
- 93 Gahrton G, Robert 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.
- 94 Einhorn S, Burvall K, Juliusson G, Gahrton G, Meeker T. Molecular Analysis of chromosome 12 in chronic lymphocytic leukemia. *Leukemia* 1989; **3**: 871–874.
- 95 Perez Losada A, Wessman M, Tiainen M, Hopman AHN, Willard HF, Solé F, Caballín MR, Woessner S, Knuutila S. Trisomy 12 in

- chronic lymphocytic leukemia: an interphase cytogenetic study. *Blood* 1991; **78**: 775–779.
- 96 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.
- 97 Raghoebar S, Kibbelaar RE, Kleiverda K, Kluijn-Nelemans JC, van Krieken JHJM, Kok F, Kluijn PM. Mosaicism of trisomy 12 in chronic lymphocytic leukemia detected by non-radioactive *in situ* hybridisation. *Leukemia* 1992; **6**: 1220–1226.
- 98 Escudier SM, Pereira-Leahy JM, Drach JW, Weier HU, Goodacre AM, Cork MA, Trujillo JM, Keating MJ, Andreeff M. Fluorescence *in situ* hybridization and cytogenetic studies of trisomy 12 in chronic lymphocytic leukemia. *Blood* 1993; **81**: 2702–2707.
- 99 Que TH, Garcia Marco J, Ellis J, Matutes E, Brito-Babapulle V, Boyle S, Catovsky D. Trisomy 12 in chronic lymphocytic leukemia detected by fluorescence *in situ* hybridization: analysis by stage, immunophenotype, and morphology. *Blood* 1993; **82**: 571–575.
- 100 Criel A, Wlodarska I, Meeus P, Stul M, Louwagie A, van Hoof A, Hidajat M, Mecucci C, van den Berghe H. Trisomy 12 is uncommon in typical chronic lymphocytic leukaemias. *Br J Haematol* 1994; **87**: 523–528.
- 101 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.
- 102 Hjalmar V, Kimby E, Matutes E, Sundstrom C, Jacobsson B, Arvidsson I, Hast R. Trisomy 12 and lymphoplasmacytoid lymphocytes in chronic leukemic B-cell disorders. *Haematologica* 1998; **83**: 602–609.
- 103 Merup M, Juliusson G, Wu X, Jansson M, Stellan B, Rasool O, Roijer E, Stenman G, Gahrton G, Einhorn S. Amplification of multiple regions of chromosome 12, including 12q13–15, in chronic lymphocytic leukaemia. *Eur J Haematol* 1997; **58**: 174–180.
- 104 Dierlamm J, Wlodarska I, Michaux L, Vermeesch JR, Meeus P, Stul M, Criel A, Verhoef G, Thomas J, Delannoy A, Louwagie A, Cassiman JJ, Mecucci C, Hagemeijer A, Van den Berghe H. 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 Chromos Cancer* 1997; **20**: 155–166.
- 105 Solinas-Toldo S, Lampel S, Stilgenbauer S, Nickolenko J, Benner A, Döhner H, Cremer T, Lichter P. Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. *Genes Chromos Cancer* 1997; **20**: 399–407.
- 106 Pinkel D, Seagraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y, Dairkee SH, Ljung BM, Gray JW, Albertson DG. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet* 1998; **20**: 207–211.
- 107 Robèrt KH, Gahrton G, Friberg K, Zech L, Nilsson B. Extra chromosome 12 and prognosis in chronic lymphocytic leukaemia. *Scand J Haematol* 1982; **28**: 163–168.
- 108 Auer RL, Bienz N, Neilson J, Cai M, Waters JJ, Milligan DW, Fegan CD. The sequential analysis of trisomy 12 in B-cell chronic lymphocytic leukaemia. *Br J Haematol* 1999; **104**: 742–744.
- 109 Hjalmar V, Kimby E, Hast R. Long-term follow-up of trisomy 12 by FISH in chronic lymphocytic leukemia. *Blood* 2001; **98** (Suppl. 1): Abstr. 1362.
- 110 Gaidano G, Ballerini P, Gong JZ, Inghirami G, Neri A, Newcomb EW, Magrath IT, Knowles DM, Dalla-Favera R. p53 mutations in human lymphoid malignancies: association with Burkitt lymphoma and chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 1991; **88**: 5413–5417.
- 111 Fenaux P, Preudhomme C, Lai JL, Quiquandon I, Jonveaux P, Vanrumbek M, Sartaix C, Morel P, Loucheux-Lefebvre MH, Bauters F, Berger R, Kerckaert P. Mutations of the p53 gene in B-cell chronic lymphocytic leukemia: a report on 39 cases with cytogenetic analysis. *Leukemia* 1992; **6**: 246–250.
- 112 El Rouby S, Thomas A, Costin D, Rosenberg CR, Potmesil M, Silber R, Newcomb EW. 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.
- 113 Gaidano G, Newcomb EW, Gong JZ, Tassi V, Neri A, Cortelezzi A, Calori R, Baldini L, Dalla-Favera R. Analysis of alterations of oncogenes and tumor suppressor genes in chronic lymphocytic leukemia. *Am J Pathol* 1994; **144**: 1312–1319.
- 114 Döhner H, Fischer K, Bentz M, Hansen K, Benner A, Cabot G, Diehl D, Schlenk R, Coy J, Stilgenbauer S, Volkman M, Galle PR, Poustka A, Hunstein W, Lichter P. 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.
- 115 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): Abstr. 4463.
- 116 Thornton PD, Gruszka-Westwood AM, Hamoudi R, Atkinson S, Kaczmarek P, Morilla R, Matutes E, Catovsky D. Detection and characterization of p53 abnormalities in chronic lymphocytic leukemia (CLL). *Blood* 2001; **98** (Suppl. 1): Abstr. 1501.
- 117 Stilgenbauer S, Scherer K, Kröber A, Bullinger L, Höchsmann B, Mayer-Steinacker R, Bunjes D, Döhner H. Campath-1H in refractory B-CLL – complete remission despite p53 gene mutation. *Blood* 2001; **98** (Suppl. 1) Abstr. 3211.
- 118 Johansson B, Mertens F, Mitelman F. Cytogenetic deletion maps of hematologic neoplasms: circumstantial evidence for tumor suppressor loci. *Genes Chromos Cancer* 1993; **8**: 205–218.
- 119 Offit K, Parsa NZ, Gaidano G, Filippa DA, Louie D, Pan D, Jhanwar SC, Dalla-Favera R, Chaganti RSK. 6q deletions define distinct clinico-pathologic subsets of non-Hodgkin's lymphoma. *Blood* 1993; **82**: 2157–2162.
- 120 Offit K, Louie DC, Parsa NZ, Filippa D, Gangi M, Siebert R, Chaganti RSK. Clinical and morphologic features of B-cell small lymphocytic lymphoma with del(6)(q21q23). *Blood* 1994; **83**: 2611–2618.
- 121 Merup M, Moreno TC, Heyman M, Rönnerberg K, Grandér D, Detlofsen R, Rasool O, Liu Y, Söderhäll S, Juliusson G, Gahrton G, Einhorn S. 6q deletions in acute lymphoblastic leukemia and non-Hodgkin's lymphomas. *Blood* 1998; **91**: 3397–4000.
- 122 Stilgenbauer S, Bullinger L, Benner A, Wildenberger K, Bentz M, Döhner K, Ho AD, Lichter P, Döhner H. Incidence and clinical significance of 6q deletions in B-cell chronic lymphocytic leukemia. *Leukemia* 1999; **13**: 1331–1334.
- 123 Zhang Y, Matthiesen P, Harder S, Siebert R, Castoldi G, Calasanz MJ, Wong KF, Rosenwald A, Ott G, Atkin NB, Schlegelberger B. A 3-cM commonly deleted region in 6q21 in leukemias and lymphomas delineated by fluorescence *in situ* hybridization. *Genes Chromos Cancer* 2000; **27**: 52–58.
- 124 Tilly H, Rossi A, Stamatoullas A, Lenormand B, Bigorgne C, Kunlin A, Monconduit M, Bastard C. Prognostic value of chromosomal abnormalities in follicular lymphoma. *Blood* 1994; **84**: 1043–1049.
- 125 Rosenberg CL, Wong E, Petty EM, Bale AE, Tsujimoto Y, Harris NL, Arnold A. PRAD1, a candidate BCL1 oncogene: mapping and expression in centrocytic lymphoma. *Proc Natl Acad Sci USA* 1991; **88**: 9638–9642.
- 126 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.
- 127 Bosch F, Jares P, Campo E, Lopez-Guillermo A, Piris MA, Villamor N, Tassies D, Jaffe SE, Montserrat E, Rozman C, Cardesa A. PRAD-1/Cyclin D1 gene overexpression in chronic lymphoproliferative disorders: a highly specific marker of mantle cell lymphoma. *Blood* 1994; **84**: 2726–2732.
- 128 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.
- 129 Tsujimoto Y, Jaffe E, Cossman J, Gorham J, Nowell PC, Croce CM. Clustering of breakpoints on chromosome 11 in human B-cell neoplasms with the t(11;14) chromosome translocation. *Nature* 1985; **315**: 343–345.
- 130 Meeker TC, Grimaldi JC, O'Rourke R, Louie E, Juliusson G, Einhorn S. An additional breakpoint in the BCL-1 locus associated with the t(11;14)(q13;q32) translocation of B-lymphocytic malignancy. *Blood* 1989; **74**: 1801–1806.

- 131 Rechavi G, Katzir N, Brok-Simoni F, Holtzman F, Mandel M, Gurfinkel N, Givol D, Ben-Bassat I, Ramot B. A search for *bcl1*, *bcl2*, and *c-myc* oncogene rearrangements in chronic lymphocytic leukemia. *Leukemia* 1988; **3**: 57–60.
- 132 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.
- 133 Raghoebar S, van Krieken JHJM, Kluin-Nelemans JC, Gillis A, van Ommen GJB, Ginsberg AM, Raffeld M, Kluin PM. Oncogene rearrangements in chronic B-cell leukemia. *Blood* 1991; **77**: 1560–1564.
- 134 Newman RA, Peterson B, Davey FR, Brabyn C, Collins H, Brunetto VL, Duggan DB, Weiss RB, Royston I, Millard FE, Miller AA, Bloomfield CD. Phenotypic markers and BCL1 rearrangements in B-cell chronic lymphocytic leukemia: a cancer and leukemia group B study. *Blood* 1993; **82**: 1239–1246.
- 135 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.
- 136 Adachi M, Tefferi A, Greipp PR, Kipps TJ, Tsujimoto Y. Preferential linkage of *bcl-2* to immunoglobulin light chain gene in chronic lymphocytic leukemia. *J Exp Med* 1990; **171**: 559–564.
- 137 Hanada M, Delia D, Aiello A, Stadtmayer E, Reed JC. *bcl-2* gene hypomethylation and high-level expression in B-cell chronic lymphocytic leukemia. *Blood* 1993; **82**: 1820–1828.
- 138 Crossen PE, Morrison MJ. Lack of 5'*bcl2* rearrangements in B-cell leukemia. *Cancer Genet Cytogenet* 1993; **69**: 72–73.
- 139 Dyer MJS, Zani VJ, Lu WZ, O'Byrne A, Mould S, Chapman R, Heward JM, Kayano H, Jadayel D, Matutes E, Catovsky D, Oscier DG. *BCL2* translocations in leukemias of mature B cells. *Blood* 1994; **83**: 3682–3688.
- 140 Michaux L, Mecucci C, Stul M, Wlodarska I, Hernandez JM, Meeus P, Michaux JL, Scheiff JM, Noël H, Louvagie A, Criel A, Boogaerts M, Van Orshoven A, Cassiman JJ, Van Den Berghe H. *BCL3* rearrangements and t(14;19)(q32;q13) in lymphoproliferative disorders. *Genes Chromos Cancer* 1996; **15**: 38–47.
- 141 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.
- 142 McKeithan TW, Ohno H, Diaz M. Identification of a transcriptional unit adjacent to the breakpoint in the 14;19 translocation of chronic lymphocytic leukemia. *Genes Chromos Cancer* 1990; **1**: 247–255.
- 143 McKeithan TW, Takimoto GS, Ohno H, Bjorling VS, Morgan R, Hecht BK, Dubé I, Sandberg AA, Rowley JD. *BCL3* rearrangements and t(14;19) in chronic lymphocytic leukemia and other B-cell malignancies: a molecular and cytogenetic study. *Genes Chromos Cancer* 1997; **20**: 64–72.
- 144 Kerr LD, Duckett CS, Wamsley P, Zhang Q, Chiao P, Nabel G, McKeithan T, Baewerle P, Verma I. The proto-oncogene *bcl-3* encodes an I kappa B protein. *Genes Dev* 1992; **6**: 2352–2363.
- 145 Willis TG, Dyer MJ. The role of immunoglobulin translocations in the pathogenesis of B-cell malignancies. *Blood* 2000; **96**: 808–822.
- 146 Stranks G, Height SE, Mitchell P, Jadayel D, Yuille MAR, De Lord C, Clutterbuck RD, Treleaven JG, Powles RL, Nacheva E, Oscier DG, Karpas A, Lenoir GM, Smith SD, Millar JL, Catovsky D, Dyer MJS. Deletions and rearrangement of *CDKN2* in lymphoid malignancy. *Blood* 1995; **85**: 893–901.
- 147 Quesnel B, Preudhomme C, Philippe N, Vanrumbeke M, Dervite I, Lai JL, Bauters F, Wattel E, Fenaux P. p16 gene homozygous deletions in acute lymphoblastic leukemia. *Blood* 1995; **85**: 657–663.
- 148 Ogawa S, Hangaishi A, Miyawaki S, Hirosawa S, Miura Y, Takeyama K, Kamada N, Ohtake S, Uike N, Shimazaki C, Toyama K, Hirano M, Mizoguchi H, Kobayashi Y, Furusawa S, Saito M, Emi N, Yazaki Y, Ueda R, Hirai H. Loss of the cyclin-dependent kinase 4-inhibitor (p16; MTS1) gene is frequent in and highly specific to lymphoid tumors in primary human hematopoietic malignancies. *Blood* 1995; **86**: 1548–1556.
- 149 Schröder M, Mathieu U, Dreyling MH, Bohlander SK, Hagemeyer A, Beverloo BH, Olopade OI, Stilgenbauer S, Fischer K, Bentz M, Lichter P, Döhner H. *CDKN2* gene deletion is not found in chronic lymphoid leukemias of B- and T-cell origin, but is frequent in acute lymphoblastic leukemia. *Br J Haematol* 1995; **91**: 865–870.
- 150 Wessendorf S, Schwaenen C, Barth TFE, Doerfel J, Kohlhammer H, Nesslering M, Wrobel G, Fritz B, Moeller P, Doehner H, Lichter P, Bentz M. Automated genomic profiling using microarray-based hybridization (Matrix-CGH) – a powerful technique for the detection of DNA-amplifications in aggressive lymphoma. *Blood* 2001; **98** (Suppl. 1): Abstr. 1940.
- 151 Schwänen C, Nesslering M, Wessendorf S, Göttel D, Wrobel G, Fritz B, Bentz M, Döhner H, Stilgenbauer S, Lichter P. Automated genomic profiling in chronic lymphocytic leukemia using microarray-based hybridization (Matrix-CGH). *Blood* 2001; **98** (Suppl. 1): Abstr. 3178.
- 152 Meeker TC, Grimaldi JC, O'Rourke R, Loeb J, Juliusson G, Einhorn S. Lack of detectable somatic hypermutation in the V region of the Ig H chain gene of a human chronic B lymphocytic leukemia. *J Immunol* 1988; **141**: 3994–3998.
- 153 Pratt LF, Rassenti L, Larrick J, Robbins B, Banks PM, Kipps TJ. Ig V region gene expression in small lymphocytic lymphoma with little or no somatic hypermutation. *J Immunol* 1989; **143**: 699–705.
- 154 Kuppers R, Gause A, Rajewsky K. B cells of chronic lymphatic leukemia express V genes in unmutated form. *Leuk Res* 1991; **15**: 487–496.
- 155 Duke V, Gandini D, Sherrington P, Lin K, Heelan B, Prentice G, Hoffbrand V, Foroni L. Immunoglobulin (Ig) VH gene usage in B-CLL patients: different VH gene profiles in unmutated compared to mutated CLL and normal controls. *Blood* 2001; **98** (Suppl. 1): Abstr. 4870.
- 156 Johnson TA, Rassenti LZ, Kipps TJ. Ig VH1 genes expressed in B cell chronic lymphocytic leukemia exhibit distinctive molecular features. *J Immunol* 1997; **158**: 235–246.
- 157 Kröber A, Bühler A, Kienle D, Benner A, Lichter P, Döhner H, Stilgenbauer S. Analysis of VDJ rearrangement structure and VH mutation status in chronic lymphocytic leukemia. *Blood* 2001; **98** (Suppl. 1): Abstr. 1509.
- 158 Stevenson F, Sahota S, Zhu D, Ottensmeier C, Chapman C, Oscier D, Hamblin T. Insight into the origin and clonal history of B-cell tumors as revealed by analysis of immunoglobulin variable region genes. *Immunol Rev* 1997; **162**: 247–259.
- 159 Oscier DG, Thompson A, Zhu D, Stevenson FK. Differential rates of somatic hypermutation in V(H) genes among subsets of chronic lymphocytic leukemia defined by chromosomal abnormalities. *Blood* 1997; **89**: 4153–4160.
- 160 Schettino EW, Cerutti A, Chiorazzi N, Casali P. Lack of intraclonal diversification in Ig heavy and light chain V region genes expressed by CD5⁺IgM⁺ chronic lymphocytic leukemia B cells: a multiple time point analysis. *J Immunol* 1998; **160**: 820–830.
- 161 Migliazza A, Martinotti S, Chen W, Fusco C, Ye BH, Knowles DM, Offit K, Chaganti RS, Dalla-Favera R. Frequent somatic hypermutation of the 5' noncoding region of the BCL6 gene in B-cell lymphoma. *Proc Natl Acad Sci USA* 1995; **92**: 12520–12524.
- 162 Ye BH, Rao PH, Chaganti RS, Dalla-Favera R. Cloning of *bcl-6*, the locus involved in chromosome translocations affecting band 3q27 in B-cell lymphoma. *Cancer Res* 1993; **53**: 2732–2735.
- 163 Bardwell VJ, Treisman R. The POZ domain: a conserved protein-protein interaction motif. *Genes Dev* 1994; **8**: 1664–1677.
- 164 Ye BH, Lista F, Lo Coco F, Knowles DM, Offit K, Chaganti RS, Dalla-Favera R. Alterations of a zinc finger-encoding gene, BCL-6, in diffuse large-cell lymphoma. *Science* 1993; **262**: 747–750.
- 165 Ye BH, Chaganti S, Chang CC, Niu H, Corradini P, Chaganti RS, Dalla-Favera R. Chromosomal translocations cause deregulated BCL6 expression by promoter substitution in B cell lymphoma. *EMBO J* 1995; **14**: 6209–6217.
- 166 Ye BH, Cattoretti G, Shen Q, Zhang J, Hawe N, de Waard R, Leung C, Nouri-Shirazi M, Orazi A, Chaganti RS, Rothman P, Stall AM, Pandolfi PP, Dalla-Favera R. The BCL-6 proto-oncogene controls germinal-center formation and Th2-type inflammation. *Nat Genet* 1997; **16**: 161–170.
- 167 Pasqualucci L, Migliazza A, Fracchiolla N, William C, Neri A, Baldini L, Chaganti RS, Klein U, Kuppers R, Rajewsky K, Dalla-Favera R. BCL-6 mutations in normal germinal center B cells: evidence of somatic hypermutation acting outside Ig loci. *Proc Natl Acad Sci USA* 1998; **95**: 11816–11821.
- 168 Shen HM, Peters A, Baron B, Zhu X, Storb U. Mutation of BCL-

- 6 gene in normal B cells by the process of somatic hypermutation of Ig genes. *Science* 1998; **280**: 1750–1752.
- 169 Peng HZ, Du MQ, Koulis A, Aiello A, Dogan A, Pan LX, Isaacson PG. Nonimmunoglobulin gene hypermutation in germinal center B cells. *Blood* 1999; **93**: 2167–2172.
- 170 Pasqualucci L, Neri A, Baldini L, Dalla-Favera R, Migliazza A. BCL-6 mutations are associated with immunoglobulin variable heavy chain mutations in B-cell chronic lymphocytic leukemia. *Cancer Res* 2000; **60**: 5644–5648.
- 171 Capello D, Fais F, Vivenza D, Migliaretti G, Chiorazzi N, Gaidano G, Ferrarini M. Identification of three subgroups of B cell chronic lymphocytic leukemia based upon mutations of BCL-6 and IgV genes. *Leukemia* 2000; **14**: 811–815.
- 172 Sahota SS, Davis Z, Hamblin TJ, Stevenson FK. Somatic mutation of bcl-6 genes can occur in the absence of V(H) mutations in chronic lymphocytic leukemia. *Blood* 2000; **95**: 3534–3540.
- 173 Yan XJ, Albesiano E, McGuire P, Peterson D, Allen SL, Vinciguerra V, Asutosh G, Rai KR, Ferrarini M, Chiorazzi N. The characteristics of the ongoing mutations in the CD79 genes of B-CLL clones are not typical of Ig V gene hypermutation. *Blood* 2001; **98** (Suppl. 1): Abstr. 1975.
- 174 Pascual V, Liu YJ, Magalski A, de Bouteiller O, Banchereau J, Capra JD. Analysis of somatic mutation in five B cell subsets of human tonsil. *J Exp Med* 1994; **180**: 329–339.
- 175 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.
- 176 Thunberg U, Johnson A, Roos G, Thorn I, Tobin G, Sallstrom J, Sundstrom C, Rosenquist R. CD38 expression is a poor predictor for VH gene mutational status and prognosis in chronic lymphocytic leukemia. *Blood* 2001; **97**: 1892–1894.
- 177 Damle RN, 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.
- 178 Stilgenbauer S, Kröber A, Seiler T, Benner A, Bullinger L, Brückle E, Lichter P, Döhner H. VH mutation status, CD38 expression level, genomic aberrations, and survival in chronic lymphocytic leukemia. *Blood* 2001; **98** (Suppl. 1): Abstr. 1971.
- 179 Meyer R, Brockman S, Paternoster S, Bone N, James CD, Jelinek D, Tschumper R, Geyer S, Hanson CA, Pruthi R, Witzig T, Kay N, Dewald G. Fluorescence-labeled DNA probes to study indolent and aggressive B-CLL: comparison to Rai stage, level of clonal B-cells, IgV_H mutation status and conventional cytogenetics. *Blood* 2001; **98** (Suppl. 1): Abstr. 1515.
- 180 Maloum K, Davi F, Merle-Beral H, Pritsch O, Magnac C, Vuillier F, Dighiero G, Troussard X, Mauro FF, Benichou J. Expression of unmutated VH genes is a detrimental prognostic factor in chronic lymphocytic leukemia. *Blood* 2000; **96**: 377–379.
- 181 Lange A, Ritgen M, Brüggemann M, Schmitz N, Kneba M, Dreger P. Unmutated VH gene status retains its adverse prognostic influence after autologous stem cell transplantation (SCT) for chronic lymphocytic leukemia (CLL). *Blood* 2001; **98** (Suppl. 1): Abstr. 3574.
- 182 Ibrahim S, Keating M, Do KA, O'Brien S, Huh YO, Jilani I, Lerner S, Kantarjian HM, Albitar M. CD38 expression as an important prognostic factor in B-cell chronic lymphocytic leukemia. *Blood* 2001; **98**: 181–186.
- 183 Del Poeta G, Maurillo L, Venditti A, Buccisano F, Epiceno AM, Capelli G, Tamburini A, Suppo G, Battaglia A, Del Principe MI, Del Moro B, Masi M, Amadori S. Clinical significance of CD38 expression in chronic lymphocytic leukemia. *Blood* 2001; **98**: 2633–2639.
- 184 Stratowa C, Löffler G, Lichter P, Stilgenbauer S, Haberl P, Schweifer N, Döhner H, Wilgenbus KK. CDNA microarray gene expression analysis of B-cell chronic lymphocytic leukemia proposes potential new prognostic markers involved in lymphocyte trafficking. *Int J Cancer* 2001; **91**: 474–480.
- 185 Rosenwald A, Alizadeh AA, Widhopf G, Simon R, Davis RE, Yu X, Yang L, Pickeral OK, Rassenti LZ, Powell J, Botstein D, Byrd JC, Grever MR, Cheson BD, Chiorazzi N, Wilson WH, Kipps TJ, Brown PO, Staudt LM. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. *J Exp Med* 2001; **194**: 1639–1647.
- 186 Klein U, Tu Y, Stolovitzky GA, Mattioli M, Cattoretti G, Husson H, Freedman A, Inghirami G, Cro L, Baldini L, Neri A, Califano A, Dalla-Favera R. 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.