Genetics of B-Cell Chronic Lymphocytic Leukemia

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1. INTRODUCTION

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

2. GENOMIC ABERRATIONS IN B-CLL

Two major subjects can be differentiated with respect to the genetic analysis of B-CLL: on the one hand, genomic aberrations, which, as acquired changes, may be involved in the initiation and progression of the disease, and, on the other hand, the mutation status of the variable segments of immunoglobulin heavy chain genes (VH), which may reflect the cellular origin of B-CLL. Since the early 1980s, chromosome banding analyses of malignant B-cells have been performed using B-cell mitogens (11–18). Up to the early 1990s, clonal aberrations could be dem-
onstrated in only 40–50% of B-CLL cases using chromosome banding (19–21). Frequently, and despite the use of B-cell mitogens, nonclonal T-cells without chromosomal aberration were analyzed (22). More recently, the development of molecular cytogenetic techniques like fluorescence in situ hybridization (FISH) has led to considerable improvement in the diagnostics of genetic aberrations in tumor cells (23,24). FISH allows sensitive detection of specific sequences in the genome using cloned DNA fragments as probes. Signal number and location reflect numerical and structural changes of the corresponding chromosomal regions. The ability to detect aberrations not only on metaphase chromosomes but also in interphase cell nuclei is of great importance, especially in B-CLL (interphase cytogenetics; Fig. 3) (25). Interphase cytogenetic studies using FISH showed that the incidence of genomic aberrations in B-CLL was markedly

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

Fig. 2. Estimated survival times of B-CLL subgroups according to Rai stage (from ref. 6, with permission).
underestimated in banding studies (9). In B-CLL cases with abnormal karyotype by banding analysis but clonal aberrations by interphase FISH, the metaphase cells are derived from nonclonal T-cells and therefore do not reflect the karyotype of the malignant B-CLL cells.

Among the genomic aberrations whose incidence has been underestimated in B-CLL in banding studies are particularly the deletions of bands 13q14 and 11q22-q23, (9); while trisomy 12, which was originally described as the most frequent aberration of B-CLL in studies using chromosome banding, has been rated the third most frequent aberration by interphase FISH (9,26,27).

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

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

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

The structural chromosome aberration most frequently found in cytogenetic studies of B-CLL is deletion of band 13q14 (18,28–31). Recurrent deletion of a chromosomal region indicates the existence of a tumor suppressor gene, whose inactivation is caused by the loss of an allele and the mutation in the remaining allele (two-hit hypothesis). The retinoblastoma tumor suppressor
gene (RB1) is a candidate gene localized in band 13q14 that codes for a nuclear phosphoprotein involved in cell cycle regulation and transcription control. Its inactivation is involved in the pathogenesis of numerous tumors (32). The deletion of an allele of RB1 was detected using molecular cytogenetic techniques in about one-fourth of all B-CLL cases (33–36). However, the inactivation of both RB1 gene copies by deletion and/or mutation could only be extremely rarely detected, which raises questions about the pathogenetic role of RB1 in B-CLL.

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

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

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

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<th>Aberration</th>
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<td>Clonal abnormalities</td>
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From ref. 9, with permission.
be demonstrated for the first time in human tumors in T-prolymphocytic leukemia (T-PLL) (56,57). Because of a lack of ATM protein expression, the involvement of ATM in B-CLL was also postulated, and inactivation of ATM by deletion and/or mutation could actually be demonstrated (58–61). It was shown that ATM mutant B-CLL cases exhibited a deficient ATM-dependent response of p21 to γ-irradiation, failure to upregulate tumor necrosis factor-related apoptosis-inducing ligand receptor 2 (TRAIL-R2), and inability to repair induced chromosomal breaks (62). An association of deletion 11q with a more aggressive clinical course of B-CLL was suggested in a chromosome banding study (63). Interestingly, all ATM mutant cases showed absence of somatic V_{H} hypermutation (see also Subheading 2.8. below), indicating that ATM may play a role at the pregerminai center stage of B-cell maturation and may lead to the development of B-CLL derived from pregerminai center cells (10,64). However, mutation of the remaining ATM allele was found only in 5 of 22 B-CLL cases with 11q22-q23 deletion of our series, which indicates a possible involvement of additional genes in this region in B-CLL (59). By contrast, in mantle cell lymphoma, in which the 11q22-q23 deletion occurs in nearly half the cases (65,66), mutation of the remaining allele could be demonstrated in all cases with deletion of an ATM allele (67). Thus, ATM appears to be the tumor suppressor gene inactivated in connection with 11q22-q23 deletions in T-PLL, mantle cell lymphoma, and some cases of B-CLL. Elucidation of the situation in B-CLL cases with 11q deletion without mutation in the remaining ATM allele is currently in progress.

2.3. Trisomy 12 As Recurrent Aberration in B-CLL

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

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

2.4. Deletion 6q in Lymphatic Neoplasms

Among the most frequent aberrations in both acute lymphoblastic leukemia and aggressive as well as indolent lymphoma are deletions involving the long arm of chromosome 6 (90). In B-CLL, 6q deletions were found in 6% of the evaluable cases by means of chromosome banding, whereby bands 6q15 and 6q23 were most often affected (21). In an extensive analysis of various types of lymphoma, at least two independent deletion regions were identified, one in bands 6q21-q23 and...
another in bands 6q25-q27 (91). Deletion 6q21-q23 was associated with the subgroup of lymphomas with lymphocytic differentiation, which may be considered as nonleukemic correlates to B-CLL (92).

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

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

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

Fig. 4. Matrix CGH in B-CLL. Hybridization of DNA derived from a patient with a 13q14 deletion (labeled in green) vs human control DNA (labeled in red). Inset: PAC clone localized in band 13q14 exhibits a dominant red fluorescence signal after hybridization, indicating the deletion of this region (arrow) (89).
17p13 deletions were found in 4–9% in B-CLL \((9,101)\). To examine the relationship of 17p13 deletion and \(p53\) inactivation by mutation in the remaining allele, 110 B-CLL cases were analyzed \((102)\). Fifteen showed mutations in the \(p53\) gene, of which half were biallelic aberrations. Among the cases with deletion, most showed mutations in the remaining \(p53\) allele, whereas among the cases without 17p13 deletion, \(p53\) mutation occurred only rarely. The high rate of \(p53\) mutations in the B-CLL cases with 17p13 deletions suggests that, in the case of 17p13 deletion in B-CLL, \(p53\) is the tumor suppressor gene affected by the aberrations.

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

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

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

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

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

### 2.8. Mutation Status of the \(V_{H}\) Genes

A novel genetic parameter of B-CLL is the mutation status of the \(V_{H}\) genes \((123–125)\). Although in the past, B-CLL was considered to be a lymphoma derived from pregerminal center B-cells, somatically mutated \(V_{H}\) genes could be demonstrated in about half of the cases in these studies. Accordingly, a separation was made into two different B-CLL groups: one with unmutated \(V_{H}\) genes, assumed to originate in pregerminal center cells, and another with mutated \(V_{H}\) genes, thought to stem from postgerminal center cells. Moreover, it could be demonstrated that the \(V_{H}\) mutation status is clinically relevant. Although B-CLL with unmutated \(V_{H}\) shows an unfavorable course, with rapid disease progression, B-CLL with mutated \(V_{H}\) often shows slow progression.
In addition, there was a correlation between V<sub>H</sub> mutation status and CD38 expression of B-CLL cells as further evidence of the biological difference between the two forms (7). The relationship of the V<sub>H</sub> mutation status to genomic aberrations and the differential influence of these factors in the pathogenesis and progression of B-CLL are currently undergoing intensive examination.

3. CLINICAL IMPACT OF GENOMIC ABERRATIONS IN B-CLL

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

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

3.1. Prognostic Relevance of Deletion 11q22-q23

B-CLL cases with deletion 11q show more rapid progression of the disease and shorter survival times. In interphase FISH, deletion 11q22-q23 is the second most frequent aberration in B-CLL, with an incidence of approx 20%, and identifies a patient group with a characteristic clinical picture (26). B-CLL patients with 11q deletion present with advanced stages of disease and pronounced lymphadenopathy, reflected by large palpable peripheral, thoracic, and abdominal lymph nodes (Fig. 1). Moreover, patients with 11q deletion have a more rapid progression of disease, as measured by shorter therapy-free intervals (9 mo vs 43 mo; p < 0.001).

In the survival time analysis, 11q deletion was associated with a poor prognosis, and the effect of this aberration on the course of the disease was age-dependent. In B-CLL patients younger than 55 yr, the survival time was significantly shorter in the group with 11q deletion than in the group without 11q deletion, whereas in patients 55 yr or older, there was only a trend to shorter survival times. Another, likewise age-dependent prognostic relevance was found in an examination of ATM protein expression in B-CLL (61). The poor outcome of B-CLL with 11q deletion was confirmed in an independent series (126). In multivariate analysis, 11q deletion was found to be an independent adverse factor (26). Since the 11q deletion appears to be prognostically relevant, especially in younger B-CLL patients, this aberration could serve to identify a patient group that could benefit from modern experimental strategies, such as autologous or allogeneic blood stem cell transplantation.
3.2. Clinical Characteristics of Trisomy 12

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

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

3.3. Clinical Relevance of Deletion 6q

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

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**Fig. 5.** Survival probability in B-CLL according to risk groups defined by genomic aberrations. The estimated median survival times were as follows: 17p deletion, 32 mo; 11q deletion, 79 mo; normal karyotype, 111 mo; 12q trisomy, 114 mo; and 13q deletion as single abnormality, 133 mo. (From ref. 9, with permission.)
FISH study on 285 B-CLL patients revealed a correlation between deletion 6q and greater tumor mass, measured by leukocyte count (median $49.3 \times 10^9/L$ vs $31.7 \times 10^9/L$; $p = 0.036$) and lymphadenopathy (95). The sum of the products of the largest cervical, axillary, and inguinal lymph nodes (median $7.3 \text{ cm}^2$ vs $3.0 \text{ cm}^2$; $p = 0.029$) and the longest lymph node diameter (median $4.0 \text{ cm}$ vs $2.0 \text{ cm}$; $p = 0.008$) were greater in the group with 6q deletion. There was, however, no significant difference in survival time or therapy-free intervals between the two groups (95). Thus, 6q deletion does not appear to be of prognostic relevance in B-CLL.

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

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

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

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

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

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

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

To examine the V_H mutation status in a large series ($n = 300$) of B-CLL patients, the VDJ-rearrangement of the immunoglobulin genes was amplified by PCR from genomic DNA, and the
mutation status of the V_H genes was determined by DNA sequencing (10,132). Taking the classical cutoff value of 98% homology to the nearest related germline gene to differentiate between mutated and unmutated VH genes, 132 cases (44%) showed mutated and 168 cases (56%) unmutated VH genes. The method of maximally selected log rank statistics was applied to test the prognostic relevance of the V_H mutation status. A corrected p value (p_cor) for the best possible separation of two subgroups with different survival probabilities was found at a VH homology to the nearest related germline gene of 97% (p_cor < 0.001; 95% confidence interval 96–98%) (10).

With a cutoff value of 97% homology to the nearest related germline gene, 112 cases (37%) showed mutated and 188 cases (63%) unmutated VH. The Kaplan-Meier estimate of the median survival time in the two VH subgroups differed both for the overall group (n = 300) and within the subgroup of patients in Binet stage A at the time of diagnosis (n = 189) (Fig. 6) (10).

4.2. Structure of the VDJ Rearrangement

In addition to the V_H mutation status, the study of the structure of the VDJ rearrangement and the character of the mutations with respect to biological factors of disease etiology, like antigen selection, is of interest. Until recently, there were only studies available on small numbers of cases (123,125,133,134, and references therein). In a large B-CLL series, at least one clonal VDJ rearrangement of genomic DNA could be amplified in all 300 cases (132). Cases with mutated V_H showed a different VDJ rearrangement structure than cases with unmutated V_H. Genes of the V_H3 and V_H4 families were over-represented in the mutated V_H subgroup, whereas the V_H1 family was found more frequently in the VH unmutated subgroup. Specific VH genes were responsible for the differences, and these imbalances were in line with previous studies (123,125,133,134, and references therein). The mean length of the CDR3 region differed significantly between the V_H mutated and unmutated subgroups. The median mutation rates and ratios of replacement/silent (R/S) mutations were greater within the VH subregions in the CDRs than in the FRs. Cases with less than 98% homology to the nearest related germline gene were examined by means of the algorithm of Chang and Casali (135) for evidence of an antigen selection in the mutation pattern of the V_H gene. In 43 cases, mutation patterns consistent with antigen selection were
found, whereas no such patterns could be recognized in 41 cases (132). The survival probabilities did not differ significantly between the two groups. Taking these data together, there are differences in the biological background of VH mutated vs unmutated B-CLL; however, the pathogenetic role of external stimuli still needs to be confirmed.

4.3. CD38 Expression in Relationship to the VH Mutation Status

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

4.4. Distribution of Genomic Aberrations in the VH Subgroups

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

4.5. Prognostic Relevance of Genomic Aberrations and VH Mutation Status

To examine the individual prognostic value of genomic aberrations, the VH mutation status, and other clinical and laboratory features, a multivariate analysis was made of the survival time by means of a Cox regression (10). The VH mutation status, 17p deletion, 11q deletion, age, leukocyte count and lactate dehydrogenase levels were identified as independent prognostic factors in this analysis. When the VH mutation status, and 11q and 17p aberrations were included, the clinical stage of disease according to the systems of Rai or Binet was not identified as an independent prognostic factor. Similar results, demonstrating a very strong prognostic impact of the VH mutation status and genomic aberrations, were independently found in two other B-CLL series (140,141). Based on this model, four subgroups with widely differing survival probabilities can be defined by the VH mutation status, 11q deletion and 17p deletion (Fig. 7).
These studies show that genomic aberrations and VH mutation status appear to have complementary relevance in estimating the prognosis in B-CLL. Unmutated VH and genomic aberrations were among the strongest prognostic factors and gives us insight into the biological bases of the clinical heterogeneity of B-CLL. For this reason, genomic aberrations and VH mutation status are currently being tested in relation to other clinical and laboratory factors in prospective multicenter studies of the German CLL Study Group (GCLLSG). If these factors allow us to predict the course of disease in individual patients at the time of diagnosis, independent of the stage, they could serve as the basis for future risk-adapted treatment strategies.

Table 2

<table>
<thead>
<tr>
<th>Aberration</th>
<th>Mutated (homology &lt; 98%) [n = 132 (44%)]</th>
<th>Unmutated (homology &gt; 98%) [n = 168 (56%)]</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonal aberrations</td>
<td>80</td>
<td>84</td>
<td>0.37</td>
</tr>
<tr>
<td>13q deletion</td>
<td>65</td>
<td>48</td>
<td>0.004</td>
</tr>
<tr>
<td>13q deletion single</td>
<td>50</td>
<td>26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trisomy 12</td>
<td>15</td>
<td>19</td>
<td>0.44</td>
</tr>
<tr>
<td>11q deletion</td>
<td>4</td>
<td>27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>17p deletion</td>
<td>3</td>
<td>10</td>
<td>0.03</td>
</tr>
<tr>
<td>17p or 11q deletion</td>
<td>7</td>
<td>35</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

aFisher’s exact test.

From ref. 10, with permission.

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

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

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

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