High prevalence of adverse prognostic genetic aberrations and unmutated *IGHV* genes in small lymphocytic lymphoma as compared to chronic lymphocytic leukemia

Patricia J. T. A. Groenen · Reinier Raymakers · Paul D. M. Rombout ·
Martijn de Prenter · Linda Kossen · Corine Philipsen-Jansen · Ton Feuth ·
Jos W. R. Meijer · Ad Geurts van Kessel · Johan H. J. M. van Krieken ·
Marian Stevens-Kroef

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Abstract According to the WHO classification, chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL) are considered the same entity. However, the clinical presentations are different. As yet, detailed molecular–genetic comparisons between CLL and SLL are scarce. In this study, we evaluated the presence of genetic abnormalities and (immunoglobulin heavy chain variable

genes) *IGHV* use and mutation status in lymph node samples of patients presenting with lymphadenopathy alone and, therefore, classified as SLL ($n=21$) or with persisting lymphadenopathy and absolute lymphocyte counts of $\geq 5 \times 10^9/L$ and, therefore, classified as CLL/SLL ($n=17$). In addition, blood samples of CLL patients were evaluated ($n=82$). The majority of lymph node samples from SLL patients (66%) showed unmutated *IGHV* genes. This occurrence was significantly higher than in CLL (29%). Poor risk genetic aberrations (11q-, 17p-, and +12) were more prevalent in the SLL (45%) as compared to CLL (22%). Samples from CLL/SLL patients exhibited almost equal frequencies of poor risk genetic aberrations (53%), but lower frequencies of unmutated *IGHV* genes (35%), as compared to SLL. In summary, SLL not only differs clinically from CLL, but also genetically, with a higher propensity of adverse molecular parameters in SLL. CLL and SLL likely encompass a phenotypic spectrum within one disease entity.

P. J. T. A. Groenen (✉) · P. D. M. Rombout · M. de Prenter ·
C. Philipsen-Jansen · J. H. J. M. van Krieken
Department of Pathology,
Radboud University Nijmegen Medical Centre,
Geert Grooteplein Zuid 10,
6525 GA Nijmegen, The Netherlands
e-mail: p.groenen@pathol.umcn.nl

R. Raymakers
Department of Hematology,
Radboud University Nijmegen Medical Centre,
Nijmegen, the Netherlands

R. Raymakers
Department of Hematology, UMC Utrecht,
Utrecht, the Netherlands

L. Kossen · A. G. van Kessel · M. Stevens-Kroef
Department of Human Genetics,
Radboud University Nijmegen Medical Centre,
Nijmegen, The Netherlands

T. Feuth
Department of Epidemiology, Biostatistics and HTA,
Radboud University Nijmegen Medical Centre,
Nijmegen, The Netherlands

J. W. R. Meijer
Department of Pathology, Rijnstate Hospital Arnhem,
Arnhem, The Netherlands

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Introduction

According to the World Health Organization (WHO) classification system, chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL) are considered the same entity. The definition of an entity in this system is primarily based on clinical, morphologic, phenotypic, and genetic criteria. CLL and SLL patients differ at least clinically, i.e., SLL patients present with

only lymphadenopathy and/or splenomegaly. The absolute lymphocyte count (ALC) in peripheral blood does not exceed $5 \times 10^9/L$ in SLL patients, whereas in CLL patients ALC is defined as $\geq 5 \times 10^9/L$. In contrast, lymph node morphology and immunophenotype are similar in CLL and SLL patients. As yet, detailed molecular–genetic comparisons are scarce.

The clinical course and the overall survival rate of patients with CLL/SLL are largely related to molecular parameters, such as specific genetic abnormalities, mutation status of the immunoglobulin heavy chain variable genes (*IGHV*), and the use of specific *VH* genes [1–7]. Patients with unmutated *IGHV* gene usage exhibit a relatively poor prognosis with overall survival times between 8 and 15 years. The use of *IGHV3-21* represents an additional adverse prognostic factor, independent of its mutation status [8–13]. Genetic abnormalities may provide additional prognostic information. CLL/SLL patients with deletions of the 11q22 (*ATM*) and/or 17p13 (*TP53*) regions, for example, exhibit a significantly shorter overall survival time than those with other genetic abnormalities (such as deletions in 13q) or patients without detected abnormalities. CLL/SLL patients with a trisomy of chromosome 12 exhibit a relatively poorer outcome and, as such, appear to represent an intermediate risk group [3, 5, 14–16]. The prognostic information obtained from *IGHV* gene use and mutation status on one hand and the genetic status on the other hand is complementary [7, 17–19]. In the HOVON-68 study [20], high-risk CLL/SLL is defined by the presence of unmutated *IGHV* genes, mutated *IGHV* genes with use of *IGHV3-21* [1, 2, 8, 11, 13], chromosome 17p or 11q deletions, and/or trisomy of chromosome 12 [3, 4, 14, 16]. We have used these criteria in the present study.

As of yet, only a few molecular–genetic studies in SLL have been reported [21–25], which may be due to the fact that the prevalence of SLL is relatively rare. In addition, lymph node samples from SLL patients are routinely examined in pathology laboratories, instead in immunology/hematology laboratories where testing of CLL-associated molecular–genetic prognostic parameters mostly takes place. Additionally, the use of fixed and paraffin-embedded tissues, which is routine in pathology laboratories, may hamper molecular–genetic analyses due to the suboptimal DNA quality obtained from them [26]. For the evaluation of genetic aberrations in CLL, fluorescence in situ hybridization (FISH) with target-specific probes used on fresh blood samples is widely used [14]. FISH can be applied to small-sized clinical samples as well [27]. More recently, also multiplex ligation-dependent probe amplification (MLPA [28]) has been used as a comprehensive tool to detect genetic abnormalities in CLL/SLL [29]. Both FISH and MLPA have also been applied to SLL [21–25]. Biased and stereotypic use of heavy chain complementary

determining region 3 has been observed in CLL/SLL, particularly for the *IGH3-21* and *IGH4-34 V*-genes, which suggests the occurrence of antigen selection during the course of the disease [30–34]. A large study on stereotypic use in SLL cases has, to the best of our knowledge, not been performed.

Here, we have assessed prognostic relevant risk molecular–genetic parameters (a) in lymph node samples of SLL patients who did not show blood involvement, (b) in lymph node samples of CLL/SLL patients with persistent lymphadenopathy and lymphocyte counts exceeding the cutoff levels provided by the WHO, and (c) in peripheral blood samples of CLL patients (comprising all Rai stages, [35]). We found that the combination of *VH* mutation status, *IGHV3-21* use, and poor prognosis genetic aberrations yielded a higher prevalence in the SLL patients as compared to CLL patients. In order to generate a broader overview on the occurrence of adverse genetic aberrations and unmutated *IGHV* genes in SLL, we have reviewed cases reported in the literature as well.

Methods and materials

Patients and tissue samples

In our studies, we included blood samples obtained from 82 patients diagnosed with CLL using standard morphologic and immunophenotypic criteria. The samples were collected before treatment of the patients. Based on the *IGH* gene clonality profiles, the percentage of monoclonal CLL cells of all the included cases was estimated to be high (>90%). From these patients, 27 were referred to the Radboud University Nijmegen Medical Centre (RUNMC) and 55 to several regional hospitals in the Netherlands. In addition, we included diagnostic frozen lymph node samples histologically classified as CLL/SLL ($n=38$). These lymph node samples were obtained from the archives of the Departments of Pathology of the RUNMC and Rijnstate Hospital Arnhem, the Netherlands. All samples were reviewed by an expert pathologist panel for lymphomas. The tumor load of all cases was estimated to be at least 60%. Handling and assessment of the tissues used in this study were performed according to the code of adequate secondary use of human tissue [36]. The lymph node samples were categorized as either SLL (21 cases) or CLL/SLL (17 cases), based on ALC using a cutoff value of $5 \times 10^9/L$ (according to the WHO classification). The CLL/SLL lymph nodes were derived from patients who presented predominantly with lymphadenopathy (8/17 cases) or from known CLL patients with persisting or increasing lymphadenopathy (8/17 cases). From one patient, no clinical information was available, except the ALC count. In all these latter patients,

the ALC counts exceeded $\geq 5 \times 10^9/\text{L}$. Lymphadenopathy was used as an indication for excision and, according to the WHO guidelines, its diagnosis was confirmed through histopathologic evaluation.

DNA extraction procedures

Frozen tissue sections (5–20 μm) from lymph nodes were used to extract DNA via standard procedures, including proteinase K treatment and ethanol precipitation. From the blood samples, DNAs were extracted using a QIAcube robotic workstation, according to the instructions of the manufacturer (Qiagen, Venlo, The Netherlands). DNA sample concentration and quality were assessed by measurement of 260/280 nm ratio (using a NanoDrop2000 spectrophotometer; Isogen Life Sciences) and by polymerase chain reaction (PCR) amplification using the BIOMED-2 control gene primer set [35]. All frozen tissue and blood samples yielded PCR-amplifiable DNAs of at least 600 nucleotides in length.

Determination of IGH mutation status and VH use

Clonality assays were performed through the amplification of *IGH-VJ* (FR1) and the *IGH*-leader using the multiplex BIOMED-2 PCR protocol [37] and the primers described by Fais et al. [38], respectively. PCR products were monitored through fluorescent gene scan analysis on an ABI 3730 platform (Life Technologies, Foster City, CA, USA). Subsequently, sequencing was performed on an ABI 3730 platform (Life Technologies), using at least two independently generated PCR products. The *VDJ* nucleotide sequences were aligned using the IMGT-IgBLAST database [39, 40]. A sequence homology cutoff of 98% was used to define the *VH* mutation status. Homology to the nearest germline *VH* gene was calculated starting at the first nucleotide 3' from the FR1 forward primer to the last codon (CDR3-IMGT codon 105/106/107, depending on exonuclease trimming) of the *IGHV* gene [41, 42]. In cases where the percentage of identity was close to 98%, the percentage identity was determined from the first (FR1-IMGT codon 1) to the last codon as described above (using the *IGH*-leader PCR product). Care was taken that length of the sequenced PCR fragment corresponded to the length evaluated by gene scanning.

Detection of genetic aberrations by FISH and MLPA

DNAs extracted from the lymph node and blood samples were assessed using MLPA [28]. In some cases, cells were assessed by FISH as well. For FISH, commercially available probes for *ATM* (11q22), centromere 12,

D13S319 (13q14), and *TP53* (17p13) were used according to the manufacturer's specifications (Abbott Molecular, Des Plaines, IL) and at least 200 interphase nuclei were scored by two independent investigators. MLPA was carried out according to the manufacturer's instructions (MRC-Holland Amsterdam, The Netherlands) using two specifically designed sets of probes for testing genetic aberrations in CLL, i.e., P037 and P038 [28]. Amplified products were analyzed by capillary electrophoresis on an ABI 3730 genetic analyzer (Life Technologies). In a previous study [29], we showed a perfect correlation between FISH and MLPA, provided that the abnormal clone was present in at least 10–20% of the cells.

Statistical analysis

A two-sided Fisher's exact test and exact linear trend test were used to identify significant differences between the three groups under investigation, i.e., lymph node samples of patients with SLL ($n=21$), lymph node samples of patients with CLL/SLL ($n=17$), and blood samples of patients with CLL ($n=82$), with respect to putative adverse prognostic factors, as defined by the HOVON-68 study [20]. High-risk CLL/SLL is defined by the presence of unmutated *IGHV* genes, mutated *IGHV* genes with use of *IGHV3-21*, chromosome 17p or 11q deletions, and/or trisomy of chromosome 12.

Results

IGHV mutation status differs significantly among SLL and CLL samples

The *IGHV* mutation status of the different test samples is presented in Table 1 and Fig. 1a. This analysis revealed that a high percentage (66%) of lymph node samples in the SLL group exhibited unmutated *VH* genes, and that this percentage was different from the lower percentage (29%) observed in the blood samples of the CLL patients. The CLL/SLL-derived lymph node samples yielded intermediate values (35%) ($P=0.003$ based on the exact linear trend test, two sided). Note that there is no overlap in cases between the SLL/CLL and the CLL patient groups. From one case included in the SLL/CLL group, a lymph node specimen and a later blood sample were available. In both samples, identical mutation rates and identical genetic aberrations were observed, indicating a full concordance between the lymph node and blood samples in this patient. Due to lack of blood samples of the other patients with SLL and CLL/SLL, concomitantly testing of blood and lymph node samples was not possible.

Table 1 Molecular prognostic parameters in patients with SLL and CLL

Molecular parameter	SLL ^c	CLL/SLL	CLL	P value
Unmutated <i>IGH</i> status ^a	66% (14/21)	35% (6/17)	29% (24/82)	0.002 ^d
Unmutated <i>IGH</i> status and/or V _H 3–21 usage	66% (14/21)	35% (6/17)	34% (28/82)	0.012 ^d
Adverse prognostic factors				
Unmutated <i>IGH</i> and/or V _H 3–21 usage and/or poor risk genetic aberrations	71% (15/21)	65% (11/17)	43% (35/82)	0.011 ^e
Poor risk genetic aberrations ^b	45% (9/20)	53% (9/17)	22% (18/82)	0.005 ^e
del(17p)	10% (2/20)	24% (4/17)	4% (3/82)	N.d.
del(11q)	20% (4/20)	6% (1/17)	9% (7/82)	N.d.
Trisomy 12	15% (3/20)	24% (4/17)	11% (9/82)	N.d.
del(13q)	55% (11/20)	71% (12/17)	51% (42/82)	N.d.
Other genetic aberrations (e.g., gain 8q24, +19, loss 6q25–26)	20% (4/20)	0% (0/17)	2% (2/82)	0.013 ^d
No genetic aberrations ^f	10% (2/20)	0% (0/17)	37% (30/82)	<0.001 ^e

SLL small lymphocytic lymphoma, CLL chronic lymphocytic leukemia

^a Unmutated *IGHV* genes, defined using the cutoff of higher or equal than 98% homology to the germline sequence

^b Poor risk genetic aberrations, as defined by the deletion of 11q, 17p and/or trisomy12

^c From one SLL case (*IGH* unmutated), there was no material left for analysis of the genetic aberrations by MLPA

^d P value of comparison between the SLL and CLL group

^e P value of comparison between the CLL group and the SLL, SLL/CLL groups

^f As measured by MLPA

Poor risk genetic aberrations prevail in CLL/SLL and SLL lymph node samples

Genetic aberrations associated with adverse outcome in CLL (i.e., deletion of 11q, 17p, and/or trisomy of chromosome 12, according to the criteria of the HOVON study [20]) occurred in about half of the lymph nodes from both the SLL and the CLL/SLL patients. No significant differences were observed between these two groups. Their incidence was, however, significantly different from those observed in the CLL blood samples (Table 1). In all except one case with a trisomy 12 and del(17p), the adverse genetic aberrations were found to be isolated, i.e., not combined with other known adverse genetic aberrations. Interestingly, in the CLL/SLL group, there were more cases with 17p loss as compared to the other two patient groups, as exemplified in Fig. 1b. Loss of 13q was commonly observed, but we found no significant differences in its incidence between the three patient groups studied (Table 1 and Fig. 1b). The loss of 13q was seen both as an isolated genetic abnormality and in combination with adverse genetic abnormalities and/or an unmutated *IGH* gene status (data not shown). Other recurrent genetic aberrations, such as gain of 8q24, trisomy of chromosome 19, and loss of 6q25–26, were more frequently observed in lymph nodes of the SLL patients as compared to the CLL/SLL cases. A lack of genetic abnormalities was significantly more frequent in CLL samples than in the lymph node samples derived from

the SLL or CLL/SLL group (Table 1). From these data, we conclude that the lymph node specimens from SLL patients and CLL/SLL patients may represent a more advanced stage of disease and display pronounce genetic instability, which will most likely result from the inactivation of DNA damage response pathways (such as deletion of 11q22 that encompasses the *ATM* gene).

VH1 gene use is high in SLL samples

Subsequent analysis of *IGHV* gene use revealed a high percentage of *VH1* gene use in the SLL lymph node samples as compared to the other two groups (Table 2). This high percentage of *VH1* gene use can mainly be attributed to the high percentage of cases exhibiting *VH1-69* use (78%). Cases that exhibited *VH1-69* use were mostly, but not exclusively, associated with an unmutated *IGHV* status [43]. In the SLL group and in the CLL/SLL group, seven and three cases with *VH1-69* use were found, respectively, all unmutated. Within the CLL group, 9 out of 11 *VH1-69* cases (including one case with bi-allelic *VH1-69* unmutated rearrangements) were found to be unmutated.

Also, *VH4* gene use occurred frequently in the SLL samples. Specifically, the use of *VH4-39* was frequently seen in the SLL samples, while this use was rarely observed in the CLL samples studied (Table 2). Of interest is that the *VH4-39* SLL cases displayed different *D* and *J* segment use (data not shown). The use of *VH4-34* was absent in the 21

Fig. 1 a Occurrence of mutational status and combined molecular prognostic parameters in SLL and CLL. Poor risk genetic aberrations are defined in our study as the deletion of 11q, 17p, and/or trisomy 12. Poor risk factors (*IGH* + genetic) are defined by unmutated *IGH* status and/or *IGHV*3-21 usage and/or poor risk genetic aberrations. **b** Occurrence of prognostic genetic parameters in SLL and CLL presented as compound bar chart

SLL samples studied, while biased stereotypic use of *VH4-34* expressing cases is found in CLL [31]. In contrast to the CLL group, no *VH3-21* use was observed nor in the SLL nor in the CLL/SLL group (Tables 1 and 2). Rearrangement of the second allele is a well-known phenomenon in leukemia and lymphoma [44, 45] and occurred in the three patient groups as well (Table 2).

Adverse genetic aberrations and *IGHV* mutations prevail in SLL samples

Combining the prognostic molecular parameters, i.e., *IGH* mutation status and *V*-gene use, with the poor risk genetic aberrations, revealed a remarkably high occurrence in SLL as compared to CLL (Table 1). In the SLL group, 13 of the 14 *IGH*-unmutated cases were evaluated by MLPA, 8 of these cases showed adverse genetic aberrations (Table 3). In contrast, only one out of seven *IGHV*-mutated cases showed adverse genetic aberrations, which was trisomy 12 (intermediate risk [14]). Del(17p) and del(11q), which are

categorized as unfavorable markers, are found in the unmutated SLL cases. Also, in the other two groups, adverse genetic aberrations were specifically observed in the *IGHV*-unmutated cases. Overall, most of the *VH1-69*-unmutated cases showed adverse genetic aberrations (Table 3). Although the number of cases studied is admittedly small, our findings indicate that the occurrence of adverse genetic aberrations is correlated to the mutation status, or even to *V*-gene use.

Discussion

We have evaluated the occurrence of prognostic molecular-genetic parameters in lymph node samples of patients with SLL/CLL. We categorized the samples in those that were derived from patients with predominant blood involvement (CLL), those that came from patients with both lymph node and blood involvement (CLL/SLL), and those that came from patients with predominantly lymph node involvement

Table 2 Details on VH gene usage in SLL and CLL

Molecular parameter	SLL	CLL/SLL	CLL
VH1 usage	43% (9/21)	17% (3/18)	30% (26/87)
V _H 1–69 usage	78% (7/9)	100% (3/3)	46% (12/26)
VH2 usage	10% (2/21)	0% (0/18)	6% (5/87)
V _H 2–5 usage	100% (2/2)	0% (0/0)	60% (3/5)
VH3 usage	19% (4/21)	67% (12/18)	43% (37/87)
V _H 3–21 usage	0% (0/4)	0% (0/12)	19% (7/37)
VH4 usage	29% (6/21)	17% (3/18)	20% (17/87)
V _H 4–34 usage	0% (0/6)	66% (2/3)	41% (7/17)
V _H 4–39 usage	50% (3/6)	0% (0/3)	6% (1/17)
VH5 usage	0% (0/21)	0% (0/18)	1% (1/87)
VH6 usage	0% (0/21)	0% (0/18)	1% (1/87)
Bi-allelic rearrangements	24% (5/21)	18% (3/17)	17% (14/82)

SLL small lymphocytic lymphoma, CLL chronic lymphocytic leukemia

The counts for V usage were based on the alleles with an Open Reading Frame (functional alleles) as such identified from the IMGT database. In two out of three (CLL/SLL lymph nodes) and in 9 out of 14 (regular CLL) bi-allelic cases, one of the two alleles was a nonproductive rearrangement. In the remaining cases (one CLL/SLL case and five CLL cases) both functional alleles were either both mutated or both unmutated. One of the CLL cases has bi-allelic *IGHV*1–69 rearrangements, both unmutated

[lymphocytosis $<5 \times 10^9/L$ (SLL)]. An integrated analysis of *IGHV* mutation status, *IGHV* use, and the occurrence of recurrent adverse genetic aberrations were performed. Together, our data indicate that the SLL group exhibits a significantly higher prevalence of adverse prognostic

Table 3 Many adverse genetic aberrations in unmutated cases

Adverse genetic aberrations and mutational status	SLL	CLL/SLL	CLL
11q- in <i>mutated</i> cases	0% (0/7)	0% (0/11)	0% (0/56)
17p- in <i>mutated</i> cases	0% (0/7)	9% (1/11)	2% (1/56)
+12 in <i>mutated</i> cases	14% (1/7)	27% (3/11)	9% (5/56)
11q- in <i>unmutated</i> cases	31% (4/13)	16% (1/6)	29% (7/24) ^a
17p- in <i>unmutated</i> cases	15% (2/13)	50% (3/6)	7% (2/24) ^{a,b}
+12 in <i>unmutated</i> cases	15% (2/13)	16% (1/6)	17% (4/24) ^{a,b}
11q- in <i>IGHV</i> 1–69 <i>unmutated</i> cases	28% (2/7)	33% (1/3)	44% (4/9)
17p- in <i>IGHV</i> 1–69 <i>unmutated</i> cases	0% (0/7)	33% (1/3)	11% (1/9)
+12 in <i>IGHV</i> 1–69 <i>unmutated</i> cases	14% (1/7)	33% (1/3)	11% (1/9)

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^a Unmutated cases include the unmutated *IGHV*3–21 and *IGHV*1–69 cases (but not the mutated *IGHV*3–21 cases, or *IGHV*1–69 cases)

^b One case displayed both trisomy 12 and del(17p)

molecular–genetic markers as compared to CLL. This striking difference can mainly be attributed to the high prevalence of *IGHV*-unmutated cases in the SLL group. We also found a high frequency of adverse genetics aberrations in lymph node samples of the SLL, which was comparable to the CLL/SLL group.

As yet, only limited information is available on the molecular–genetic makeup of lymph nodes of SLL patients, which is mainly due to the fact that the occurrence of SLL is rare. The M.D. Anderson Cancer Center reviewed retrospectively CLL/SLL patient records on characteristics at presentation, treatment modalities, and clinical outcome [22]. It was found that patients with SLL exhibited lower rates of unmutated *IGHV* genes, which are discordant with our data. This difference in outcome is most likely due to the limited number of cases included in both studies. The higher percentage of genetic abnormalities that we observed in lymph node samples of both the SLL and CLL/SLL groups, as compared to those present in the blood CLL samples, was not noted by Flanagan et al. [46]. Instead, they found similar frequencies of these genetic abnormalities in lymphoid tissues as compared to peripheral blood or bone marrow samples. They did, however, not make a distinction between patients with SLL and CLL. The relatively high frequency of genetic alterations in the lymph node samples of both the SLL and the SLL/CLL cases might be a reflection of the compartment or microenvironment in which they arise in. It is noteworthy in this respect that not all genetic alterations tested are increased in the lymph node specimens, as exemplified by 13q- deletions and to a lesser extent trisomy 12 (Fig. 1b). Genetic markers and *IGHV* mutation status were recently assessed in patients with SLL and CLL by the group of Bastard, using methods comparable to ours [23]. They found that trisomy 12 was the most frequent genetic aberration in a cohort of 22 SLL cases. Taking our data and those reported in the literature together (Table 4), it can be concluded that there is no specific increase in trisomy 12 frequency in these patient groups.

Deletion of 17p is the less frequently occurring adverse genetic aberration in SLL. In the CLL/SLL lymph node group in the present study, however, a high number of 17p- cases (24%) was observed as compared to the 4% of 17p- found in CLL patients (which is in line with 7% of 17p- found by Döhner et al. [14]). The high prevalence of 17p- in the CLL/SLL group is most likely due to the fact that these patients were either known as CLL patients with persistent or increasing lymphadenopathy, or as patients predominantly presenting with lymphadenopathy (and an ALC of $\geq 5 \times 10^9/L$). The latter category most likely represents CLL patients with a more advanced stage of disease and discerns them from the classical CLL patients. The high prevalence of 17p-

Table 4 Literature review of the genetic aberrations and *IGHV* mutation status in SLL

SLL	Aoun et al. [25] ^a ; Bahler et al. [21] ^b	Tsimberidou et al. [22]	Daudignon et al. [23]	Garcia et al. [24]	This study
del 17p	1/14	3/44	1/22	1/27	2/20
del 11q	1/14	7/44	4/22	7/27	4/20
Trisomy12	2/14	8/44	8/22	6/27	3/20
del 13q isolated	1/14	N.d.	2/22	13/27	9/20
<i>IGHV</i> mutated	4/10	25/33	11/17	10/26	8/21
<i>IGHV</i> unmutated	6/10	8/33	6/17	16/26	13/21

Note that lymph node specimens were evaluated by Aoun et al. [25], Daudignon et al. [23], Garcia et al. [24], and the current study. Bone marrow biopsies were evaluated by Tsimberidou et al. [22]. In this table, the FISH data (and not the cytogenetics data) of the Tsimberidou study were included because of reasons of comparison to the other studies

^a The study that reports the SLL data on the genetic aberrations

^b The study that reports the SLL data on the *IGHV* mutation status

supports the findings reported by the group of Trbusek, who demonstrated that up to 20% of treated CLL patients acquired new *TP53* mutations [47, 48].

Only a limited number of *IGHV* mutation studies have been performed in SLL (Table 4). Some of these studies reported an overrepresentation of mutated cases [21, 22], whereas other studies have reported an overrepresentation of unmutated SLL cases [20, 23]. In a study reported by Leuenberger et al. [49], using lymph node samples from both patients with SLL and CLL, frequent unmutated cases as well as a frequent use of *VH3* followed by *VH4* and *VH1* respectively were observed. *VH1-69* was, similar to the SLL cases included in our study, the most prevalent *VH* gene detected. Taken together with the literature data (listed in Table 4), it is evident that, as yet, only a limited number of SLL cases has been studied in detail at the molecular–genetic level, which may explain the (partly) contradictory results outlined above, and may preclude an in-depth evaluation of the occurrence of relevant prognostic molecular–genetic parameters in SLL.

Whether stereotypic patterns of somatic hypermutation specifically occur in SLL is not thoroughly investigated yet. In CLL, multiple subsets of patients appear to exist that display similar immunoglobulin gene use with almost identical complementary determining regions [31]. In our SLL study group, we did not observe *VH3-21* or *VH4-34* use, whereas CLL cases with this *V* gene use often display characteristic stereotypic patterns of hypermutation [32]. *IGHV1-69* use is frequently observed in CLL, often in an unmutated form. *IGHV1-69* use was also frequently encountered in our SLL patient group. Of the seven unmutated *IGHV1-69* SLL cases, five exhibited specific *D* and *J* segment use, which is also seen in “stereotypic” CLL patients. This latter result indicates that stereotypy may be involved in SLL as well. For a better evaluation of *VH* use and the occurrence of stereotypic patterns of

somatic hypermutation in SLL, a combined study effort is needed.

In summary, we have assessed the occurrence of several molecular–genetic prognostic factors in clinically distinct groups of CLL/SLL patients. By doing so, we found that the prevalence of adverse prognostic genetic aberrations and unmutated *IGHV* genes is significantly higher in patients with SLL as compared to those with CLL. Our data support the current WHO classification and illustrate that CLL and SLL likely encompass a phenotypic spectrum within one disease entity.

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