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Chronic Lymphocytic Leukemia with t(14;18)(q32;q21)

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Abstract

The t(14;18)(q32;q21) is a cytogenetic hallmark of follicular lymphoma and also occurs in approximately 20% of diffuse large B-cell lymphomas of follicle center cell origin. Relatively few cases of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) with t(14;18) have been reported previously. We report the clinicopathologic, cytogenetic, and molecular genetic features of 12 patients with CLL associated with t(14;18). There were nine men and three women, with a median age of 51 years at diagnosis. To date, 11 patients have required chemotherapy, six before coming to our institution. At last follow-up five patients have died of disease. Karyotypic analysis showed that ten cases had t(14;18) in the stemline and two cases in the sideline; t(14;18) was the sole abnormality in the stemline in two cases. In 11 cases, other abnormalities were identified in the stemline or sidelines, most commonly trisomy 12 in six cases. Trisomy 12 was associated with atypical morphology and immunophenotype. Seven out of eight cases tested showed somatically mutated *IGHV* genes. We conclude that the t(14;18) in CLL is associated with relatively young age at diagnosis, mutated *IGHV* genes, and a clinical course that usually requires chemotherapy. The cytogenetic findings, in particular, t(14;18) in the stemline in ten cases, and as the sole karyotypic abnormality in two cases, suggests that t(14;18) is an early pathogenetic event in this small subset of CLL cases.

Keywords

CLL; t(14;18)(q32;q21); *BCL2*; *IGHV* somatic mutation; trisomy 12

INTRODUCTION

Chronic lymphocytic leukemia (CLL), the most common adult leukemia in Western countries, affects ~2-6 per 100,000 persons per year in the United States, with a mean age at diagnosis of 65 years [1]. The diagnosis of CLL is based on characteristic morphologic and immunophenotypic features. The neoplastic cells are generally small lymphocytes that express CD5, CD19, and CD23, dimly express CD20 and surface immunoglobulin, and are negative for FMC-7 [2]. In 60-80% of patients, recurrent genomic abnormalities can be

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identified, such as deletions in chromosomes 6q21, 11q22, 13q14, 17p13, and trisomy 12 (+12) [3,4]. Chronic lymphocytic leukemia has a variable clinical course and long-term outcome. Deletions in chromosome 13q14, the most frequent aberration (~50% of cases), have been associated with a favorable long-term outcome when present as a sole abnormality [4,5]. In contrast, deletions in chromosome 11q22 (~10-20% of cases), which includes the *ataxia-telangiectasia mutated* (*ATM*) gene, and 17p13 (~3-5% of cases), which includes the *tumor protein p53* (*TP53*) gene, are independent predictors of rapid disease progression, treatment resistance, and inferior survival [4,5].

Chromosomal translocations are uncommon in CLL, identified in fewer than 5% of all cases [6-9]. The t(14;18)(q32;q21), involving *IGH@* and *BCL2*, is characteristic of follicular lymphomas and is found in ~90% of cases [10]. It is unusual in CLL, and is found in, at most, 1-2% of cases [6,11-17]. The prognostic significance of t(14;18) in CLL is not clear. Several studies have suggested that translocations involving the immunoglobulin heavy chain are associated with a poorer prognosis in CLL patients [6,7,9]. However, other studies failed to demonstrate a strong association between the t(14;18) and an inferior outcome [11,13,16,17]. The pathophysiologic role, if any, of t(14;18) in initiation and evolution of CLL is also unclear. In this study we report the clinical, morphologic, immunophenotypic, cytogenetic, and molecular genetic features of 12 cases of CLL associated with t(14;18)(q32;q21).

MATERIAL AND METHODS

Case selection

We searched the files of the Clinical Cytogenetics Laboratory, Department of Hematopathology, The University of Texas M. D. Anderson Cancer Center (MDACC) for cases of CLL with t(14;18)(q32;q21) between 1996 to 2010. We identified 12 cases (<1% of all cases) classified as CLL/SLL based on morphologic and immunophenotypic criteria. Two cases, cases 8 and 10, have been reported previously [11]. We obtained the clinical and laboratory data by review of medical records.

Morphological examination

We reviewed peripheral blood, bone marrow (BM) aspirate smears, touch imprints, aspirate clot and core biopsy specimens for all cases. Peripheral blood smears were stained with May–Grünwald–Giemsa, and a manual 100-cell differential white blood cell (WBC) count was performed. Differential cell counts of 500 cells were performed either on BM touch imprints or aspirate smears stained with Wright–Giemsa. Particular attention was paid to the cytology of lymphocytes with respect to atypical morphological features, including indented or cleaved nuclei, plasmacytoid features, and the presence of prolymphocytes. Bone marrow aspirate clot and decalcified core biopsy specimens were routinely processed and stained with hematoxylin and eosin (H&E). The BM cellularity was estimated (to the nearest 5%) and the pattern of lymphocytic infiltration was classified as nodular, interstitial, diffuse, or a combination of these patterns.

Immunophenotypic analysis by flow cytometry

A BM aspirate specimen from each patient was assessed by three- or four-color flow cytometry as described previously [8]. The lymphocyte population was gated using right angle light scatter and CD45 expression. The panel of monoclonal antibodies used included reagents specific for: CD5, CD10, CD11c, CD19, CD20, CD22, CD23, CD38, CD45, CD79b, FMC7 and immunoglobulin light chains. Ten thousand events were acquired to a FACSCalibur (Becton Dickinson, San Jose, CA, USA) and the data were analyzed using CellQuest software (Becton Dickinson). For each case the CLL score was calculated according to the system of Matutes and co-workers [2], subsequently modified by Moreau and co-workers [18]. Scores were based on five variables: expression of dim surface immunoglobulin, CD5, CD23, dim or absent CD22/CD79b, and absent FCM-7. Cases with a score of 4-5 were considered to have typical immunophenotypes; cases that deviated from this pattern of antigen expression were considered to have atypical immunophenotypes.

Immunohistochemical analysis for ZAP70 (1:100; Upstate Cell Signaling Systems, Lake Placid, NY, USA) was performed using formalin-fixed and paraffin-embedded (FFPE) tissue sections of BM core biopsy or aspirate clot specimens, as described previously [19].

Somatic mutation analysis of the immunoglobulin heavy chain variable region genes

Sequence analysis of the immunoglobulin heavy chain variable region (*IGHV*) genes was performed using total RNA extracted from BM aspirate material or DNA extracted from formalin-fixed, paraffin-embedded (FFPE) BM aspirate clot sections, as described previously [8]. The *IGHV* somatic mutation status was designated as unmutated if there was <2% deviation or as mutated if there was ≥2% deviation from germline sequences [20].

Conventional cytogenetic and fluorescence in situ hybridization analyses

Conventional cytogenetic analysis was performed on metaphase cells prepared from BM aspirate specimens cultured for 24 hours without mitogens, or for 72 hours with lipopolysaccharide, using standard techniques. Twenty Giemsa-banded metaphases were analyzed and the results were reported using the International System for Human Cytogenetic Nomenclature (ISCN 2009) [21].

Fluorescence in situ hybridization (FISH) for common abnormalities associated with (but not specific for) CLL was performed on interphase nuclei obtained from cultured BM cells using a multi-color probe panel designed to detect deletions of 11q22.3 (*ATM*), 13q14.3 (*D13S319*), 13q34 (*LAMP1*), 17p13.1 (*TP53*) and trisomy 12 (*12p11.1-q11*) according to the manufacturer's instructions (Abbott Molecular, Abbott Park, IL). FISH analysis for t(14;18)(q32;q21) was performed using the LSI *IGH@BCL2* dual-color, dual fusion translocation probe (Abbott Molecular, Abbott Park, IL) on interphase nuclei obtained from cultured BM cells or on FFPE BM aspirate clot sections.

RESULTS

Clinical findings

The clinical and laboratory data are summarized in Table 1. There were nine men and three women. At diagnosis of CLL, the patients were of median age of 51 years (range, 41-78 years). All patients had low Rai stage disease (stage 0, n=7; stage I, n=5). In the five patients (cases 3, 4, 7, 10, 12) with lymphadenopathy at presentation (Rai stage I), the lymph node involvement was localized. Subsequently, at presentation to our institution, their median age was 67 years (range, 46-79). Nine had progressed to advanced stages (stage III, n=2; stage IV, n=7); one patient had progressed from stage 0 to stage I and two showed no evidence of progression. The median interval between initial diagnosis and presentation at MDACC was 52.5 months (range, 12-349).

At our institution, physical examination demonstrated lymphadenopathy in seven patients (cases 1-4, 6, 7, 11) and splenomegaly in four patients (cases 1, 2, 10, and 11). To date, three patients (cases 5, 8, and 9) have not developed lymphadenopathy. Eleven out of 12 patients (cases 1-10, 12) had an absolute lymphocytosis (median, $18.95 \times 10^9/L$; range $0.25-98 \times 10^9/L$). Ten patients (cases 1-5, 7, 8, 10-12) had anemia, with a hemoglobin level ranging from 7.7 to 14.4 g/dl with a median of 11.8 g/dl (reference range, 14-18 g/dl). Seven patients (cases 1, 2, 4, 5, 7, 10, 11) had thrombocytopenia (median of $125 \times 10^9/L$; range $23-255 \times 10^9/L$; [reference range $140-440 \times 10^9/L$]). Eight patients (cases 1, 2, 4, 6-8, 10, 11) reported "B" symptoms, particularly fatigue and night sweats. Serum lactate dehydrogenase (LDH) levels were increased in five patients (cases 1, 3, 9, 10, 12) (median 612.5, range 389-817 IU/L; [reference range: 313-618 IU/L]). All patients showed an elevated serum beta-2 microglobulin (B2M) level (median 3.95, range 2.0-10.4 mg/L; [reference range: 0.7-1.8 mg/L]; in 5 patients (cases 2, 3, 7, 10, 11) the level was 4.0 mg/L, which is associated with a poor prognosis [22].

Eleven patients subsequently received chemotherapy for CLL (cases 1-8, 10-12), six before presentation to MDACC (cases 1-4, 7, 10). One patient also received an allogeneic stem cell transplant (case 2). The interval between initial diagnosis and chemotherapy ranged from 0 months (cases 3 and 4) to 30 years (case 11), with a median treatment free survival of 24 months (range, 0-349 months). One patient has not yet required treatment 28 month after diagnosis (case 9). With a median follow-up of eight years (range, 2-29 years), five patients have died of disease (one with Richter's transformation, case 4), and seven patients are alive (six with persistent CLL and one in clinical remission), with a median overall survival of 10 years.

Morphologic and immunophenotypic findings

The morphological features are summarized in Table 2. Eight cases (cases 1-2, 5-7, 9, and 11-12) showed typical CLL morphology. Three cases (cases 3, 4, and 8) were cytologically atypical, with irregular nuclear contours and/or plasmacytoid differentiation (Figure 1 A, B). One case (case 10) showed increased prolymphocytes (24%). The cellularity of the BM ranged from 50-95% (median, 80%), and the neoplastic lymphoid cells comprised of 50-90% of the BM cellular elements, with an interstitial, nodular, diffuse, or mixed pattern;

none showed a paratrabeular pattern of involvement. Proliferation centers were noted in bone marrow one case (case 8).

The results of immunophenotypic analysis performed by flow cytometry and immunohistochemistry on bone marrow specimens are summarized in Table 3. Using the modified Matutes system, seven cases (cases 1, 2, 6, 7, 9, 11, 12) showed typical immunophenotypes and five cases (cases 3-5, 8, 10) showed atypical immunophenotypes (score ≥ 3), without expression of CD5 in one case (case 3), absent or weak expression of CD23 in three cases (cases 3-5), positivity for FMC-7 in two cases (cases 4 and 5), moderate to bright expression of immunoglobulin light chain in four cases (cases 3, 4, 10, 12), and bright CD20 expression in three cases (cases 3, 5, 8). Five cases were positive for CD38 (cases 2-5, 8). CD10 was negative in all 10 cases tested (cases 1-5, 7-9, 11-12). Nine cases were tested for ZAP70; seven were negative (cases 1, 5, 7-10, 12) and two were positive (cases 3 and 11).

Two patients (cases 3 and 4) subsequently underwent fine needle aspiration of enlarged retroperitoneal lymph nodes for suspected clinical progression/transformation to diffuse large B-cell lymphoma, 11 and 8 years after diagnosis, respectively. Although the CLL cells in bone marrow in case 3 were morphologically and immunophenotypically atypical (Tables 2 and 3), the CLL cells in the lymph node aspirate showed typical morphology and immunophenotype, i.e., small round lymphoid cells positive for CD5, 19, 20, 23, and monotypic surface lambda light chain, and negative for CD10 by flow cytometry. In case 4, the fine needle aspirate, performed at presentation to our institution, showed typical CLL morphology. Interestingly, a biopsy specimen obtained from a rib lesion two years later revealed anaplastic large cell lymphoma (ALCL) [11].

Somatic mutation status of the IGHV genes

Sequence analysis of the *IGHV* genes was performed in the eight cases with material available for analysis, using total RNA extracted from BM aspirate material (cases 6, 8, 9, 11) or DNA extracted from FFPE tissue sections obtained from BM aspirate clot sections (cases 1, 3, 5, 7) (Table 2). Seven cases were mutated (cases 1, 3, 5-9) and one was unmutated (case 11). Seven cases used *IGHV3* family genes (cases 1, 5-9, 11), and one case used *IGHV1-8* (case 3). Expression of ZAP70 was concordant with somatic mutation status (mutated and ZAP70 negative, or unmutated and ZAP70 positive) in six cases (cases 1, 5, 7-9, 11) and was discordant (mutated and ZAP70 positive) in one case (case 3).

Cytogenetic findings

The results of conventional karyotypic and FISH analyses are summarized in Table 4. All patients underwent conventional cytogenetic analysis upon presentation at MDACC. In each patient the neoplasm carried t(14;18)(q32;q21), including 10 cases in the stemline (cases 2, 4-12) and two cases in a sideline (cases 1 and 3). By conventional cytogenetic analysis, t(14;18) was the sole cytogenetic abnormality in the stemline in four cases (cases 6, 7, 9, 12). The most common abnormality in addition to the t(14;18) was +12, in the stemline in 5 cases (cases 3-5, 8, 10) and in a sideline in 1 case (case 1) (Figure 1C). *IGH@/BCL2* rearrangement was confirmed by FISH analysis in nine of 10 cases with material available

(cases 1-3, 6-9, 11, 12) (Figure 1D) and by PCR assay in one case (case 10). Conventional cytogenetic analysis of the initial diagnostic material was available in two cases (cases 9 and 10) and both showed t(14;18) in the stemline.

We also performed FISH analysis on cultured bone marrow cells in seven cases with material available for analysis (cases 3, 5, 6, 8, 9, 11) to assess for common abnormalities in CLL, i.e., deletions of 11q22.3, 13q14.3, 13q34, and 17p13.1 and +12. In all cases, the results of conventional cytogenetic and FISH analyses for +12 and del(17)(p13.1) were concordant. However, FISH analysis also demonstrated del(13q14.3) in four cases (case 5, 112/200 interphase nuclei; case 6, 156/200 interphase nuclei; case 9, 102/200 interphase nuclei; case 11, 191/200 interphase nuclei) in which it was not apparent by conventional cytogenetic analysis.

DISCUSSION

The t(14;18)(q32;q21), involving the *IGH@* and *BCL2* genes, is a hallmark of follicular lymphoma and occurs in ~20% of diffuse large B-cell lymphomas, but is rare in CLL [6,16,17]. We identified 12 cases with CLL and t(14;18) in the past 15 years. All patients presented to our institution after the diagnosis of CLL had been established, six of whom had received treatment. Conventional cytogenetic analysis demonstrated the t(14;18)(q32;q21), ten in the stemline, and two as the sole abnormality in the stemline. The t(14;18) as a sole abnormality in the stemline has been reported previously in a subset of CLL cases associated with t(14;18) [6,9,16,17]. This raises the possibility that t(14;18) may be an early pathogenetic event in these cases.

Trisomy 12, the most common numeric chromosomal abnormality in CLL, is identified in ~10-20% of cases by conventional cytogenetic analysis [3,4,23]. Consistent with previous reports of CLL with t(14;18) [11,13,16,17], half of our cases also demonstrated +12. Chronic lymphocytic leukemia cases with +12 frequently show atypical morphology and immunophenotype [24]. In our study, all five cases that were morphologically and/or immunophenotypically atypical showed trisomy 12, in the stemline in four cases and in a sideline in one case; none that lacked +12 were atypical. Thus, it appears that in CLL cases with t(14;18), atypical features may be associated with the +12 abnormality rather than the t(14;18). An association has also been described between +12 and other translocations in CLL [8]. The biological basis of this association is unknown.

The presence of the t(14;18), particularly in CLL cases with an atypical immunophenotype, raises the possibility that these cases represent CD5-positive follicular lymphomas rather than CLL. CD-5 positive follicular are rare, with fewer than 40 cases reported in the literature [25]. Several findings argue against the possibility that our cases are follicular lymphomas. All patients in this study presented with lymphocytosis, which would be unusual for follicular lymphoma, particularly in the patients without lymphadenopathy (Rai stage 0) at diagnosis. None of the cases showed a paratrabecular pattern of bone marrow involvement nor did any express CD10. Both the morphology and the immunophenotype were typical for CLL in seven case. All five cases that were morphologically and/or immunophenotypically atypical for CLL showed trisomy 12. Thus, we believe that the cases

we report are best classified as CLL. However, in cases with an atypical immunophenotype, the t(14;18), and lymphadenopathy, a lymph node biopsy may be necessary to exclude a diagnosis of follicular lymphoma.

The del(13)(q14.3) is the most common cytogenetic abnormality reported in CLL, identified in at least 50% of cases by FISH analysis [3,26]. This region contains the microRNA genes, *miR-15a* and *miR-16-1*, whose products are important regulators of BCL2 expression, and are believed to play an important role in the pathogenesis of CLL cases that harbor this deletion [26]. Most importantly, del(13)(q14.3) is associated with a better prognosis than other cytogenetic subtypes [3]. In our cohort, four of seven cases tested (57%) showed del(13)(q14.3) by FISH, which is comparable the frequency expected in CLL in general [3]. These deletions were not detected by conventional cytogenetic analysis.

Molecular genetic analysis of the *IGHV* somatic mutation status in CLL patients has identified two prognostic subtypes; patients whose CLL cells lack somatic mutations (~50% of patients) have a poorer prognosis than patients whose CLL cells contain somatic mutations, with a median survival of 8 and 24 years respectively [27,28]. Most of the cases in the current study (87.5%) were mutated. Approximately three quarters of these cases used the *VH3* family and none used a *VH* family known to be associated with a poor prognosis, such as *VH3-21* [29]. Similarly, Put and co-workers [16] in a study of 25 cases of CLL associated with t(14;18) found that approximately 90% of cases were mutated and used the *VH3* family in approximately 75% of cases, with none using *VH3-21*. Since CLL cases are generally evenly divided between somatically mutated and unmutated [28,30], these findings suggest that there is an association between the t(14;18) and *IGHV* somatic mutation. In follicular lymphomas the *IGHV* genes are heavily mutated [31], with *VH3* family as the most common one (found in approximately 70% of cases) [32]. Expression of ZAP70 protein in CLL has been used as a surrogate marker for *IGHV* somatic mutation status [19,33]. In general, unmutated cases are ZAP70 positive and mutated cases are ZAP70 negative. In our cohort, only one case showed discordance between the somatic mutation status and ZAP70 expression, a mutated case that was ZAP70 positive.

Although the number of cases is small, our results suggest that t(14;18) in CLL is associated with a poor prognosis. Eleven of 12 patients in this study required chemotherapy, and five have died of disease, ranging from 111-377 months after diagnosis. As most of these cases were mutated, a group with an expected median survival of 293 months [28], it seems that we have identified an important subset within the mutated group that may require more aggressive therapy. However, we acknowledge that 6 patients in this study had trisomy 12, which also may have contributed to the poorer prognosis in our study group [3]. In addition, since we identified cases based on the results of conventional cytogenetic analysis, there is a selection bias for cases that divide in culture, which has been associated with a poorer prognosis in CLL [34].

In summary, the t(14;18) in CLL is associated with a relatively young age at diagnosis, somatically mutated *IGHV* genes, trisomy 12, requirement for chemotherapy, and possibly poorer survival. Thus, our results have identified a subset of patients in the mutated CLL group that may not be indolent clinically. In addition, the finding of t(14;18) in the stemline

in ten cases, as a sole abnormality in two cases, raises the possibility that the t(14;18) is an early pathogenetic event in this subset of CLL cases.

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References

1. Rozman C, Montserrat E. Chronic lymphocytic leukemia. *N Engl J Med.* 1995; 333:1052–1057. [PubMed: 7675049]
2. Matutes E, Owusu-Ankomah K, Morilla R, et al. The immunological profile of B-cell disorders and proposal of a scoring system for the diagnosis of CLL. *Leukemia.* 1994; 8:1640–1645. [PubMed: 7523797]
3. Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med.* 2000; 343:1910–1916. [PubMed: 11136261]
4. Juliusson G, Oscier DG, Fitchett M, et al. Prognostic subgroups in B-cell chronic lymphocytic leukemia defined by specific chromosomal abnormalities. *N Engl J Med.* 1990; 323:720–724. [PubMed: 2201915]
5. Goorha S, Glenn MJ, Drozd-Borysiuk E, Chen Z. A set of commercially available fluorescent in-situ hybridization probes efficiently detects cytogenetic abnormalities in patients with chronic lymphocytic leukemia. *Genet Med.* 2004; 6:48–53. [PubMed: 14726809]
6. Lu G, Kong Y, Yue C. Genetic and immunophenotypic profile of IGH@ rearrangement detected by fluorescence in situ hybridization in 149 cases of B-cell chronic lymphocytic leukemia. *Cancer Genet Cytogenet.* 2010; 196:56–63. [PubMed: 19963136]
7. Mayr C, Speicher MR, Kofler DM, et al. Chromosomal translocations are associated with poor prognosis in chronic lymphocytic leukemia. *Blood.* 2006; 107:742–751. [PubMed: 16179374]
8. Huh YO, Schweighofer CD, Ketterling RP, et al. Chronic lymphocytic leukemia with t(14;19) (q32;q13) is characterized by atypical morphologic and immunophenotypic features and distinctive genetic features. *Am J Clin Pathol.* 2011; 135:686–696. [PubMed: 21502423]
9. Cavazzini F, Hernandez JA, Gozzetti A, et al. Chromosome 14q32 translocations involving the immunoglobulin heavy chain locus in chronic lymphocytic leukaemia identify a disease subset with poor prognosis. *Br J Haematol.* 2008; 142:529–537. [PubMed: 18547320]
10. Yunis JJ, Oken MM, Kaplan ME, Ensrud KM, Howe RR, Theologides A. Distinctive chromosomal abnormalities in histologic subtypes of non-Hodgkin's lymphoma. *N Engl J Med.* 1982; 307:1231–1236. [PubMed: 7133054]
11. Sen F, Lai R, Albitar M. Chronic lymphocytic leukemia with t(14;18) and trisomy 12. *Arch Pathol Lab Med.* 2002; 126:1543–1546. [PubMed: 12456221]
12. Kern W, Haferlach T, Schnittger S, Schoch C. Detection of t(14;18)(q32;q21) in B-cell chronic lymphocytic leukemia. *Arch Pathol Lab Med.* 2005; 129:410–411. [PubMed: 15737042]
13. Kojima K, Taniwaki M, Yoshino T, et al. Trisomy 12 and t(14;18) in B-cell chronic lymphocytic leukemia. *Int J Hematol.* 1998; 67:199–203. [PubMed: 9631588]
14. Jonveaux P, Hillion J, Bennaceur AL, et al. t(14;18) and bcl-2 gene rearrangement in a B-chronic lymphocytic leukaemia. *Br J Haematol.* 1992; 81:620–621. [PubMed: 1390255]
15. Haferlach C, Dicker F, Schnittger S, Kern W, Haferlach T. Comprehensive genetic characterization of CLL: a study on 506 cases analysed with chromosome banding analysis, interphase FISH, IgV(H) status and immunophenotyping. *Leukemia.* 2007; 21:2442–2451. [PubMed: 17805327]
16. Put N, Meeus P, Chatelain B, et al. Translocation t(14;18) is not associated with inferior outcome in chronic lymphocytic leukemia. *Leukemia.* 2009; 23:1201–1204. [PubMed: 19295547]
17. Baseggio L, Geay MO, Gazzo S, et al. In non-follicular lymphoproliferative disorders, IGH/BCL2-fusion is not restricted to chronic lymphocytic leukaemia. *Br J Haematol.* 2012

18. Moreau EJ, Matutes E, A'Hern RP, et al. Improvement of the chronic lymphocytic leukemia scoring system with the monoclonal antibody SN8 (CD79b). *Am J Clin Pathol*. 1997; 108:378–382. [PubMed: 9322589]
19. Admirand JH, Rassidakis GZ, Abruzzo LV, Valbuena JR, Jones D, Medeiros LJ. Immunohistochemical detection of ZAP-70 in 341 cases of non-Hodgkin and Hodgkin lymphoma. *Mod Pathol*. 2004; 17:954–961. [PubMed: 15133473]
20. Fais F, Ghiotto F, Hashimoto S, et al. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. *J Clin Invest*. 1998; 102:1515–1525. [PubMed: 9788964]
21. Shaffer LG, Slovak ML, Campbell LJ, editors. ISCN(2009): An International System for Human Cytogenetics Nomenclature. S.Karger; Basel: 2009.
22. Wierda WG, O'Brien S, Wang X, et al. Prognostic nomogram and index for overall survival in previously untreated patients with chronic lymphocytic leukemia. *Blood*. 2007; 109:4679–4685. [PubMed: 17299097]
23. Escudier SM, Pereira-Leahy JM, Drach JW, et al. Fluorescent in situ hybridization and cytogenetic studies of trisomy 12 in chronic lymphocytic leukemia. *Blood*. 1993; 81:2702–2707. [PubMed: 7683927]
24. Matutes E, Oscier D, Garcia-Marco J, et al. Trisomy 12 defines a group of CLL with atypical morphology: correlation between cytogenetic, clinical and laboratory features in 544 patients. *Br J Haematol*. 1996; 92:382–388. [PubMed: 8603004]
25. Sekiguchi Y, Imai H, Wakabayashi M, et al. CD5-positive follicular lymphoma: a case report and literature review. *Intern Med*. 50:899–904. [PubMed: 21498939]
26. Nicoloso MS, Kipps TJ, Croce CM, Calin GA. MicroRNAs in the pathogeny of chronic lymphocytic leukaemia. *Br J Haematol*. 2007; 139:709–716. [PubMed: 18021085]
27. Damle RN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood*. 1999; 94:1840–1847. [PubMed: 10477712]
28. 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. [PubMed: 10477713]
29. Krober A, Bloehdorn J, Hafner S, et al. Additional genetic high-risk features such as 11q deletion, 17p deletion, and V3-21 usage characterize discordance of ZAP-70 and VH mutation status in chronic lymphocytic leukemia. *J Clin Oncol*. 2006; 24:969–975. [PubMed: 16418492]
30. Krober A, Seiler T, Benner A, et al. V(H) mutation status, CD38 expression level, genomic aberrations, and survival in chronic lymphocytic leukemia. *Blood*. 2002; 100:1410–1416. [PubMed: 12149225]
31. Aarts WM, Bende RJ, Steenbergen EJ, et al. Variable heavy chain gene analysis of follicular lymphomas: correlation between heavy chain isotype expression and somatic mutation load. *Blood*. 2000; 95:2922–2929. [PubMed: 10779440]
32. Bahler DW, Campbell MJ, Hart S, Miller RA, Levy S, Levy R. Ig VH gene expression among human follicular lymphomas. *Blood*. 1991; 78:1561–1568. [PubMed: 1909196]
33. Crespo M, Bosch F, Villamor N, et al. ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. *N Engl J Med*. 2003; 348:1764–1775. [PubMed: 12724482]
34. Juliusson G, Gahrton G. Abnormal/normal metaphase ratio and prognosis in chronic B-lymphocytic leukemia. *Cancer Genet Cytogenet*. 1985; 18:307–313. [PubMed: 3877560]

Figure 1.

Case 8. **A.** The bone marrow core biopsy shows an interstitial and nodular infiltrate of small lymphoid cells (H&E $\times 100$). **B.** Lymphoid cells in the bone marrow aspirate smear show irregular nuclear contours and nuclear clefts (Wright-Giemsa $\times 1000$). **C.** Conventional

cytogenetic analysis demonstrates a complex karyotype that includes the t(14;18) and +12, as well der(8;17)(q10;q10), which results in deletion of the short arm of chromosome 17. **D.** FISH analysis for *IGH@/BCL2* rearrangement. The *IGH@* locus on chromosome 14q32 is labeled with SpectrumGreen and the *BCL2* locus on chromosome 18q21 is labeled with SpectrumOrange. The fusion genes are indicated by the yellow fusion signals. The interphase nuclei on the left are negative for rearrangement; the interphases on the right are positive. The metaphase in the center demonstrates green and red signals on the uninvolved chromosomes 14 and 18, and fusion signals on the derivative chromosomes 14 and 18.

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Table 1

Clinical features

Case / Gender	Age / Stage at Dx	Age / Stage at MDACC	Months between Dx & Tx	Hgb (g/dL)	Lymph Count (×10 ⁹ /L)	Platelet Count (×10 ⁹ /L)	“B” symptoms	LDH (IU/L)	B2M (mg/L)	LN	SP	Prior Therapy	Therapy at MDACC	Outcome
1 / F	45 / 0	69 / IV	243	11.7	98	57	Yes	817	5.8	Yes	Yes	Yes	FCR	DOD
2 / F	55 / 0	65 / IV	28	9.5	0.25	31	Yes	517	10.4	Yes	Yes	Yes	CHOP, FCR, SCT	DOD
3 / F	69 / I	79 / III	0	11.6	20	167	No	780	9.3	Yes	No	Yes	FC	DOD
4 / M	64 / I	72 / IV	88	9.9	12.5	23	Yes	502	3.4	Yes	No	Yes	FC	DOD
5 / M	44 / 0	46 / IV	26	13.6	95	127	No	499	2.7	No	No	No	FCR	AWD
6 / M	52 / 0	54 / I	54	14.4	20	247	Yes	389	2.0	Yes	No	No	FCR	AWD
7 / M	49 / I	52 / IV	1	13.2	13	124	Yes	608	4.6	Yes	No	Yes	FCR	ACR
8 / M	50 / 0	51 / 0	52	13.1	6.3	255	Yes	426	2.5	No	No	No	FCR	AWD
9 / M	78 / 0	79 / 0	NA	14.1	17.9	164	No	625	2.0	No	No	No	No	AWD
10 / M	50 / I	52 / IV	3	11.3	10.9	79	Yes	721	4.3	No	Yes	Yes	FCR	AWD
11 / M	41 / 0	70 / IV	349	7.7	33.5	102	Yes	617	8.0	Yes	Yes	No	FCR	AWD
12 / M	70 / I	76 / III	121	11.9	44	135	No	677	3.6	No	No	No	FCR	DOD

Abbreviations: F, female; M, male; Dx, diagnosis; Tx, treatment; Hgb, hemoglobin; Lymph, lymphocyte; LDH, lactate dehydrogenase; B2M, beta-2 microglobulin; LN, lymphadenopathy; SP, splenomegaly; F, fludarabine; C, cyclophosphamide; R, rituximab; CHOP, cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone; SCT, allogeneic stem cell transplant; AWD, alive with disease; ACR, alive in complete remission; NA, not applicable

Table 2Morphologic findings and *IGHV* somatic mutation status

Case	PB and BM lymphocyte cytology	BM cellularity; Pattern of involvement; % CLL	<i>IGHV</i> SM status; <i>VH</i> family; % deviation from germline;
1	Typical CLL morphology	80%; D, I; 60%	Mutated; <i>VH</i> -30; 8.5%
2	Typical CLL morphology	90%; D; 80%	ND
3	Heterogeneous, plasmacytoid differentiation, 12% prolymphocytes	90%; D, N; 90%	Mutated; <i>VH</i> -18; 6.9%
4	Heterogeneous, nuclear irregularities, plasmacytoid differentiation, 6% prolymphocytes	85%; I, N; 90%	ND
5	Typical CLL morphology	50%; I; 50%	Mutated; <i>VH</i> -48; 8%
6	Typical CLL morphology	80%; N, I; 80%	Mutated; <i>VH</i> -3; 4.1%
7	Typical CLL morphology	95%; I; 90%	Mutated; <i>VH</i> -53; 9.6%
8	Heterogeneous, nuclear irregularities, 10% prolymphocytes	80%; I; 55%	Mutated; <i>VH</i> -62; 14%
9	Typical CLL morphology	70%; N; 60%	Mutated; <i>VH</i> -07; 7%
10	Increased prolymphocytes, 24%	80%; D; 85%	ND
11	Typical CLL morphology	95%; I, N; 90%	Unmutated; <i>VH</i> -07; 0.2%
12	Typical CLL morphology	85%; I, N; 90%	ND

Abbreviations: PB, peripheral blood; BM, bone marrow; *IGHV*, immunoglobulin heavy chain variable region; SM, somatic mutation; D, diffuse; I, interstitial; N, nodular; ND, not determined

Table 3

Bone Marrow Immunophenotype

Case	CD5	CD23	FMIC7	CD79b/CD22	Surface light chain	CLL score	CD10	CD20	sIgM/D	CD38	ZAP70
1	Pos	Pos	Neg	Weak/Neg	Weak (κ)	5	Neg	Mod	Pos	Neg	Neg
2	Pos	Pos	Neg	Neg/Neg	Weak (κ)	5	Neg	Weak	Neg	Pos	ND
3*	Neg	Weak	Weak	Weak/Neg	Mod (λ)	2	Neg	Bright	Pos	Pos	Pos
4	Pos	Weak	Pos	Neg/Neg	Mod (λ)	2	ND	Mod	Neg	Pos	ND
5	Pos	Neg	Pos	Pos/Neg	Weak (κ)	2	Neg	Bright	Neg	Pos	Neg
6	Pos	Pos	Neg	Weak/Neg	Weak (κ)	5	ND	Mod	Pos	Neg	ND
7	Pos	Pos	Neg	Neg/Neg	Weak (λ)	5	Neg	Weak	Neg	Neg	Neg
8	Pos	Pos	Weak	Pos/Pos	Weak (κ)	3	Neg	Bright	Pos	Pos	Neg
9	Pos	Pos	Weak	Weak/Neg	Weak (λ)	5	Neg	Mod	ND	Neg	Neg
10	Pos	Pos	Neg	Pos/Weak	Mod (λ)	3	ND	Weak	Pos	Neg	Neg
11	Pos	Pos	Neg	Neg/Neg	Weak (κ)	5	Neg	Weak	ND	Neg	Pos
12	Pos	Pos	Neg/Weak	Neg/Neg	Mod (κ)	4	Neg	Mod	Pos	Neg	Neg

* Immunophenotypic analysis performed by flow cytometry on a subsequent lymph node aspirate specimen was typical for CLL.

Abbreviations: Pos, positive; Neg, negative; ND, not determined

Table 4

Cytogenetic findings upon presentation to MDACC

Case	Conventional Karyotype	CLL FISH Panel	IGH/BCL2 Rearrangement
1	46,XX,del(13)(q12q14)[3]/47,idem,+12,t(14;18)(q32;q21)[7]/46,XX[10]	ND	FISH Positive
2	46,XX,inv(6)(p21q21),t(14;18)(q32;q21)[7]/46,XX,t(14;18)(q32;q21)[7]/46,XX[6]	ND	FISH Positive
3	47,XX,+12[5]/47,XX,t(6;7)(q15;p15),t(14;18)(q32;q21.3),+12[3]/46,XX[11]	+12	FISH Positive
4	46,X,-Y,+12,t(14;18)(q32;q21)[6]/46,XY[8]	ND	ND
5	47,XX,+12,t(14;18)(q32;q21)[7]/46,XY[13]	+12, del(13q14.3)	ND
6	46,XX,t(14;18)(q32;q21.3)[6]/46,XY[8]	del(13q14.3)	FISH Positive
7	46,XX,t(14;18)(q32;q21)[16]	Negative	FISH Positive
8	46,XX,der(8;17)(q10;q10),+12,t(14;18)(q32;q21.3)[4]/47,XX,add(8)(p21),+12,t(14;18)(q32;q21.3)[1]/46,XY[8]	+12, del(17p13.1)	FISH Positive
9	46,XY[20]*	del(13q14.3)	FISH Positive
10	47,XX,+12,t(14;18)(q32;q21)[5]/47,idem,t(4;13)(q35;q21)[1]/46,XY[24]	ND	PCR Positive for <i>BCL-2</i> MBR
11	46,XX,t(3;11)(q27;q22),add(4)(p16),add(6)(p23),t(14;18)(q32;q21.3)[8]/46,XX,t(3;11)(q27;q22),add(6)(p23),t(14;18)(q32;q21.3)[cp2]/46,XY[3]	del(13q14.3)	FISH Positive
12	46,XX,t(14;18)(q32;q21)[12]/45,X,-Y,t(14;18)(q32;q21)[3]/45,X,-Y[8]/46,XY[4]	ND	FISH Positive

* Conventional cytogenetic analysis performed at diagnosis demonstrated t(14;18)(q32;q21.3).

Abbreviations: FISH, fluorescence *in situ* hybridization analysis; PCR, polymerase chain reaction assay; MBR, major breakpoint cluster region; ND, not determined.