Original Article
Specific chromosomal IG translocations have different prognoses in chronic lymphocytic leukemia

Florence Nguyen-Khac[1,2], Elise Chapiro[1,2], Claude Lesty[1], Aurore Grelier[1], Isabelle Luquet[3], Isabelle Radford-Weiss[4], Christine Lefebvre[5], Sandra Fert-Ferrer[6], Evelyne Callet-Bauchu[7], Eric Lippert[8], Victoria Raggueneau[9], Lucienne Michaux[10], Carole Barin[11], Marie-Agnes Collonge-Rame[12], Francine Mugneret[13], Virginie Eclache[14], Sylvie Taviaux[15], Nicole Dastugue[16], Steven Richebourg[17], Stéphanie Struski[16], Pascaline Talmant[17], Laurence Baranger[18], Nathalie Gachard[19], Carine Gervais[20], Benoit Quilichini[21], Catherine Settegrana[1], Karim Maloum[1], Frederic Davi[1], Hélène Merle-Béral[1,2], on the behalf of the Groupe Francophone de Cytogénétique Hématologique

[1]Service d'Hématologie Biologique, Hôpital Pitié-Salpêtrière, APHP, Université Pierre et Marie Curie-Paris 6, France [2]INSERM U872; Université Pierre et Marie Curie-Paris 6, France, [3]Laboratoire de Cytogénétique, Hôpital de Reims, France, [4]Laboratoire de Cytogénétique, Hôpital Necker-Enfants Malades, France, [5]Laboratoire de Cytogénétique, Hôpital de Grenoble, France, [6]Laboratoire de Cytogénétique, Hôpital de Chambéry, France, [7]Laboratoire de Cytogénétique, Hôpital Lyon-Sud, France, [8]Laboratoire d'Hématologie, CHU Bordeaux, and Laboratoire hématopoïèse leucémique et Cibles Thérapeutiques, INSERMU876, Université Bordeaux2, France, [9]Laboratoire de Cytogénétique, Hôpital A Mignot, Versailles, France, [10]Center for Human Genetics, KULeuven, Belgium, [11]Laboratoire de Cytogénétique, Hôpital de Tours, France, [12]Laboratoire de Cytogénétique, CHU Besançon, France, [13]Laboratoire de Cytogénétique, Dijon, France, [14]Hématologie Biologique, Hôpital Avicenne, Université Paris 13, Bobigny, France, [15]Laboratoire de Cytogénétique, Montpellier, France, [16]Génétique des Hémopathies, CHU Toulouse, France, [17]Laboratoire de Cytogénétique, Hôpital de Nantes, France, [18]Laboratoire de Cytogénétique, CHU Angers, France, [19]Laboratoire de Cytogénétique, Limoges, France, [20]Laboratoire de Cytogénétique, CHU Strasbourg, France, [21]Laboratoire Biomnis, Lyon, France

Abstract: Background: Chromosomal translocations are usually analyzed as a single entity, and are associated with a poor outcome in chronic lymphocytic leukemia. Translocations involving immunoglobulin genes are recurrent, but uncommon (<5%), and their individual prognosis is not clear. The two most frequent partners are *BCL2* (18q21) and *BCL3* (19q13). Designs and methods: Herein, 75 cases are reported of chronic lymphocytic leukemia and t(14;18) (*BCL2*-CLLs). Our series benefits from morphological, immunological and cytogenetical reviews. The *IGHV* status analyses were performed by referring laboratories. Comparison was made with our previously published series of chronic lymphocytic leukemia patients with t(14;19) (*BCL3*-CLLs, n=29). *Results:* Compared with *BCL3*-CLLs, lymphocytosis was lower in *BCL2*-CLLs (p<0.008), and splenomegaly was less frequent (p<0.0001). There were more "typical" morphologies (p<0.005) and Matutes scores ≥4 (p<0.001) in the *BCL2*-CLLs group, and less CD38 expression (p<0.04). More variant *BCL2*-translocations were observed (t(18;22), n=11; 2t(2;18), n=2; p<0.02), and *BCL2*-translocation was frequently single (p<0.002). Complex karyotypes (p<0.02), trisomy 12 (p<0.03), 6q deletion (p<0.002) and *TP53* deletion (p<0.02) were less frequent in *BCL2*-CLLs, whereas 13q deletion was more frequent (p<0.005). The *IGHV* gene was frequently mutated in *BCL2*-CLLs (p<0.0001). Treatment-free survival was longer in *BCL2*-CLLs (p<0.0001). Conclusions: *BCL2*-CLLs express CD5 and lack expression of CD38, and have a Matutes score ≥4, frequent trisomy 12, no *ATM* and 6q deletions, and a mutated *IGHV* status. Compared to *BCL3*-CLLs, *BCL2*-CLLs are much less aggressive; indicating that identifying individual translocations and cytogenetic partners would allow improved patient stratification.

Keywords: Chronic lymphocytic leukemia, *IGH*, *BCL2*, chromosomal translocation

Introduction

Chromosomal translocations involving the immunoglobulin (*IG*) loci are found in some, but not all, forms of B-cell malignancies. Some *IG* translocations are seen in almost all cases of a specific disease subgroup and, therefore, may be useful as a diagnostic marker. In contrast,

cytogenetically identical translocations may be found in several types of disease. Chromosomal translocations are usually associated with poor prognosis in chronic lymphocytic leukemia (CLL) [1-3]. Translocations involving *IG* genes are recurrent, but uncommon (<5%) in CLL. The result of these translocations is deregulated expression of the partner genes. Recurrent known partner genes in CLL include *BCL2* (18q21), *BCL3* (19q13), *BCL11A* (2p11), and *MYC* (8q24), with *BCL2* and *BCL3* being the two most frequent [4]. In most studies, CLL cases with *IG* translocations are analyzed as one group; consequently, the significance of these translocations remains poorly understood.[5, 6] The target gene that becomes overexpressed may be relevant to the outcome. We have recently observed that the t(14;19) translocation, which involves the *BCL3* gene, is associated with an aggressive subgroup of atypical CLLs [7], in accordance with previous publications [8-10].

Herein, we report a clinical and biological study of 75 cases where CLL harbors a t(14;18), or its variants (*BCL2*-CLLs), the t(2;18)(p11;q21) and t(18;22)(q21;q11). This is the largest reported series to date, and is compared with our previously published series of 29 cases with CLL and t(14;19) (*BCL3*-CLLs).

Design and methods

The Groupe Francophone de Cytogénétique Hématologique (GFCH) collected data from 80 B chronic lymphoproliferative disorders with circulating blood cells, excluding follicular lymphoma. The cases had t(14;18) or variant *BCL2*-translocations, and were diagnosed between 1985 and 2009. All patients gave their informed consent in agreement with the Helsinki declaration, and the Institutional Ethics Committee at Pitie-Salpetriere Hospital approved this study. Morphological review was performed for 62 cases by two cytologists (KM and CS), an immunological review was performed for all cases by HM-B. Cytogenetic analyses were performed on peripheral blood or bone-marrow lymphocytes cultured for 72h with 12-O-tetradecanoylphorbol-13-acetate (TPA) or CpG-oligonucleotides + interleukin 2 (IL-2). All karyotypes were reviewed by members of the GFCH. Chromosomes were classified according to the International System for Human Cytogenetic Nomenclature (ISCN 2009). Fluorescence *in situ* hybridization (FISH) was performed on inter-

phase nuclei and metaphases, following standard procedures and using specific probes: IGH, BCL2, IGL, IGK (Dako, Trappes, France), CEP12, 13q14 (D13S319), ATM, p53, 6q21, MALT1, (Abbott, Rungis, France). Analyses of the mutational status of the variable region of the immunoglobulin gene (*IGHV*) were performed by the referring laboratories. Statistical analyses were carried out using Fisher's exact test, and continuous data were analyzed using the Mann-Whitney test. The chi^2-test was used to compare our data with that from the literature. An effect was considered statistically significant if the *p* value was 0.05 or less. Overall survival (OS) and treatment-free survival (TFS) calculated from diagnoses were estimated using Kaplan-Meier methodology, and statistical significance was determined using a log-rank test. All tests were two-sided.

Results

The 80 patients had a gender ratio of 61 males and 19 females. Their median age at diagnosis was 66 years (range 32-83). Seventeen out of 66 (26%) patients had lymphnodes, and four (6%) had splenomegaly. The median level of lymphocytosis was 13.9×10^9/l (range 5.4-106.2×10^9/l). Of the 62 cases that had a cytological review, 29 (47%) had "typical" CLL, 28 (45%) had "atypical" CLL, with more than 10% of lymphoplasmacytoid cells and/or large cells, 4 (6%) had marginal zone lymphoma (MZL), and 1 (2%) had unclassified low-grade lymphoma. The 18 cases that could not be reviewed were classified as CLLs by referring laboratories. In total, there were 75 CLLs and 5 lymphomas. Of the 68 CLLs with available data at diagnosis, 63 (93%) were classified as Binet stage A, 4 (6%) as Binet B, and 1 (1%) as Binet C (Supplementary Table 1).

Regarding CLLs, all tested cases (58/58) were CD10 negative, 69/73 (94%) were CD5 positive and 61/70 (87%) were CD23 positive. Of the 68 CLL cases with an available Matutes score, 57 (84%) had a score ≥4, seven (10%) a score of 3, and four (6%) a score <3. Of 64 analyzed cases, 45 (70%) were CD38 negative. We observed 62 t(14;18) translocations, and 13 variant translocations 11 t(18;22), 2 t(2;18) (**Table 1** and Supplementary **Table 1**). The involvement of *IG* was confirmed by FISH in all tested cases (54 *IGH*, 6 *IGL*), and the involvement of *BCL2* was confirmed by FISH in 69/70 (98%) of tested cases.

Table 1. Morphologic, immunophenotypic, cytogenetic and molecular data

No	Morphology	Matutes score	CD38	Treated before K	Time Diagnosis-K (months)*	Sample	Karyotype (according to ISCN2009)	IGHV mutation % identity	V rearrangement
1	Atypical CLL	3	95%	No	98	Pb	47,XX,+12,t(14;18)(q32;q21)[13]/47,idem,?del(3)(p14p22),add(9)(q34),del(10)(q23),add(17)(q23)[6]	nd	nd
2	CLL	4	1%	No	4	Pb	46,XY,t(14;18)(q32;q21)[15]/46,XY[16]	nd	nd
3	CLL	2	1%	No	0	Pb	47,XX,+12,t(14;18)(q32;q21)[2]/46,XX[13]	nd	nd
4	CLL	nd	2%	No	80	Pb	46,XY,del(13)(q13q22),t(14;18)(q32;q21)[20]	nd	nd
5	CLL	5	2%	No	30	Pb	46,XY,t(14;18)(q32;q21)[9]/46,XY[5]	nd	nd
6	CLL¤	4	0%	No	18	Pb	46,XY,t(14;18)(q32;q21)[16]/46,XY[3]	M	V3-23
7	CLL	4	5%	No	5	Pb	46,XX,t(14;18)(q32;q21)[7]/46,idem,del(10)(q23q24)[7]/46,idem,der(1)t(1;1)(p12;q34),add(5)(p15),del(20)(q12)[7]/46,XX[4]	M	V3-23
8	CLL	4	0%	No	0	Pb	47,XY,+12,t(14;18)(q32;q21)[10]/46,XY[13]	M	V4-4
9	CLL	5	26%	No	39	Pb	46,XX,t(14;18)(q32;q21)[15]/47,idem,+12[5]	nd	nd
10	CLL	4	57%	No	102	Pb	47,XY,?add(1)(p36),+12,t(14;18)(q32;q21)[19]/46,XY[2]	nd	nd
11	CLL	4	nd	No	0	Pb	46,XY,t(14;18)(q32;q21)[2]/46,XY[20]	nd	nd
12	Atypical CLL	5	0%	No	0	Pb	47,XY,+12,?del(13)(q14q21)[2]/47,idem,t(14;18)(q32;q21)[7]/46,XY[10]**	nd	nd
13	Atypical CLL	3	Pos^	No	0	Pb	47,XY,+12,t(14;18)(q32;q21)[16]/46,XY[4]	M	V2-5
14	CLL	4	Neg^	No	0	Pb	47,XY,+X,t(14;18)(q32;q21)[18]/47,idem,der(5)del(5)(p13p14)del(5)(q12q2?1),der(12)(12p13->p12::12q13->p11::12q14->q24::?)[4]	M	V3-9
15	Atypical CLL	4	10%	No	0	Bm	47,XY,+X,t(14;18)(q32;q21)[16]/46,XY[4]	nd	nd
16	Atypical CLL	5	Neg^	No	0	Pb	46,XY,t(14;18)(q32;q21)[16]/46,idem,?del(13)(q14q24)[2]/46,XY[14]**	nd	nd
17	Atypical CLL	5	nd	Yes	165	Pb	47,XY,+12,t(14;18)(q32;q21)[11]/46,XY[9]	nd	nd
18	Atypical CLL	4	Neg^	No	0	Pb	46,XY,t(14;18)(q32;q21)[2]/46,XY[18]	nd	nd
19	Atypical CLL	4	Neg^	No	0	Pb	46,XY,t(14;18)(q32;q21)[3]/46,XY[18]	nd	nd
20	CLL¤	nd	nd	Un	40	Pb	47,XY,+12,t(18;22)(q21;q11)[19]/46,XY[1]	nd	nd
21	CLL¤	nd	nd	No	36	Pb	46,XY,t(18;22)(q21;q11)[17]/46,XY[3]	nd	nd
22	Atypical CLL	5	2%	No	90	Pb	46,XY,t(14;18)(q32;q21)[17]/46,idem,del(13)(q14q21)[3]/46,XY[2]	M	V3-33
23	CLL¤	4	11%	No	78	Pb	46,XY,t(14;18)(q32;q21)[4]/46,idem,t(13;22)(q14;p11)[9]/46,XY[9]	nd	nd
24	CLL¤	4	65%	Yes	28	Pb	46,XX,t(1;14)(q31;q32),t(2;14)(p12;q32),t(18;22)(q21;q11)[20]	NM	V3-30
25	Atypical CLL	4	7%	Yes	188	Pb	46,XY,t(14;18)(q32;q21)[2]/46,idem,del(5)(q13q31),inv(12)(q13q24)[11]/46,idem,add(4)(p11),add(10)(q22),-11,+mar[8]/46,XY[1]	M	V2-5
26	CLL¤	nd	95%	Yes	211	Pb	47,XX,+12,t(14;18)(q32;q21)[2]/47,XX,+X[2]/46,XX[39]	M	V5-51
27	Atypical CLL	nd	92%	No	6	Pb	46,XX,t(14;18)(q32;q21)[1]/47,idem,+12[1]/46,XX[40]	M	V3-74
28	CLL	3	0%	No	15	Pb	46,XY,t(14;18)(q32;q21)[3]/46,XY[14]/47,idem,+X[5]/46,XY[1]	M	V3-23
29	CLL¤	2	9%	No	0	Bm	46,XX,t(14;18)(q32;q21)[5]/6,XX[16]	M	V1-69
30	CLL¤	4	84%	No	0	Pb	46,XY,t(14;18)(q32;q21)[10]/46,idem,r(7)(?)[4]/47,idem,+12[2]/46,XY[1]	NM	V1-69
31	Atypical CLL	4	95%	No	0	Pb	47,XY,+12,t(14;18)(q32;q21)[19]/46,XY[6]	nd	nd
32	Atypical CLL	5	6%	No	0	Bm	46,XY,t(14;18)(q32;q21)[11]/46,XY[9]	nd	nd
33	CLL	4	3%	No	19	Pb	47,XY,+12,t(14;18)(q32;q21)[20]	nd	nd
34	CLL¤	4	8%	Yes	12	Pb	46,XY,t(14;18)(q32;q21)[20]	nd	nd
35	CLL¤	5	0%	Yes	69	Pb	46,XY,t(6;17)(p21;p13),t(14;18)(q32;q21)[20]	nd	nd
36	CLL¤	4	nd	No	17	Pb	46,XY,del(13)(q13q22),t(14;18)(q32;q21)[17]/46,XY[3]	nd	nd
37	CLL	4	7%	No	7	Pb	45,XY,der(1)t(1;8)(q41;q13),del(7)(p15p22),-8,t(18;22)(q21;q11)[20]	nd	nd
38	CLL	5	7%	No	40	Pb	46,XX,t(14;18)(q32;q21)[11]/46,XX[16]	M	V5-51
39	CLL	4	nd	No	46	Pb	46,XY,t(18;22)(q21;q11)[19]/46,XY[2]	M	V4-39

IG translocations in chronic lymphocytic leukemia

40	CLL	4	3%	No	41	Pb	46,XY,t(14;18)(q32;q21)[4]/46,XY[13]	M	*V4-34*
41	CLL	4	5%	No	4	Pb	46,XY,t(2;18)(p11;q21)[9]/46,XY[2]	M	*V3-9*
42	CLL	5	nd	No	56	Pb	47,XX,+12,t(14;18)(q32;q21)[13]/46,XX[1]	M	*V3-30*
43	CLL	2	90%	No	26	Pb	46,XY,t(14;18)(q32;q21)[6]/46,XY[11]£	nd	nd
44	Atypical CLL	5	nd	No	47	Pb	47,XY,+12[21]/47,idem,t(14;18)(q32;q21)[4]	NM	*V3-7*
45	Atypical CLL	4	3%	No	5	Pb	47,XY,+12,t(18;22)(q21;q11)[16]/46,XY[1]	NM	*V2-70*
46	CLL	5	0%	No	0	Pb	46,XY,t(14;18)(q32;q21)[17]/46,idem,del(13)(q14q24)[2]	nd	nd
47	CLL	4	7%	No	0	Bm	46,XY,t(14;18)(q32;q21)[9]/46,XY[1]	M	*V3-48*
48	CLL	5	13%	No	88	Pb	46,XY,t(14;18)(q32;q21)[7]/46,XY[3]	M	*V5-51*
49	CLL	4	0%	No	81	Pb	46,XX,-4,add(8p)(p21),der(14)del(14)(q21q24)t(14;18)(q32;q21),der(18)t(14;18),+mar[10]	M	*V3-48*
50	Atypical CLL	5	1%	No	0	Pb	46,XY,t(14;18)(q32;q21)[17]/46,XY[3]	M	*V3-23*
51	CLL	5	1%	Un	27	Pb	46,XY,del(13)(q14q2?1),t(18;22)(q21;q11)[12]/46,XY[3]	M	*V4-34*
52	Atypical CLL	5	4%	Yes	145	Pb	46,XY,t(14;18)(q32;q21)[11]/46,idem,del(13)(q14q3?1)[6]/46,XY[2]	M	*V3-07*
53	Atypical CLL	4	61%	No	5	Pb	47,XX,+12,t(14;18)(q32;q21)[7]/46,XX[13]	NM	*V6-1*
54	CLL¤	5	61%	Un	32	Pb	47,XY,+12[18]/47,idem,t(18;22)(q21;q11)[2]	NM	*V1-69*
55	Atypical CLL	5	4%	No	1	Pb	47,XY,+12[2]/47,idem,t(14;18)(q32;q21)[14]/46,XY[5]	nd	nd
56	Atypical CLL	4	47%	No	31	Pb	46,XX,t(18;22)(q21;q11)[8]/46,idem,t(4;20)(q13;q11)[16]/47,idem,+12[2]	M	*V3-7*
57	Atypical CLL	3	nd	No	6	Pb	46,XY,t(14;18)(q32;q21)[10]/46,XY[10]	M	*V3-30*
58	CLL	5	1%	No	0	Pb	46,XY,t(14;18)(q32;q21)[2]/46,XY[18]	M	*V3-74*
59	Atypical CLL	5	70%	No	6	Pb	47,XY,+12,t(14;18)(q32;q21)[4]/46,XY[13]	M	*V3-23*
60	Atypical CLL	3	nd	No	16	Pb	47,XX,+12,t(14;18)(q32;q21)[2]/46,XX[18]	M	*V4-34*
61	Atypical CLL	4	20%	Yes	22	Pb	47,XY,+12[15]/47,idem,t(14;18)(q32;q21)[6]/46,XX[1]	NM	*V1-69*
62	CLL¤	5	59%	No	34	Pb	47,XY,+12,t(14;18)(q32;q21)[2]/46,XY[18]	NM	*V3-11*
63	Atypical	nd	nd	No	26	Pb	47,XY,add(4)(q?34),+12,t(14;18)(q32;q21)[16]/46,XY[4]	nd	nd
64	Atypical CLL	nd	3%	Un	Un	Pb	46,XY,t(2;18)(p11;q21)[8]/49,idem,+6,+12,+21[3]/46,XY[9]	nd	nd
65	Atypical CLL	5	0%	No	0	Pb	46,XY,t(14;18)(q32;q21)[6]/46,XY[24]	M	*V3-53*
66	CLL¤	4	2%	No	84	Pb	46,XY,t(18;22)(q21;q11)[27]/46,XY[3]	nd	nd
67	CLL	4	0%	No	98	Pb	46,XY,t(14;18)(q32;q21)[20]	M	*V3-23*
68	CLL	5	0%	No	26	Pb	46,XY,t(14;18)(q32;q21)[18]/46,XY[2]	nd	nd
69	CLL	4	1%	No	0	Pb	47,XX,+12,t(14;18)(q32;q21)[11]/47,idem,del(13)(q11)[2]/46,XX[7]	nd	nd
70	CLL	3	79%	No	12	Pb	47,XY,+12,t(14;18)(q32;q21)[8]	M	*V3-7*
71	CLL¤	4	3%	No	1	Pb	46,XY,t(14;18)(q32;q21)[10]	nd	nd
72	CLL¤	5	4%	No	29	Pb	46,XY,t(14;18)(q32;q21)[5]/47,idem,+12[7]	nd	nd
73	Atypical CLL	2	85%	No	0	Pb	Failure££	M	*V3-48*
74	CLL¤	3	59%	No	188	Pb	47,XY,+12,del(13)(q13q21),t(18;22)(q21;q11)[5]/47,idem,del(10)(q24)[6]/47,idem,t(3;12)(p21;q24)[5]/47,idem,t(2;11)(q22;q22)[4]	M	*V4-34*
75	CLL¤	4	8%	No	10	Pb	46,XX,t(14;18)(q32;q21)[5]/46,idem,del(13)(q13q22)[3]/46,XX[10]	M	*V1-69*
76	MZL	1	99%	No	0	Pb	47,XX,+12,t(14;18)(q32;q21)[12]/46,XX[8]	M	*V3-23*
77	MZL	3	38%	No	4	Pb	47,XY,t(2;18)(p11;q21),+12[11]/46,XY[9]	nd	nd
78	MZL	1	1%	No	0	Pb	47,XY,+12,t(14;18)(q32;q21)[5]/46,XY[15]	nd	nd
79	MZL	2	95%	No	0	Pb	47,XY,+12,t(14;18)(q32;q21)[12]/46,XY[7]	M	*V3-23*
80	MZL	0	83%	No	33	Pb	47,XY,+12,t(14;18)(q32;q21)[18]/46,XY[1]	nd	nd

CLL: chronic lymphocytic leukemia. MZL: marginal zone lymphoma. ¤: not rewieved. nd: not done. Un: unknown.^: % not communicated, the cut off being 20%.of cells. Bm: bone marrow. Pb: peripheral blood. K:karyotype. NM : unmutated, M: mutated.*: time between Diagnosis and Karyotype.£: Using FISH, trisomy 12 was detected in a sub-clone. ££: Using FISH, trisomy 12 was present.**: No 13q14 deletion was detected using FISH.

Table 2. Comparison between *BCL2*-CLLs and *BCL3*-CLLs

Patients	*BCL2*-CLLs n=75	*BCL3*-CLLs n=29	*p*
Morphology			
CLL	47 (63%)	9 (31%)	<0.005
Atypical CLL	28 (37%)	20 (69%)	
Gender ratio: M/F	57/18	18/11	0.22
Age at diagnosis	66 [32-83]	62 [39-79]	0.14
Binet stage	63A/4B/1C	16A/5B/4C	0.37
Splenomegaly	3/61 (5%)	13/28 (46%)	<0.0001
Lymphocytes count x10⁹/l (median, range)	14.6 [5.4–106.2]	24.4 [5.5–514]	<0.008
Matutes score			
≥4	57/68 (84%)	5/20 (25%)	<0.001
=3	7/68 (10%)	5/20 (25%)	
<3	4/68 (6%)	10/20 (50%)	
CD5 positive	69/73 (94%)	27/29 (93%)	0.99
CD38 negative	45/64 (70%)	1/5 (20%)	<0.04
Karyotype / FISH			
Variant translocation	13/75* (17%)	0/29	<0.02
Complex (≥3 abnormalities)	15/74 (20%)	13/28 (46%)	<0.02
Single translocation	25/74 (34%)	1/28 (4%)	<0.002
Trisomy 12	33/75 (44%)	20/29 (69%)	<0.03
13q14 deletion	32/68 (47%)	4/27 (15%)	<0.005
TP53 deletion	1/72 (1%)	4/23 (17%)	<0.02
ATM deletion	0/72 (0%)	2/24 (8%)	0.06
6q deletion	0/59 (0%)	5/25 (20%)	<0.002
Mutated cases	33/41 (80%)	2/20 (10%)	<0.0001
IGHV4-39	1/41 (2%)	5/20 (25%)	<0.02
Treated cases	35/64 (55%)	21/28 (75%)	0.1
TFS interval (median +/- SE¤)	48 +/-12 months	1.2 +/- 1 months	<0.0001

CLL: chronic lymphocytic leukemia. TFS: treatment-free survival.*: 11 t(18;22), 2 t(2;18). M: male. F: female. ¤SE: standard error.

Of note, the case without a proven *BCL2*-rearrangement displayed a t(18;22), with a rearrangement of *IGL* in 6% of the interphase nuclei, and did not involve the *MALT1* gene. The median percentage of interphase nuclei carrying the *BCL2*-rearrangement was 81% (range 15-100%). The t(14;18) or variant-t was observed as the sole cytogenetic aberration in 25/74 (34%) cases. When associated with other chromosomal abnormalities in the karyotype, t(14;18) (or variant-t) was the primary change in 15/49 (31%) cases, in the same clone in 29/49 (59%) of cases, and as the subclone change in 5/49 (10%) cases. Trisomy 12 was the primary change in these five latter cases. The karyotype was complex (≥3 abnormalities) in 15/74 (20%) cases. There were 33/75 (44%) cases with trisomy 12, 32/68 (47%) with 13q14 deletion (9 observed by karyotype and FISH, 23 detected by FISH only), 1 (out of 72) (1%) *TP53* deletion, no (0/72) *ATM* deletion, and no (0/59) 6q21 deletion. Of note, the majority of cytogenetic analyses was ob-

tained before any treatment (63/71, 89%).

IGHV mutation analysis was performed in 41 patients. Based on a conventional 98% identity cutoff level, 33/41 (80%) cases were mutated. None showed use of the *VH3-21* gene. After a median follow up of 48 months (range 4-264 months), 2 of the 62 patients who had available data had died from disease (at 48 and 204 months after diagnosis) (Supplementary **Table 1**). The median TFS interval was 48 months (+/-12 months), and the median OS was not reached. Finally, there was no clinical or biological difference between t(14;18)-CLLs and variant-CLLs (data not shown).

Comparisons between the 75 *BCL2*-CLLs and the 29 previously published *BCL3*-CLLs showed no difference in gender ratio, age or Binet stages (**Table 2**). Lymphocytosis was lower in *BCL2*-CLLs (*p*<0.008), and splenomegaly was less frequent (*p*<0.0001). There were more "typical" morphologies in the *BCL2*-CLL group
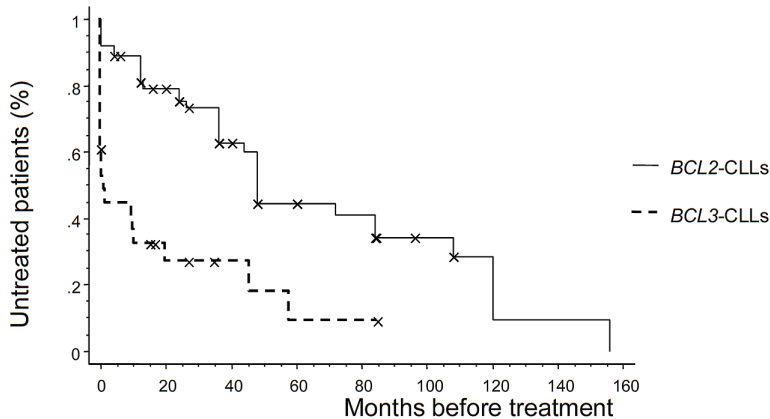
Figure 1. Treatment-free survival (TFS) time from diagnosis for 92 CLLs : 64 *BCL2*-CLLs and 28 *BCL3*-CLLs. The median (+/- standard error) TFS for the *BCL2*-CLLs and *BCL3*-CLLs were 48 (+/-12) and 1.2 (+/-1) months respectively. X: censored case.

($p<0.005$), more Matutes score ≥ 4 ($p<0.001$), and more CD38-negative cases ($p<0.04$). There were more variant *BCL2*-r: translocations compared with variant *BCL3*-translocations ($p<0.02$). *BCL2*-translocations were more frequently observed as single aberrations ($p<0.002$). There were less complex karyotypes ($p<0.02$), more 13q14 deletions ($p<0.005$), and less occurrences of trisomy 12 ($p<0.03$), 6q deletion ($p<0.002$), and *TP53* deletions ($p<0.02$) in *BCL2*-CLLs. The *IGHV* status of *BCL2*-CLLs was more frequently mutated ($p<0.0001$). *BCL2*-CLLs less frequently expressed *IGHV4-39* genes ($p<0.02$). The TFS interval was longer in *BCL2*-CLLs ($p<0.0001$, median 48 *vs* 1.2 months) (**Figure 1**).

We also compared our FISH and molecular data with common CLL. Compared to the referent series of 325 CLLs published by Dohner et al., *BCL2*-CLLs displayed significantly more trisomy 12 (33/75 [44%] vs. 53/325 [16%], $p<0.00001$), and less *ATM* deletions (0/72 [0%] vs. 58/325 [22%], $p<0.0001$) and 6q deletion (0/59 [0%] vs. 21/325 [6%], $p<0.05$) [11]. Regarding *IGHV* status, *BCL2*-CLLs were mutated more frequently than a series of 2662 common CLLs (33/41 [80%] vs. 1426/2662 [54%], $p<5.10^{-4}$) [12]. Moreover, *BCL2*-CLLs used the *VH5-51* gene (3/41, 7%) more frequently than common CLLs (3/41 [7%] vs. 53/2662 [2%], $p=0.05$).

Finally, regarding the five patients with lym-

phoma (four MZLs, one unclassified lymphoma), all were CD10 negative, and four were CD5 positive (**Table 1** and Supplementary **Table 1**). The Matutes score was 3 for one MZL, and <3 for the remaining lymphoma cases. Four of these five cases were CD38 positive. We observed four t (14;18) translocations and one variant t(2;18). In two cases [one t(14;18), one t(2;18)] an atypical signal pattern was observed, consistent with a 5' side breakpoint of the *BCL2* gene. These were analyzed with dual-color *IG* probes (*IGH* and *IGK*), and displayed atypical pattern, with a breakpoint in the constant region of *IG*. (Of note, one t(14;18)-CLL case harbored the same atypical signal with the BCL2 probe.) All lymphoma cases were simple karyotypes, and trisomy 12 was always associated with the *BCL2*-translocation.

Discussion

The t(14;18)(q32;q21) translocation occurs in more than 80% of follicular lymphoma (FL) cases, in about 20% of diffuse large B-cell lymphoma cases, and, more rarely (<5%), in CLL. Whereas *IG* heavy-chain genes (*IGH* on 14q32) are often analyzed in CLL, the *IG* light-chain genes (*IGkappa* on 2p11 and *IGlambda* on 22q11) loci are not commonly investigated using FISH. Consequently, the frequency of variant translocations could be under estimated. These translocations result in the juxtaposition of *BCL2* to *IG*. About 75% of *BCL2* breakpoints are clustered in the major breakpoint region (MBR) and the minor cluster region (mcr), whereas the remaining breakpoints are scattered between these clusters, or at the 5' side (variant cluster region or vcr) of the *BCL2* gene. The breakpoints in the *BCL2* gene of variant translocations, t(2;18) and t(18;22), map consistently to vcr. The vcr rearrangement implies that translocation must be associated with inversion, with a juxtaposition of the *BCL2* and *IG* genes in a head-to-head configuration, and suggests a different pathogenic mechanism [13]. Actually, in three patients [2 t(14;18), 1 t(2;18)], FISH signals were consistent with a 5' breakpoint at the cytogenetic level. Variant translocations are

significantly more frequent in *BCL2*-CLLs than in *BCL3*-CLLs, this could be because, in these cases, *BCL2* is activated at a mature B-cell stage, when *IGL* or *IGK* is rearranged, whereas *BCL3* is activated at a more immature stage when *IGH* is rearranged. In a few cases, in both *BCL2-* and *BCL3*-CLLs, translocation presents as a subclonal change, suggesting that at least in some cases, it may represent a secondary aberration. It is interesting to note that both *BCL2*-translocations and *BCL3*-translocations are frequently associated with trisomy 12, suggesting oncogenic cooperation. Although 13q14 deletion is more frequent in *BCL2*-translocations, *BCL3*-translocations are more frequently associated with a complex karyotype, an *ATM* deletion, and a *TP53* deletion, which are all poor cytogenetic prognostic factors. We and others have already observed that the *IGHV4-39/D6-13/J5* rearrangement is frequent in *BCL3*-CLLs [7, 9, 14]. Although *BCL2*-CLLs use the *VH5-51* gene more frequently than common CLLs, we have not observed any recurrent combination. Finally, *BCL2*-CLLs are more frequently mutated, and have longer TFS than *BCL3*-CLLs: this supports the hypothesis that *BCL2*-CLLs and *BCL3*-CLLs do not have the same prognosis, and need to be identified and distinguished.

Surprisingly, comparison with common CLL showed that *BCL2*-CLLs have significant differences, and are associated with good prognostic markers. Even if *BCL2*-CLLs are frequently associated with trisomy 12, they lack *ATM* and 6q deletions, *ATM* deletion being a poor prognostic factor. It is intriguing that none of the 72 cases tested actually showed a deletion of *ATM*, whereas the deletion is not as rare in CLL. This could suggest that both *BCL2*-translocation and *ATM* deletion could be exclusive oncogenic events. Accordingly, Put et al. observed no 11q deletions in a series of 40 cases with CLL and *BCL2* rearrangements [15]. Among cell-surface markers, CD38 expression is associated with a poor clinical outcome [16, 17]. The majority of *BCL2*-CLLs (70%) in our series were CD38 negative. Finally, the majority of *BCL2*-CLLs (80%) were mutated, without any *VH3-21* usage: these two last criteria are associated with a good prognosis.[18] The median TFS of *BCL2*-CLLs is 48 months, which is very close to the median TFS (49 months) of the Dohner subgroup with a "normal karyotype" [11]. Our result contrasts with a previously published series of eight

t(14;18)-CLLs, with a median progression-free survival of only 20.6 months [19]. However, another study showed a TFS of 48 months in a series of 40 *BCL2*-CLLs [15]. Regarding the patients with a *BCL2*-translocation and trisomy 12 (*n*=33) in our series, the presence of the *BCL2*-translocation remains a favorable marker, even in patients who also exhibit markers of intermediate prognosis, such as trisomy 12. Actually, the median TFS of this group is longer than the median TFS of the reference group with trisomy 12 as found by Dohner et al. (48 vs. 33 months). Of note, there is no significant difference between *BCL2*-CLLs with trisomy 12 and *BCL2*-CLLs without trisomy 12 with regard to median TFS in our series. Thus, we extend in our large series of independent data, a recently published series which concluded that t(14;18) was not associated with an inferior outcome in CLL [15]. However, our results contrast with previous data which reported CLL patients with translocations or 14q32 rearrangements. Mayr et al. found a median TFS of 24 months, and a median OS of 94 months in CLL with translocations [1]. Juliusson et al. and Cavazzini et al. reported short TFS and/or OS in CLL with 14q32 abnormalities [5, 6]. Haferlach et al. found a significant correlation between CD38 expression and *IGH* rearrangement [2]. Also, Cavazzini et al. observed 8/14 (57%) unmutated CLLs with a 14q32 rearrangement [6]. Our data underline the fact that these chromosomal abnormalities need further characterization and may not be considered as a whole group. In the same way, Yin et al. have recently reported six cases of CLL/SLL, with a t(2;14)(p16;q32) translocation involving *BCL11A* and *IGH* genes, which have been characterized with atypical morphological features and unmutated *IGHV* genes [20].

Finally, MZL could be associated with t(14;18), this is in contrast to a recent publication that reported 29/239 MZL cases that had abnormal karyotypes that carried a translocation involving *IGH*, but not the *BCL2* gene [21].

In conclusion, different *IG*-translocations have different prognoses. Therefore, screening and identification of partner genes may be warranted in CLL. Variant translocations involving *IGL* and *IGK* are not as rare, but could be missed by classical FISH analyses using only *IGH* probes. The majority of *BCL2*-CLLs are characterized by CD5+, the CD38- immunopheno-

type, a Matutes score ≥ 4, no deletion of *ATM*, and a mutated *IGHV* status. Although frequently associated with trisomy 12, *BCL2*-translocations may be associated with a good prognosis.

Authorship and disclosures

Acknowledgements

Address correspondance to: Florence Nguyen-Khac (MD-PhD), Service Hématologie Biologique, Groupe Hospitalier Pitié-Salpêtrière, 47-83, Bvd de l'Hôpital, 75013 Paris, France. Tel: 33 (1) 42162451; Fax: 33 (1)42162453; E-mail: Florence.nguyen-khac@psl.aphp.fr

References

[1] Mayr C, Speicher MR, Kofler DM, Buhmann R, Strehl J, Busch R, et al. Chromosomal translocations are associated with poor prognosis in chronic lymphocytic leukemia. Blood 2006; 107(2):742-51.

[2] 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(12):2442-51.

[3] Van Den Neste E, Robin V, Francart J, Hagemeijer A, Stul M, Vandenberghe P, et al. Chromosomal translocations independently predict treatment failure, treatment-free survival and overall survival in B-cell chronic lymphocytic leukemia patients treated with cladribine. Leukemia 2007; 21(8):1715-22.

[4] Mitelman F, Johansson B, Mertens F, (eds). Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer. 2010. http://cgap.nci.nih.gov/Chromosomes/Mitelman.

[5] Juliusson G, Oscier DG, Fitchett M, Ross FM, Stockdill G, Mackie MJ, et al. Prognostic subgroups in B-cell chronic lymphocytic leukemia defined by specific chromosomal abnormalities. N Engl J Med 1990; 323(11):720-4.

[6] Cavazzini F, Hernandez JA, Gozzetti A, Russo Rossi A, De Angeli C, Tiseo R, 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 (4):529-37.

[7] Chapiro E, Radford-Weiss I, Bastard C, Luquet I, Lefebvre C, Callet-Bauchu E, et al. The most frequent t(14;19)(q32;q13)-positive B-cell malignancy corresponds to an aggressive subgroup of atypical chronic lymphocytic leukemia. Leukemia 2008; 22(11):2123-7.

[8] Michaux L, Dierlamm J, Wlodarska I, Bours V, Van den Berghe H, Hagemeijer A. t(14;19)/BCL3 rearrangements in lymphoproliferative disorders: a review of 23 cases. Cancer Genet Cytogenet 1997; 94(1):36-43.

[9] Martin-Subero JI, Ibbotson R, Klapper W, Michaux L, Callet-Bauchu E, Berger F, et al. A comprehensive genetic and histopathologic analysis identifies two subgroups of B-cell malignancies carrying a t(14;19)(q32;q13) or variant BCL3-translocation. Leukemia 2007; 21 (7):1532-44.

[10] Huh YO, Abruzzo LV, Rassidakis GZ, Parry-Jones N, Schlette E, Brito-Bapabulle V, et al. The t (14;19)(q32;q13)-positive small B-cell leukaemia: a clinicopathologic and cytogenetic study of seven cases. Br J Haematol 2007; 136 (2):220-8.

[11] Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000; 343(26):1910-6.

[12] Darzentas N, Hadzidimitriou A, Murray F, Hatzi K, Josefsson P, Laoutaris N, et al. A different ontogenesis for chronic lymphocytic leukemia cases carrying stereotyped antigen receptors: molecular and computational evidence. Leukemia 2010; 24(1):125-32.

[13] Willis TG, Dyer MJ. The role of immunoglobulin translocations in the pathogenesis of B-cell malignancies. Blood 2000; 96(3):808-22.

[14] Schweighofer CD, Huh YO, Luthra R, Sargent RL, Ketterling RP, Knudson RA, et al. The B cell antigen receptor in atypical chronic lymphocytic leukemia with t(14;19)(q32;q13) demonstrates remarkable stereotypy. Int J Cancer 2010.

[15] Put N, Meeus P, Chatelain B, Rack K, Boeckx N, Nollet F, et al. Translocation t(14;18) is not associated with inferior outcome in chronic lymphocytic leukemia. Leukemia 2009; 23

(6):1201-4.

[16] Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999; 94(6):1840-7.

[17] Shanafelt TD, Rabe KG, Kay NE, Zent CS, Jelinek DF, Reinalda MS, et al. Age at diagnosis and the utility of prognostic testing in patients with chronic lymphocytic leukemia. Cancer; 116 (20):4777-87.

[18] Tobin G, Thunberg U, Johnson A, Thorn I, Soderberg O, Hultdin M, et al. Somatically mutated Ig V(H)3-21 genes characterize a new subset of chronic lymphocytic leukemia. Blood 2002; 99 (6):2262-4.

[19] Nowakowski GS, Dewald GW, Hoyer JD, Paternoster SF, Stockero KJ, Fink SR, et al. Interphase fluorescence in situ hybridization with an IGH probe is important in the evaluation of patients with a clinical diagnosis of chronic lymphocytic leukaemia. Br J Haematol 2005; 130(1):36-42.

[20] Yin CC, Lin KI, Ketterling RP, Knudson RA, Medeiros LJ, Barron LL, et al. Chronic lymphocytic leukemia With t(2;14)(p16;q32) involves the BCL11A and IgH genes and is associated with atypical morphologic features and unmutated IgVH genes. Am J Clin Pathol 2009; 131 (5):663-70.

[21] Salido M, Baro C, Oscier D, Stamatopoulos K, Dierlamm J, Matutes E, et al. Cytogenetic aberrations and their prognostic value in a series of 330 splenic marginal zone B-cell lymphomas: a multicenter study of the Splenic B-Cell Lymphoma Group. Blood 2010; 116(9):1479-88.

Supplementary Table 1. Clinical data: Binet stages and lymphocyte counts at diagnosis. Survival was calculated from the diagnosis.

CLL	Gender/Age	Binet Stage	Lymphocyte count x 10⁹/l	CD5/CD23	Time to first treatment (months)	Treatments	Follow up* (months)
1	F/73	C	un	+/-	108	CVP	A (108)
2	M/75	A	14.6	+/+	4	CHOP/R	A (20)
3	F/68	A	57.1	+/-	un	un	un
4	M/66	A	13.7	+/nd		no	A (84)
5	M/72	A	14	+/+		No	A (36)
6	M/69	A	13.3	-/+	12	Cb	A (72)
7	F/68	A	20.7	+/+	36	FR;R	A (48)
8	M/54	A	8.4	+/-	un	un	un
9	F/70	A	un	+/+		No	A (48)
10	M/69	A	un	+/-		No	A (108)
11	M/74	A	7.8	+/+	36	Cb;FR	A (132)
12	M/82	un	24	+/+	un	un	un
13	M/80	A	6.1	+/+	un	un	un
14	M/60	B	92.4	-/+	0	FCR	un
15	M/70	A	9.1	+/+		No	A (24)
16	M/66	A	10.2	+/+		No	A (12)
17	M/48	A	un	+/+	12	Cb;CHOP;F;FC;Alemtuzumab	D (204)
18	M/57	A	15.2	+/+		No	A (60)
19	M/62	A	6.2	+/+		No	A (48)
20	M/72	un	10.9	nd/nd		No	A (40)
21	M/52	un	un	nd/nd		No	A (36)
22	M/54	A	un	+/+	36	Cb;FCR	A (96)
23	M/69	A	un	+/+	84	Cb	A (84)
24	F/62	B	un	-/+	0	Cb;FC;CHOP/R;Alemtuzumab	D (48)
25	M/45	A	66.2	+/+	4	Cb;FC;Cb	A (216)
26	F/56	A	un	-/-	120	Cb	A (264)
27	F/74	A	6.6	+/+	120	Cb	A (144)
28	M/75	A	5.6	+/+		No	A (15)
29	F/64	A	22.4	+/-	84	Cb	A (84)
30	M/70	A	73.6	+/+		No	A (6)
31	M/64	B	9.38	+/+	0	Cb	un
32	M/54	A	5.4	+/+		No	A (12)
33	M/68	A	16.5	+/+		No	A (20)
34	M/65	A	15.6	+/+	5	RC	A (12)
35	M/57	A	15.4	+/-	12	Cb	A (72)
36	M/73	A	un	+/+		No	A (24)
37	M/82	A	15	+/+		No	A (12)
38	F/66	A	un	+/+		No	A (40)
39	M/32	A	100	+/+	48	FCR	A(48)
40	M/57	A	68.6	+/+		No	A (48)
41	M/72	A	5.6	+/+		No	A (4)
42	F/67	A	18	+/+		No	A (60)
43	M/75	A	un	+/-	48	Cb	A (72)
44	M/60	A	25	+/+	48	FC	A (48)
45	M/61	A	9.2	+/+	un	un	un
46	M/50	A	15.6	+/+	un	un	un
47	M/74	A	8.8	+/+	un	un	un
48	M/61	A	13.8	+/+	24	Cb	A (96)
49	F/78	A	5.7	+/+		No	A (84)
50	M/51	A	7.38	+/+		No	A (12)
51	M/68	un	un	+/+	36	FC	A (48)
52	M/63	A	19.4	+/+	72	CHOP;R/Pentostatine/C	A (168)
53	F/71	A	un	+/+	12	RC	A (36)
54	M/63	un	un	+/+	36	FC	A (48)
55	M/72	un	17.8	+/+	un	un	un
56	F/49	A	un	+/-		No	A (36)
57	M/70	A	9.3	+/+		No	A (108)
58	M/43	A	106.2	+/+		No	A (24)
59	M/36	A	11.9	+/+	36	Cb	A (72)
60	F/75	A	un	+/+		No	A (36)
61	F/66	A	11.1	+/+	0	Cb	A (24)
62	M/55	A	11	+/+	44	CHOP/F;FCR;ASCT	A (96)
63	M/74	A	un	+/nd	24	Cb;CHOPR	A (26)
64	M/69	un	un	+/nd	un	un	un
65	M/31	A	9.97	+/+	un	un	un

66	M/57	A	9	+/+		No	A (84)
67	M/54	B	un	+/+		No	A (98)
68	M/53	A	19.2	+/+		No	A (26)
69	F/64	A	16.1	+/+		No	A (12)
70	M/56	A	24.8	+/+	48	CHOP/F;R/DHAP	A (96)
71	M/71	A	24.6	+/+	un	un	un
72	M/75	A	un	+/+	24	Cb	A (36)
73	F/55	A	82.7	+/+	0	FRC	A (24)
74	M/56	A	un	+/+	156	Cb;FR	A (192)
75	F/57	A	21.6	+/+	12	Cb/P	A (12)
MZL							
76	F/76		12.8	+/-	24	CHOP/R	A(24)
77	M/57		8	+/+	un	un	un
78	M/76		8.8	+/-		No	A (12)
79	M/57		33	+/+	5	FCR	A (12)
80	M/65		5.7	-/-		No	A (36)

un: unknown, nd: not done, CLL: chronic lymphocytic leukemia, MZL: marginal zone lymphoma,Cb: chlorambucil, P: prednisone, CHOP: cyclophasphamide/doxorubicine/vincristine/prednisone, F: fludarabine, R: rituximab, C: cyclophosphamide, DHAP: dexamethasone, cytarabine, cisplatine, ASCT: autologous stem cell transplantation
*: defined as the time from diagnosis to last follow up; A: alive, D: dead.