Mutational and cytogenetic analyses of 188 CLL patients with trisomy 12: A retrospective study from the French Innovative Leukemia Organization (FILO) working group

Damien Roos-Weil, Florence Nguyen-Khac, Sylvie Chevret, Cyrille Touzeau, Clémence Roux, Julie Lejeune, Adrien Cosson, Stephanie Mathis, Pierre Feugier, Stéphane Leprêtre, et al.

► To cite this version:

Damien Roos-Weil, Florence Nguyen-Khac, Sylvie Chevret, Cyrille Touzeau, Clémence Roux, et al.. Mutational and cytogenetic analyses of 188 CLL patients with trisomy 12: A retrospective study from the French Innovative Leukemia Organization (FILO) working group. *Genes, Chromosomes & Cancer*, 2018, 57 (11), pp.533-540. 10.1002/gcc.22650 . hal-01972303

HAL Id: hal-01972303

<https://hal.sorbonne-universite.fr/hal-01972303>

Submitted on 7 Jan 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Mutational and cytogenetic analyses of 188 CLL patients with trisomy 12: A retrospective study from the French Innovative Leukemia Organization (FILO) working group

Damien Roos-Weil¹ | Florence Nguyen-Khac^{2,3} | Sylvie Chevret⁴ | Cyrille Touzeau⁵ | Clémence Roux⁶ | Julie Lejeune⁴ | Adrien Cosson³ | Stéphanie Mathis² | Pierre Feugier⁷ | Stéphane Leprêtre⁸ | Marie-Christine Béné⁹ | Marine Baron¹ | Sophie Raynaud⁵ | Stéphanie Struski¹⁰ | Virginie Eclache¹¹ | Laurent Sutton¹² | Claude Lesty² | Hélène Merle-Béral² | Florence Cymbalista¹³ | Loïc Ysebaert¹⁴ | Frédéric Davi² | Véronique Leblond¹ | on behalf of the FILO working group

¹Sorbonne Universités, UPMC Univ Paris 06, AP-HP, GRC-11, Groupe de recherche clinique sur les hémopathies lymphoïdes (GRECHY), Hôpital Pitié-Salpêtrière, APHP, Paris, France

²Service d'Hématologie Biologique, Hôpital Pitié-Salpêtrière, APHP, Paris, France

³Centre de Recherche des Cordeliers, INSERM UMRS 1138, Cell Death and Drug Resistance in Lymphoproliferative Disorders Team, Paris, France

⁴Département de Biostatistique et Informatique Médicale (DBIM), Hôpital Saint Louis, APHP, Paris, France

⁵Service d'Hématologie clinique, CHU de Nantes, France

⁶Laboratoire d'Hématologie, Hôpital Pasteur, CHU de Nice, Nice, France

⁷Service d'Hématologie, Hôpitaux de Brabois, Vandoeuvre Les Nancy, France

⁸Département d'Hématologie, Centre Henri Becquerel, Rouen, France

⁹Service d'Hématologie biologique, CHU de Nantes, Nantes, France

¹⁰Département d'Hématologie, CHU de Toulouse, Université de Toulouse, Centre de Recherche sur le Cancer de Toulouse (CRCT), Toulouse, France

¹¹Laboratoire d'Hématologie, Hôpital Avicenne, AP-HP, Bobigny, France

¹²Service d'Hématologie, Centre Hospitalier Victor Dupouy, Argenteuil, France

¹³Service d'Hématologie Biologique, GHUPSSD, AP-HP, U978 INSERM, Université Paris 13, Sorbonne Paris Cité, Labex Inflamex, Bobigny, France

¹⁴IUC Toulouse-Oncopole, Toulouse, France

Correspondence

Damien Roos-Weil, AP-HP, Hôpital Pitié-Salpêtrière, Service d'Hématologie Clinique, 47-83 Bd de l'Hôpital 75651 Paris Cedex, France.

Email: damien.roosweil@aphp.fr

Abstract

Trisomy 12 (tri12) is the second most frequent chromosomal aberration (15%-20%) in chronic lymphocytic leukemia (CLL). Tri12 confers an intermediate prognosis but is a heterogeneous entity. We examined whether additional mutational or chromosomal alterations might impact tri12 patient outcomes. This retrospective study, carried out by the French Innovative Leukemia Organization, included 188 tri12 patients with comprehensive information on immunoglobulin heavy chain (*IGHV*) gene status, karyotypic/FISH abnormalities, and *NOTCH1*, *TP53*, *SF3B1*, and *MYD88* mutations. The main cytogenetic abnormalities associated with tri12 were del(13q) (25%), additional trisomies (14%) (including tri19 (10%) and tri18 (4%)), 14q32 translocations (10%), del(17p) (6.5%), del(14q) (4%), and del(11q) (4%). Unmutated (UM) *IGHV*, *NOTCH1*, and *TP53* mutations were identified in respectively 66%, 25%, and 8.5% of cases. Multivariate analyses showed that additional trisomies (HR = 0.43, 95% CI = 0.23-0.78, *P* = .01) were associated with a significantly longer time to first treatment in Binet stage A patients and with a lower risk of relapse (HR = 0.37, 95% CI = 0.15-0.9, *P* = .03) in the overall tri12 population. Binet stage B/C, *TP53* disruption, and UM *IGHV* status were associated with a shorter time to next

treatment, while Binet stage B/C (HR = 4, 95% CI = 1.6-4.9, $P = .002$) and *TP53* disruption (HR = 5, 95% CI = 1.94-12.66, $P = .001$) conferred shorter overall survival in multivariate comparisons. These data indicate that additional cytogenetic and mutational abnormalities, and particularly additional trisomies, *IGHV* status, and *TP53* disruption, influence tri12 patient outcomes and could improve risk stratification in this population.

KEYWORDS

chronic lymphocytic leukemia, trisomy 12, *IGHV*, *TP53*

1 | INTRODUCTION

The clinical course of chronic lymphocytic leukemia (CLL) is highly variable, ranging from very stable disease with near-normal life expectancy to rapidly progressive disease that requires treatment.^{1,2} Features known to influence the disease course include somatic mutations of immunoglobulin heavy chain (*IGHV*) genes, recurrent chromosomal abnormalities (CAs), and gene mutations such as inactivating mutations of *TP53* and *ATM*. Recently, several large studies based on massive next-generation sequencing (NGS)³⁻¹⁰ have provided a more detailed genetic picture of CLL, revealing recurrent mutations in other genes such as *SF3B1*, *NOTCH1*, *EGR2*, *NFKBIE*, *XPO1*, and *MYD88*, some being associated with aggressive disease.^{11,12}

Among the recurrent cytogenetic abnormalities associated with CLL, trisomy 12 (tri12) occurs in approximately 15%-20% of cases.^{13,14} Other common CAs identified by means of conventional and molecular cytogenetic approaches include del (13)(q14), del (11)(q22-23), del (17)(p13), gain (2)(p), and del (6)(q21). These alterations influence survival.¹⁴⁻¹⁷ Tri12 was usually considered to be an intermediate-risk genetic lesion in newly diagnosed CLL and in the context of chemotherapy treatment,¹⁴ although recent reports suggest that it confers more complex and heterogeneous clinical behavior.¹⁸ Indeed, accompanying chromosomal aberrations such as additional trisomies (tri18 and/or tri19),¹⁸⁻²⁰ 14q32 translocations (t[14;18](q32;q21) and t[14, 19](q32;q13)),²¹⁻²⁵ and 14q deletions^{26,27} are commonly observed in tri12 CLL, and it has been suggested that patients harboring additional trisomies have better outcomes than patients with isolated tri12.¹⁸ Compared to CLL lacking this cytogenetic abnormality, tri12 CLL has a more atypical morphology and immunophenotype,^{28,29} more frequent expression of the poor prognostic markers CD49d and CD38, more frequent *NOTCH1* mutations, and unmutated (UM) *IGHV* gene status.^{27,30,31}

Recent NGS analyses show that the CLL genome displays a high degree of heterogeneity, both across patients and in a given patient.⁸⁻¹⁰ Among somatic genetic alterations, copy-number abnormalities may be the earliest events in CLL, with two distinct points of departure involving del(13q) and tri12.^{8,9} Tri12 is thus usually considered to be a clonal driver lesion occurring early in the course of CLL, and additional molecular and/or cytogenetic abnormalities could explain in part why it is a heterogeneous entity and why its prognostic value is controversial.

The aim of this study was to determine whether additional molecular and cytogenetic abnormalities could impact the outcome of tri12 CLL.

2 | MATERIAL AND METHODS

2.1 | Patients

This retrospective multicenter study conducted by the CLL French Innovative Leukemia Organization included 188 tri12 CLL patients diagnosed from 1999 to 2013. Median age at diagnosis was 64 years (range, 56-72); 129 (69%) patients were male and 59 (31%) female. CLL was defined by a Royal Marsden Hospital (Maudsley) immunophenotypic score ≥ 4 . Tri12 was diagnosed by fluorescence in situ hybridization (FISH) and/or chromosomal banding (CB), and cases were selected according to the availability of DNA for Sanger sequencing. FISH, CB, and Sanger sequencing were performed on blood samples. The patients' main clinical and biological characteristics are shown in Table 1. Written consent for blood collection and biological analyses were obtained in accordance with the Declaration of Helsinki and with approval from the local ethics committee (CPP Ile-De-France, May 21, 2014).

2.2 | Cytogenetic and mutational analyses

CB analysis was performed as previously described,²⁷ with CpG oligonucleotides and interleukin 2 stimulation. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN 2013). FISH was performed on interphase nuclei and metaphases, following standard procedures and using specific probes, namely *IGH* (Dako, Trappes, France), tri12 (CEP12), 13q14 (D13S319), *ATM*, *TP53* (Abbott, Rungis, France), and 6q21 (SEC63) (Kreatech, Strasbourg, France). *IGHV* mutation status was determined as previously described.³² Sequences were aligned to the ImMunoGeneTics sequence directory and considered unmutated if homology with the germline was $\geq 98\%$. Mutations were sought by Sanger direct sequencing in *TP53* (exons 4-10), *NOTCH1* (exon 34), *MYD88* (exons 3-5), *SF3B1* (exons 14-16), and *XPO1* (exons 14-15) genes. Known polymorphisms were ruled out using the European 1000 genomes and dbSNP137 variant databases unless also listed in the Catalogue of Somatic Mutations in Cancer (COSMIC) 65 database. All *TP53* mutations were checked for their existence in the IARC database. All *NOTCH1*, *SF3B1*, and *MYD88* mutations identified in our study were already described in CLL.

Tri12 was detected either by CB and/or FISH analyses in the 188 patients of our cohort. CB analysis was performed in 184 patients and detected tri12 in 177 of them. All 7 cases negative for tri12 by

TABLE 1 Main characteristics of tri12 patients

Characteristic	No. (%)
Total	188 (100)
Age, years	
Median (range)	64 (56-72)
Male	129 (69)
Binet stage	
A	120 (64)
B/C	68 (36)
Lymphocytosis, G/L	
Median (range)	19 (8-52)
CD38 expression	/164
>30%	96 (59)
Unmutated IGHV	124/187 (66)
Karyotype	/184
Normal*	7 (3)
Complex (3 or 4 CA)	36 (20)
Highly complex (≥5)	18 (10)
Additional trisomy (≥1)	23 (14)
FISH	
Del(13q)	45/182 (25)
Del(11q)	7/185 (4)
Del(17p)	12/184 (6.5)
Mutations	
NOTCH1	47/188 (25)
TP53	16/188 (8.5)
SF3B1	7/188 (4)
MYD88	2/188 (1)
XPO1	0/91 (0)
TP53 disrupted	24/184 (13)
Trisomy 12, sole abnormality	
By CB and FISH	61/175 (35)
By CB, FISH and mutations	42/180 (24)

Only CLL cases defined by a Royal Marsden Hospital (Matutes) immunophenotypic score ≥ 4 were included and CD5 was not expressed in 10/188 cases (5%). In the 7 cases negative for tri12 by chromosome banding, indicated by asterisk, the presence of tri12 was demonstrated by FISH analysis. Abbreviations: CA, chromosomal abnormality; CB, chromosome banding; G/L, giga per liter

CB had a FISH analysis that demonstrated the presence of tri12. Tri12 was also identified by FISH analysis in the 4 cases without CB. Cytogenetic analyses and molecular testing, performed at the same time for each patient, were carried out before treatment for all 188 patients (see the “Cytogenetic and molecular features” section).

2.3 | Statistical analysis

The following clinical and biological characteristics were included in univariate analysis of prognostic factors: age, Binet stage, lymphocytosis, LDH, beta2microglobulin (B2M), CD38 expression (defined as positive if expressed by $\geq 30\%$ of CLL cells), IGHV mutational status, percentage of interphase nuclei positive for tri12, additional FISH (del(13q), del(11q), del(17p)) or chromosomal aberrations, and complex and highly complex karyotype as a whole (≥ 3 CB abnormalities). TP53 disruption was defined by the presence of del(17p) and/or TP53

mutations. Primary and secondary endpoints were time to first treatment (TFT) for Binet stage A cases, and response to therapy, relapse rate, time to next treatment (TNT), and overall survival (OS) for the whole population. OS and TFT were calculated from the date of diagnosis until last follow-up/death and the date of initial treatment, respectively. Kaplan-Meier analysis was used to construct survival curves, and the log rank test was used to determine differences between groups. The X^2 test or Fisher's exact test was used to compare the data distribution between different cytogenetic and molecular subgroups. Significance was assumed at $P < .05$. All prognostic factors found to be significant at a level of $P < .05$ in the univariate analyses were included into stepwise regression models using Cox's proportional hazards models. All statistical analyses used SAS 9.3 (SAS Inc, Cary, North Carolina) or R 3.0.2 software (<http://www.R-project.org>).

3 | RESULTS

3.1 | Patient characteristics

The study population comprised 188 untreated patients. Respectively 120 and 68 patients had stage A and stage B/C CLL. B2M was above 4 mg/L in 46/152 patients (32%) and IGHV status was unmutated (UM) in 66% of cases (Table 1). Median follow-up of the whole cohort was 78 months (range, 1-397).

3.2 | Cytogenetic and molecular features

Tri12, either identified by CB and/or FISH (see the “Material and Methods” section), was detected at the time of diagnosis in 115 patients (61%). In the other 73 patients, cytogenetic analysis was performed before the first treatment, with a median interval of 17 months between CLL diagnosis and tri12 detection (range, 0.7-43 months). The most frequent CAs identified by CB and associated with tri12 were tri19 in 17 patients (10%), t(14;18) in 13 patients (7%), tri18 in 8 patients (4%), del(14q) in 8 patients (4%), t(14;19) in 4 patients (2.2%), and additional X in 3 patients (1.7%). Tri21 and tri22 were each detected in 1 patient ($<1\%$). An additional trisomy was detected in 23 patients (14%), with one additional trisomy in 15 patients and two in 8 patients. Tri19 was the more frequent additional trisomy ($n = 17$); it was isolated in 9 patients and associated with tri18 and tri22 in 7 and 1 patients, respectively (Supporting Information Figure S1). The median number of CA identified by karyotypes, including tri12, was 2. Complex and highly complex (defined as 3 or 4 and ≥ 5 CA, respectively) K were present in respectively 36 patients (20%, including 8 cases with only multiple trisomies) and 18 patients (9.8%). By FISH, tri12 was associated with del(13q), del(11q), and del(17p) in 45 (24.7%), 7 (3.8%), and 12 (6.5%) patients, respectively. The median percentage of interphase nuclei positive for tri12 was 58% (range, 5%-100%). The relationship between this percentage and main molecular features is summarized in Supporting Information Figure S2. Of note, this percentage was significantly higher in cases with UM compared to mutated IGHV cases while there was no

TABLE 2 Distribution of mutations in different cytogenetic groups

Cytogenetic groups	NOTCH1 mutations		TP53 disruption		IGHV, UM	
	n (%)	P	n (%)	P	n (%)	P
Additional trisomies	4/23 (17.4)	.44	2/23 (8.7)	.74	7/23 (30)	.0002
Tri19	1/17 (5.8)	.05	1/17 (5.8)	.47	3/17 (17.6)	.0001
14q32 translocations	4/19 (21)	.90	3/19 (15.8)	.51	9/19 (41)	.04
14q deletions	3/8 (37.5)	.42	0/8 (0)	.60	4/8 (50)	.44
Patients without the above CA	38/132 (28.8)	.24	18/132 (13.6)	.62	104/132 (78.8)	.24
Isolated tri12 by CB and FISH	16/61 (26.2)	.86	3/61 (4.9)	.01	47/61 (77)	.02

Statistical comparisons were performed between the population carrying the abnormality and the population without that abnormality. Differences were considered significant when *P* value was inferior or equal to .05. Abbreviations: CA, chromosomal abnormalities; CB, chromosome banding; n, number; *P*, *P* value; UM, unmutated

statistical difference for patients with or without *NOTCH1* mutations or for those with or without *TP53* disruption.

Targeted mutational analysis of *NOTCH1*, *TP53*, *SF3B1*, and *MYD88* was performed in the entire cohort. Sixty-five patients (35%) harbored at least one genomic alteration (one, two, and three concurrent mutations were observed in 59, 5, and 1 patient, respectively). *NOTCH1*, *TP53*, *SF3B1*, and *MYD88* mutations were identified in 47 (25%), 16 (8.5%), 7 (3.7%), and 2 (1%) patients, respectively. No *XPO1* mutations were detected. *TP53* was disrupted in 24/184 cases (13%). Tri12 was the sole abnormality observed by CB in 74 patients (43%), by CB and FISH in 61 patients (35%), and by CB, FISH, and genomic analyses in 42 patients (24%). The distribution of *NOTCH1* mutations, *TP53* disrupted, and UM *IGHV* cases among the different cytogenetic groups is summarized in Table 2. Of note, UM *IGHV* cases were significantly less frequent in the subgroups with additional trisomies and 14q32 translocations. *NOTCH1* mutations were associated with UM *IGHV* (respectively 31% and 14% of *NOTCH1* mutations in UM and M *IGHV* cases; *P* = .02) and there was a trend for an association between *TP53* disruption and *IGHV* status as respectively 15% and 8% of UM and mutated *IGHV* cases were *TP53* disrupted (*P* = .07).

3.3 | Outcomes

In the stage A population of 120 patients, 76 patients (63%) needed treatment, with a median TFT of 45 months (range, 1-170). In univariate analysis, a shorter TFT was significantly associated with lymphocytosis (*P* = .003), the percentage of interphase nuclei positive for tri12 by FISH (*P* = .01), both studied as continuous variables, and the presence of *NOTCH1* mutations (*P* = .03) or any mutation (*P* = .007), while the presence of additional trisomies (*P* = .002) was associated with a significantly longer TFT. In multivariate analysis, only additional trisomies (HR = 0.43, 95% CI = 0.23-0.78, *P* = .01) and one or more mutated genes (HR = 1.73, 95% CI = 1.03-2.9, *P* = .04) retained their prognostic significance for TFT (Table 3), with a respective median TFT of 90 and 30 months.

In the whole tri12 population, 143 patients (76%) required treatment, consisting of immunochemotherapy in 70% of cases. The overall response rate to first therapy was 76% among the 142 assessable patients. The only prognostic variables statistically associated with the response to therapy were advanced age (HR = 0.94, 95% CI = 0.91-0.97, *P* = .0002) and lymphocytosis (HR = 1.01, 95% CI = 1.00-1.02, *P* = .04), studied as continuous variables. Sixty-nine patients relapsed and 34 patients received a second line of treatment

TABLE 3 Univariate and multivariate analysis of time to first treatment in Binet stage a tri12 patients (n = 120)

Variable	Events/total	Univariate			Multivariate		
		HR	CI 95%	P	HR	CI 95%	P
Age	-	1.02	1-1.05	NS			-
Lymphocytosis	-	1.01	1-1.02	.003			NS
CD38 > 30%	32/62	0.68	0.51-1.36	NS			-
B2M > 4 mg/L	17/31	0.94	0.63-1.56	NS			-
% of tri12 by FISH	-	1.02	1-1.03	.01			NS
Additional trisomy present	7/14	0.29	0.13-0.64	.002	0.43	0.23-0.78	.01
Complex and highly complex karyotype	25/33	1.03	0.63-1.68	NS			-
Unmutated <i>IGHV</i>	53/80	1.20	0.78-1.72	NS			-
<i>NOTCH1</i> mutations	18/25	1.83	1.06-3.16	.02			NS
Any mutation present	25/34	2	1.21-3.22	.007	1.73	1.03-2.9	.007
<i>TP53</i> disruption	8/13	1.13	0.85-1.95	NS			NS

The second column represents for each variable the number of events and the total number of samples assessable for. Lymphocytosis, age, and percent of tri12 were studied as continuous variables. The percent of tri12 corresponds to the percentage of interphase nuclei harboring trisomy 12 by FISH. Of note, as *MYD88* mutations have been associated with good prognosis, we performed statistical analysis excluding, from the "any mutation" group, patients harboring those mutations (*n* = 2) and observed similar associations with survival (data not shown). Abbreviations: B2M, beta2microglobuline; CI, confidence interval; HR, hazard ratio; NS, non significant

TABLE 4 Univariate and multivariate analysis of time to next treatment (n = 143)

Variable	Events/total	HR	Univariate		HR	Multivariate	
			CI 95%	P		CI 95%	P
Binet stage B/C vs A	25/58	4.22	1.76-10.12	.001	5.66	2.09-15.33	.001
Age	-	1.04	0.99-1.08	NS			-
Lymphocytosis	-	1	1-1.01	NS			-
CD38 > 30%	15/29	1.39	0.56-3.42	NS			-
B2M > 4 mg/L	20/39	1.21	0.52-2.21	NS			-
Percent of tri12 by FISH	-	1.01	0.99-1.04	NS			-
Additional trisomy present	3/9	0.33	0.1-1.17	NS			-
Complex and highly complex karyotype	9/18	1.00	0.90-1.11	NS			-
Unmutated IGHV	21/37	6.25	2.04-16.66	.001	9.09	2.63-33.33	.001
NOTCH1 mutations	9/20	1.68	0.73-3.88	NS			-
Any mutation present	13/29	2.02	0.89-4.55	NS			-
TP53 disrupted	6/11	3.4	1.4-9.5	.01			.03

The second column represents for each variable the number of events and the total number of samples assessable for. Lymphocytosis, age, and percent of tri12 were studied as continuous variables. The percent of tri12 corresponds to the percentage of interphase nuclei harboring trisomy 12 by FISH. Of note, as MYD88 mutations have been associated with good prognosis, we performed statistical analysis excluding, from the "any mutation" group, patients harboring those mutations (n = 2) and observed similar associations with survival (data not shown). Abbreviations: B2M, beta2microglobuline; CI, confidence interval; HR, hazard ratio; NS, non significant

(median number of treatment lines = 2, range 0-7). In multivariate analysis, Binet stage B/C (HR = 3.4, 95% CI = 1.62-7.13, P = .001) correlated with a higher risk of relapse, while the presence of additional trisomies correlated with a lower risk of relapse (HR = 0.37, 95% CI = 0.15-0.9, P = .03). TP53 disruption was associated with a higher risk of relapse in univariate analysis (HR = 2.4, 95% CI = 1.2-2.6, P = .005) but did not retain statistical significance in multivariate analysis although there was a trend for (P = .07) (Supporting Information Table S1). Binet stage B/C and TP53 disruption were also associated with a shorter TNT in multivariate analysis, as was UM IGHV status (Table 4). The median OS for the whole tri12 population was 188 months, with 32 deaths (17%). In multivariate analysis, a shorter OS was significantly associated with Binet stage B/C (median OS = 80 months, HR = 4, 95% CI = 1.6-4.9, P = .002), TP53 disruption (median OS = 80 months, HR = 5, 95% CI = 1.94-12.66,

P < 0.001) and advanced age (HR = 1.09, 95% CI = 1.04-1.15, P = .001) (Table 5).

4 | DISCUSSION

Recurrent cytogenetic abnormalities are among the most important prognosticators in CLL, defining subgroups of patients with distinct biological features, treatment responses, and clinical outcomes.¹⁴ Tri12 is the second most frequent chromosomal aberration (15%-20% of cases) and was historically considered as an intermediate-risk genetic lesion. Tri12 is considered to be an early event in CLL evolution^{8,9} and is characterized by clinical and biological heterogeneity that could potentially be linked to additional/secondary genetic aberrations. Different cytogenetic groups have been described among CLL

TABLE 5 Univariate and multivariate analysis of overall survival (n = 188)

	Univariate			Multivariate		
	HR	CI 95%	P	HR	CI 95%	P
Binet stage B/C vs A	5.8	2.4-13	.002	4	1.5-4.6	.002
Age	1.05	1-1.09	.01	1.09	1.04-1.15	<.001
Lymphocytosis	1.01	1-1.01	NS			-
CD38 > 30%	1.7	0.74-3.94	NS			-
B2M > 4 mg/L	1.4	0.68-2.14	NS			-
Percent of tri12 by FISH	1.03	1.01-1.03	.03			NS
Additional trisomy present	0.5	0.17-1.44	NS			-
Complex and highly complex karyotype	1.6	0.77-3.32	NS			-
Unmutated IGHV	1.7	1.3-1.9	.008			NS
NOTCH1 mutations	1.32	0.61-3.1	NS			-
Any mutation present	1.92	0.95-3.87	NS			-
TP53 disrupted	2.9	1.3-6	.005	5	1.94-12.66	<.001

Lymphocytosis is studied as a continuous variable. The percent of tri12 corresponds to the percentage of interphase nuclei harboring trisomy 12 by FISH. Of note, as MYD88 mutations have been associated with good prognosis, we performed statistical analysis excluding, from the "any mutation" group, patients harboring those mutations (n = 2) and observed similar associations with survival (data not shown). Abbreviations: B2M, beta2microglobuline; CI, confidence interval; HR, hazard ratio; K, karyotype; NS, non significant

FIGURE 1 Profile of associated genomic alterations in the tri12 cohort. Each column represents an individual patient, while each row corresponds to a specific genomic alteration. Colored and white cells respectively indicate positive and negative cases; gray cells represent cases without the relevant data

patients with tri12, notably those with additional trisomies (tri18 and tri19),^{18–20} 14q32 translocations,^{21,22} or del(14q).^{26,27} Recent NGS studies have also highlighted the prognostic impact of some recurrent gene mutations, such as those affecting *TP53*, *NOTCH1*, and *SF3B1*. A study of more than 1000 newly diagnosed and untreated CLL patients showed that the integration of mutational and cytogenetic data could improve the classical FISH-based hierarchical model, by reclassifying patients who harbored *NOTCH1*, *SF3B1*, *TP53*, and/or *BIRC3* mutations into higher risk groups.³³

Studies of tri12 populations integrating comprehensive karyotype/FISH data and mutational information are scarce. Our study included exhaustive cytogenetic data, *IGHV* status, and a search for several recurrent and high-risk genetic mutations in order to refine outcome prediction in a retrospective multicentre cohort of tri12 patients. Two-thirds of the patients were UM *IGHV*, and the frequencies of the main cytogenetic abnormalities were concordant with previous reports: del(13q) (18%-25%), del(11q) (4%-8%), del(17p) (5%-7%), and additional trisomies (12%-13%).^{31,34} In agreement with previous studies, we also found that *NOTCH1* mutations were associated with UM *IGHV* and were the most recurrent mutations (25%), followed by *TP53* mutations (8.5%), while *SF3B1* and *MYD88* mutations were rare (<5%).^{18,31,34,35}

In addition to classical prognostic factors (age and Binet stage), certain cytogenetic and molecular features impacted the outcome of tri12 CLL patients in our cohort. Indeed, additional trisomies were significantly associated with a longer TFT and a lower relapse rate, while *TP53* disruption correlated with a higher risk of relapse, shorter TNT, and shorter OS.

Regarding recurrent cytogenetic abnormalities, the presence of concurrent trisomies has been linked to particular clinical-biological profiles.^{18,34,36} The two most recurrent trisomies associated with tri12 are tri18 and tri19.^{18,20} The presence of concurrent tri19 has been shown to confer a better prognosis compared to isolated tri12,¹⁸ while tri18 is rarely isolated, mostly associated with tri19 and considered as a subclonal event.^{20,36} In our series, all cases harboring tri18 except one were indeed associated with tri19. Tri19 was associated with longer TFT (HR = 0.22, 95% IC 0.08-0.62, $P = .004$), lower rate of relapse (HR = 0.14, 95% IC 0.03-0.52, $P = .007$) and a trend to longer TNT (HR = 0.13, 95% IC 0.02-1.03, $P = .053$) in univariate analysis but did not retain prognostic significance in multivariate analyses while additional trisomies taken as a whole were significantly associated with longer TFT and lower relapse rate (Table 3 and Supporting

Information Table S1). Definitive interpretation of outcomes associated with tri19 is limited by the small number of cases included in our cohort and the enrichment in mutated *IGHV* in this particular subgroup. However, these findings tend to corroborate previous reports demonstrating that numerical chromosomal aberrations in CLL may be associated with favorable outcomes.

A handful of studies have shown that *NOTCH1* mutations are enriched in tri12 CLL patients and may confer an unfavorable outcome.^{30,31,37} Although we found that *NOTCH1* mutations impacted TFT and relapse in univariate analysis, this was not the case in multivariate analysis, and we observed no impact on OS. On the other hand, *TP53* disruption, occurring in 13% of cases, was a strong independent predictor of OS, with a 5-fold higher risk of death in this population, which was mainly treated before the advent of novel therapeutic agents. *TP53* disruption is generally associated with poor outcomes in CLL but has not yet been studied extensively in the particular context of tri12.

IGHV mutational status was recently shown to be the strongest prognostic factor in a cohort of tri12 patients,³⁵ after exclusion of *TP53* disrupted and del(11q) cases. In our series, in which these latter cases were not excluded, *IGHV* mutational status impacted the relapse rate, TNT, and OS, but not TFT, in univariate analyses (Supporting Information Figure S3). However, *IGHV* mutational status only retained significance for TNT in multivariate analysis. We also examined whether additional trisomies and *NOTCH1* mutations could stratify mutated and UM *IGHV* cases, respectively (Supporting Information Figure S4). Interestingly, additional trisomies positively impacted TFT and TNT in *IGHV* mutated patients, while *NOTCH1* mutations had no impact on the outcome of UM *IGHV* patients. Thus, tri12 patients harboring mutated *IGHV* and additional trisomies display a very indolent clinical course, raising the question of the potential interest of performing CB to identify this particular population.

The differences between our results and previous reports might be due in part to differences in the exhaustiveness of the cytogenetic and molecular abnormalities examined. Of note, the distribution of mutations varies among different cytogenetic groups (Table 2 and Figure 1). The tri19 and additional trisomies groups were, for example, significantly enriched in mutated *IGHV* cases when compared to patients with isolated tri12. Patients harboring 14q32 translocations (notably t(14;18)) had more frequent *TP53* disruption than did patients with additional trisomies or isolated tri12. Comprehensive investigation of these cytogenetic and molecular abnormalities in larger

cohorts might help to decipher biological associations and potential oncogenic cooperation in tri12 CLL.

The retrospective nature of our analysis and the heterogeneity of treatments received by the patients may be considered as limitations. Nevertheless, we believe our study provides valuable information on the frequency and prognostic impact of cytogenetic and mutational abnormalities associated with tri12 CLL, which represent a heterogeneous entity in the hierarchical model of Döhner¹⁴ and emphasizes the potential usefulness of comprehensive cytogenetic and IGHV analyses in this population in order to improve risk stratification. It highlights the importance of performing CB, which can detect chromosomal translocations, gains and losses, as recommended in³⁸.

Finally, our findings need to be confirmed in clinical trials and in the context of novel treatments such as BCR and BCL2 inhibitors, given their efficacy in high-risk (TP53-disrupted, UM IGHV) CLL patients.

ACKNOWLEDGMENTS

The authors thank the French Innovative Leukemia Organization data managers and physicians who made this analysis possible. The corresponding author especially thanks Dr Laurence Simon and David Young for careful review of the article and the team of Moulin Gely in Cantal for their support during the writing process.

ORCID

Damien Roos-Weil <http://orcid.org/0000-0002-7767-755X>

REFERENCES

- Fabbri G, Dalla-Favera R. The molecular pathogenesis of chronic lymphocytic leukaemia. *Nat Rev Cancer*. 2016;16(3):145-162.
- Roos-Weil D, Nguyen-Khac F, Bernard OA. Chronic lymphocytic leukemia: time to go past genomics? *Am J Hematol*. 2016;91(5):518-528.
- Puente XS, Pinyol M, Quesada V, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. *Nature*. 2011;475(7354):101-105.
- Quesada V, Conde L, Villamor N, et al. Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. *Nat Genet*. 2011;44(1):47-52.
- Wang L, Lawrence MS, Wan Y, et al. SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. *N Engl J Med*. 2011;365(26):2497-2506.
- Fabbri G, Rasi S, Rossi D, et al. Analysis of the chronic lymphocytic leukemia coding genome: role of NOTCH1 mutational activation. *J Exp Med*. 2011;208(7):1389-1401.
- Ramsay AJ, Quesada V, Foronda M, et al. POT1 mutations cause telomere dysfunction in chronic lymphocytic leukemia. *Nat Genet*. 2013;45(5):526-530.
- Landau DA, Carter SL, Stojanov P, et al. Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. *Cell*. 2013;152(4):714-726.
- Landau DA, Tausch E, Taylor-Weiner AN, et al. Mutations driving CLL and their evolution in progression and relapse. *Nature*. 2015;526(7574):525-530.
- Puente XS, Bea S, Valdes-Mas R, et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia. *Nature*. 2015;526(7574):519-524.
- Lazarian G, Guieze R, Wu CJ. Clinical implications of novel genomic discoveries in chronic lymphocytic leukemia. *J Clin Oncol*. 2017;35(9):984-993.
- Damm F, Mylonas E, Cosson A, et al. Acquired initiating mutations in early hematopoietic cells of CLL patients. *Cancer Discov*. 2014;4(9):1088-1101.
- Dierlamm J, Michaux L, Criel A, Wlodarska I, Van den Berghe H, Hossfeld DK. Genetic abnormalities in chronic lymphocytic leukemia and their clinical and prognostic implications. *Cancer Genet Cytogenet*. 1997;94(1):27-35.
- Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med*. 2000;343(26):1910-1916.
- Van Bockstaele F, Verhasselt B, Philippe J. Prognostic markers in chronic lymphocytic leukemia: a comprehensive review. *Blood Rev*. 2009;23(1):25-47.
- Davids MS, Vartanov A, Werner L, Neuberg D, Dal Cin P, Brown JR. Controversial fluorescence in situ hybridization cytogenetic abnormalities in chronic lymphocytic leukaemia: new insights from a large cohort. *Br J Haematol*. 2015;170(5):694-703.
- Cosson A, Chapiro E, Bougacha N, et al. Gain in the short arm of chromosome 2 (2p+) induces gene overexpression and drug resistance in chronic lymphocytic leukemia: analysis of the central role of XPO1. *Leukemia*. 2017;31(7):1625-1629.
- Baliakas P, Puiggros A, Xochelli A, et al. Additional trisomies amongst patients with chronic lymphocytic leukemia carrying trisomy 12: the accompanying chromosome makes a difference. *Haematologica*. 2016;101(7):e299-e302.
- Schwaenen C, Nessling M, Wessendorf S, et al. Automated array-based genomic profiling in chronic lymphocytic leukemia: development of a clinical tool and discovery of recurrent genomic alterations. *Proc Natl Acad Sci U S A*. 2004;101(4):1039-1044.
- Sellmann L, Gesk S, Walter C, et al. Trisomy 19 is associated with trisomy 12 and mutated IGHV genes in B-chronic lymphocytic leukaemia. *Br J Haematol*. 2007;138(2):217-220.
- Sen F, Lai R, Albitar M. Chronic lymphocytic leukemia with t(14;18) and trisomy 12. *Arch Pathol Lab Med*. 2002;126(12):1543-1546.
- Martin-Subero JI, Ibbotson R, Klapper W, 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):1532-1544.
- 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(6):1201-1204.
- Baliakas P, Iskas M, Gardiner A, et al. Chromosomal translocations and karyotype complexity in chronic lymphocytic leukemia: a systematic reappraisal of classic cytogenetic data. *Am J Hematol*. 2014;89(3):249-255.
- Chapiro E, Radford-Weiss I, Bastard C, 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):2123-2127.
- Reindl L, Bacher U, Dicker F, et al. Biological and clinical characterization of recurrent 14q deletions in CLL and other mature B-cell neoplasms. *Br J Haematol*. 2010;151(1):25-36.
- Cosson A, Chapiro E, Belhouachi N, et al. 14q deletions are associated with trisomy 12, NOTCH1 mutations and unmutated IGHV genes in chronic lymphocytic leukemia and small lymphocytic lymphoma. *Genes Chromosomes Cancer*. 2014;53(8):657-666.
- 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(2):382-388.
- Athanasiadou A, Stamatoopoulos K, Tsompanakou A, et al. Clinical, immunophenotypic, and molecular profiling of trisomy 12 in chronic lymphocytic leukemia and comparison with other karyotypic subgroups defined by cytogenetic analysis. *Cancer Genet Cytogenet*. 2006;168(2):109-119.
- Balatti V, Bottoni A, Palamarchuk A, et al. NOTCH1 mutations in CLL associated with trisomy 12. *Blood*. 2012;119(2):329-331.
- Del Giudice I, Rossi D, Chiaretti S, et al. NOTCH1 mutations in +12 chronic lymphocytic leukemia (CLL) confer an unfavorable prognosis, induce a distinctive transcriptional profiling and refine the intermediate prognosis of +12 CLL. *Haematologica*. 2012;97(3):437-441.

32. Ghia P, Stamatopoulos K, Belessi C, et al. Geographic patterns and pathogenetic implications of IGHV gene usage in chronic lymphocytic leukemia: the lesson of the IGHV3-21 gene. *Blood*. 2005;105(4):1678-1685.
33. Rossi D, Rasi S, Spina V, et al. Integrated mutational and cytogenetic analysis identifies new prognostic subgroups in chronic lymphocytic leukemia. *Blood*. 2013;121(8):1403-1412.
34. Lopez C, Delgado J, Costa D, et al. Different distribution of NOTCH1 mutations in chronic lymphocytic leukemia with isolated trisomy 12 or associated with other chromosomal alterations. *Genes Chromosomes Cancer*. 2012;51(9):881-889.
35. Bulian P, Bomben R, Bo MD, et al. Mutational status of IGHV is the most reliable prognostic marker in trisomy 12 chronic lymphocytic leukemia. *Haematologica*. 2017;102(11):e443-e446.
36. Ibbotson R, Athanasiadou A, Sutton LA, et al. Coexistence of trisomies of chromosomes 12 and 19 in chronic lymphocytic leukemia occurs exclusively in the rare IgG-positive variant. *Leukemia*. 2012;26(1):170-172.
37. Balatti V, Lerner S, Rizzotto L, et al. Trisomy 12 CLLs progress through NOTCH1 mutations. *Leukemia*. 2013;27(3):740-743.
38. Hallek M, Cheson BD, Catovsky D, et al. Guidelines for diagnosis, indications for treatment, response assessment and supportive management of chronic lymphocytic leukemia. *Blood*. 2018;131:2745-2760.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.