Chromosomal aberrations and their prognostic value in a series of 174 untreated patients with Waldenström's macroglobulinemia

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ABSTRACT

Waldenström's macroglobulinemia is a disease of mature B cells, the genetic basis of which is poorly understood. Few recurrent chromosomal abnormalities have been reported, and their prognostic value is not known. We conducted a prospective cytogenetic study of Waldenström's macroglobulinemia and examined the prognostic value of chromosomal aberrations in an international randomized trial. The main aberrations were 6q deletions (30%), trisomy 18 (15%), 13q deletions (13%), 17p (*TP53*) deletions (8%), trisomy 4 (8%), and 11q (*ATM*) deletions (7%). There was a significant association between trisomy of chromosome 4 and trisomy of chromosome 18. Translocations involving the *IGH* genes were rare (<5%). Deletion of 6q and 11q, and trisomy 4, were significantly associated with adverse clinical and biological parameters. Patients with *TP53* deletion had short progression-free survival and short disease-free survival. Although rare (<5%), trisomy 12 was associated with short progression-free survival. In conclusion, the cytogenetic profile of Waldenström's macroglobulinemia appears to differ from that of other B-cell lymphomas. Chromosomal abnormalities may help with diagnosis and prognostication, in conjunction with other clinical and biological characteristics. *This trial is registered with Clinicaltrials.gov, numbers NCT00566332 and NCT00608374.*

Introduction

Waldenström's macroglobulinemia (WM) is a disease of mature B cells, the genetic basis of which is poorly understood. Few recurrent chromosomal abnormalities have been reported in WM, partly because of difficulties in obtaining tumor metaphases for karyotyping. Alternative techniques such as fluorescence *in situ* hybridization (FISH) have been used to study this disease. Deletion of the long arm (q) of chromosome 6 is the most common abnormality, being found in up to 50% of patients.[1,2] Trisomy 4 and 13q14 and 17p13 deletion have also been described.[3-5] Deletion of chromosome arm 6q is associated with features of adverse prognosis, and 13q14 and 17p13 deletions are associated with advanced disease stages. More recently, using high-array-based comparative genomic hybridization, Braggio *et al.* observed a recurrent gain in the short arm (p) of chromosome 6.[6] Most relevant studies have involved both treated and untreated patients, making it difficult to determine the prognostic value of chromosomal abnormalities.

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To address these issues, we conducted a cytogenetic study of a large series of untreated symptomatic patients enrolled in a prospective, randomized, open-label clinical trial. The WM1 study took place in 101 centers in five countries and enrolled 339 patients with WM, 37 patients with non-MALT marginal zone lymphoma (MZL), and 38 patients with lymphoplasmacytic lymphoma, who were all randomly assigned to receive chlorambucil or fludarabine.[7]

Here we report our cytogenetic findings in 174 of the WM patients enrolled in this trial.

Design and Methods

Patients and the WM1 study

Patients ≥18 years old with previously untreated WM and an Eastern Cooperative Oncology Group (ECOG) performance status score of ≤2 were eligible. To be included, patients had to have an established diagnosis of WM requiring treatment, with a $CD20^+$, $CD5^-$, $CD23^-$, $CD10^-$ tumor-cell immunophenotype. Patients were assigned to oral chlorambucil 8 mg/m^2/day (6 mg/m^2/day if >75 years old) for 10 days every 28 days for a maximum of 12 cycles, or to oral fludarabine 40 mg/m^2/day (30 mg/m^2/day if >75 years old) for 5 days every 28 days for up to six cycles. The trial was approved by a local ethics committee (CCPPRB Pitié-Salpêtrière) and conformed with the Declaration of Helsinki. All the patients gave written informed consent to their participation. *The trial is registered with Clinicaltrials.gov, numbers NCT00566332 and NCT00608374.*

Conventional cytogenetic and fluorescence in situ hybridization studies

Conventional cytogenetic and FISH analyses were performed at enrollment in the trial, between 2001 and 2009. Chromosomal studies were performed for 172 patients: these studies were done by the referring laboratories on bone marrow and/or on peripheral blood when circulating tumor cells were present. Cells were not stimulated (43 patients) or stimulated for 72 h with 12-O-tetradecanoyl-phorbol-13-acetate (56 patients) until 2006, and subsequently with CpG-oligonucleotide plus interleukin-2 (21 patients). The type of culture was unknown for 52 patients. Centralized examinations included metaphase karyotyping, and FISH analyses of 6q21 (139 patients), 11q22 (*ATM* locus) (139 patients), 13q14 (145 patients) and 17p13 (*TP53* locus) (140 patients) deletions, trisomy of chromosomes 4 (139 patients), 12 (140 patients) and 18 (117 patients), and translocations involving 14q32 (*IGH* locus) (129 patients). The following probes were used: CEP4, CEP12, 13q14 (D13S319), ATM, p53, IGH, BCL2, PAX5 (Abbott, Rungis, France), and 6q21 (Kreatech, Illkirch, France). FISH analyses were performed on unselected cultured cells for all but one patient, on interphase nuclei (minimum of 100 counted nuclei) and also on metaphases when available.

TP53 gene sequencing

TP53 variants at exons 4 to 10 were analyzed by direct sequencing in three patients with 17p deletion, as previously described, starting from DNA extracted from cytogenetic pellets.[8]

Statistical analysis

Group-wise comparisons of the distributions of clinical and laboratory variables were conducted using Wilcoxon's test (for quantitative variables) or Fisher's exact test (for qualitative variables). Overall survival was measured from enrollment to death or the last follow-up visit. Progression-free survival was measured from enrollment to disease progression, death from any cause, or the last follow-up visit. Disease-free survival was measured from the date of response until disease progression or death. Distributions of overall, progression-free and disease-free survival were estimated with the Kaplan-Meier method and compared using the log-rank test.[9,10]

All tests were two-sided. An effect was considered statistically significant if the *P* value was 0.05 or less. The SAS 9.2 (SAS, Inc., NC, USA) and R 2.10.1 (R Development Core Team, 2006) software packages were used.

Results

Clinical results

The male to female ratio was 2.16, and the mean age was 66.4 years (range, 40.0 to 84.7). The median interval between diagnosis and inclusion in the trial was 2.3 months (Q1=0.69; Q3=14.8). The median follow-up was 40.7 months (Q1=22.1; Q3=60.6). The median percentage of lymphoplasmacytic cells, calculated on bone-marrow smears, was 51.5% (Q1=29%; Q3=75%).

Conventional cytogenetic and fluorescence in situ hybridization analyses

Conventional cytogenetic analysis was successful in 141/172 cases (82%), of which 66 (47%) showed clonal abnormalities (Table 1). Twenty (30%) of the 66 cases with abnormal conventional cytogenetics were complex, with at least three chromosomal changes, and 23 (35%) showed translocations (5 balanced and 18 unbalanced). A t(11;18)(q21;q21) translocation involving *MALT1* was observed in one case. Twenty (30%) of these 66 cases with abnormal conventional cytogenetic findings included loss of sex chromosomes, and this was an isolated abnormality in six other patients. Since the loss of sex chromosomes is common in elderly patients, it may represent a non-specific finding in WM.

FISH analyses with all eight probes, performed in 113 patients, showed a mean of 0.8 (±0.91) abnormalities per patient (range, 0-5). Of the 113 patients analyzed, 63 (56%) had a FISH abnormality. Among these 63 patients, 41 had a single abnormality, 18 had two abnormalities, three had three abnormalities and one had five abnormalities (*Online Supplementary Table S1*). Combining results from conventional cytogenetic and FISH studies, the mean number of abnormalities was 1.4 (±1.77) (range, 0-11). The estimated median percentage of tumor cells carrying a FISH abnormality was 46% (Q1=31; Q3=68).

Using FISH and/or conventional cytogenetics, we observed 6q deletions in 43/141 (30%) cases, trisomy 18 in 17/117 (15%), 13q14 deletion in 19/145 (13%), trisomy 4 in 11/139 (8%), 17p13 (*TP53*) deletions in 11/140 (8%), 11q22 (*ATM*) deletions in 10/139 (7%), trisomy 12 in 6/140 (4%), and 14q32 (*IGH*) translocations in 3/129 (2%). There was one case of t(14;18)(q32;q21) and two translocations with unknown partners (neither *BCL2* nor *PAX5*). In the latter two cases the *IGH* rearrangement was observed only in interphase nuclei.

Deletion of 6q was significantly more frequent in complex karyotypes than in abnormal karyotypes with fewer than three abnormalities [5/9 (57%) *versus* 7/36 (19%), *P*=0.005] (*Online Supplementary Table S2*). There was a significant association between trisomy of chromosome 4 and trisomy of chromosome 18; among 11 cases with trisomy

18, trisomy 4 was present in four cases (36%), compared to three cases among the 85 patients (4%) without trisomy 18 (*P*=0.003) (*Online Supplementary Table S2*). Deletion of 17p was only detected by FISH in four patients (three with a normal karyotype and one with a complex karyotype with two markers) (Table 2). Deletion of 17p was observed in the dominant clone of four patients and in a subclone from one patient. Among seven patients with a translocation, three (43%) had a 17p deletion *versus* 4/86 (5%) patients without a translocation (*P*=0.03) (*Online Supplementary Table S2*). When associated with another FISH abnormality, 17p deletion affected a lower proportion of cells in four patients and the same proportion in two patients. Of note, patients with 17p deletion had a higher percentage of tumor cells in their bone marrow than patients without 17p deletion (median 77% *versus* 46%, *P*=0.01).

Table 1. Summary of cytogenetic findings in 174 WM patients.

Successful karyotyping	141/172 (82%)°
Abnormal karyotype	66/141 (47%)
Abnormalities (K)$ (n=141)	
Median	0
Mean	1.1
Range	0-11
Abnormalities (K)* (n=66)	
Median	1.5
Mean	2.4
Range	1-11
Complex karyotype*	20/66 (30%)
Translocation*	23/66 (35%)
Sex chromosome loss*	20/66 (30%)
6q deletion (K+F)	43/141 (30%)
(K)*	18/66 (27%)
(F)	40/139 (29%)
Trisomy 18 (K+F)	17/117 (15%)
(K)*	8/66 (12%)
(F)	15/117 (13%)
Trisomy 4 (K+F)	11/139 (8%)
(K)*	6/66 (9%)
(F)	10/139 (7%)
Trisomy 12 (K+F)	6/140 (4%)¤¤
(K)*	5/66 (8%)
(F)	5/140 (4%)
13q14 deletion (F)$	19/145 (13%)
17p13 (*TP53*) deletion (F)$	11/140 (8%)
11q22 (*ATM*) deletion (F)$	10/139 (7%)
14q32 (*IGH*) translocation (F)$	3/129 (2%)¤
Abnormalities (F) (n=113)	
Median	1
Mean	0.8
Range	0 - 5
Abnormalities (K+F) (n=97)$$	
Median	1
Mean	1.4
Range	0-11

*WM: Waldenström's macroglobulinemia: °Karyotyping was performed in 172 of 174 patients. *In patients with an abnormal karyotype. K: Observed by karyotyping. K +F: observed by karyotyping and/or FISH. F: observed by FISH. $Successful karyotype. $$Number of patients with successful karyotyping and analysis with all eight FISH probes to detect: 6q, 13q14, 17p13 (*TP53*), and 11q22 (ATM) deletions, trisomy 18, 4 and 12, and 14q32 (IGH) translocations. ¤One t(14;18)(q32;q21) (involving BCL2) and two unknown partners; there were two normal karyotypes and one failure. #Karyotype analyses did not detect additional cases. ¤¤One patient had 4/40 mitoses with trisomy 12 by karyotyping. Trisomy 12 was not observed using FISH.*

Correlations with prognostic factors

Cytogenetic profiles were comparable across the two treatment arms. The observed chromosomal abnormalities were analyzed with respect to the adverse characteristics included in the International Scoring System for WM (ISSWM), namely age >65 years, hemoglobin ≤11.5 g/dL, platelet count ≤100x10^9/L, neutrophil count ≤1.5x10^9/L, albumin <35 g/dL, β2-microglobulin >3 mg/L, and IgM >7 g/dL.[11] Deletion of 6q was significantly associated with β2-microglobulin >3 mg/L [28/73 (38%) *versus* 15/68 (22%), *P*=0.04], trisomy 4 with β2-microglobulin >3 mg/L [10/70 (14%) *versus* 1/69 (1%), *P*=0.009], and *ATM* deletion with age >65 years [9/79 (11%) *versus* 1/60 (2%), *P*=0.04]. Of note, 6q deletion and *ATM* deletion were also significantly associated with hypoalbuminemia (<35 g/L), another classical factor of poor prognosis [23/48 (48%) *versus* 20/103 (19%), *P*<0.0001; and 6/40 (15%) *versus* 4/99 (4%), *P*=0.03, respectively].[12] Finally, trisomy 4 was significantly associated with male gender [11 (100%) *versus* 85 (66%), *P*=0.02]. Cytogenetic profiles were comparable across the three risk groups defined by the ISSWM.[11]

We then analyzed the impact of chromosomal abnormalities on the response rate, progression-free survival, disease-free survival and overall survival. The six patients with trisomy 12 had significantly shorter progression-free survival (median 12.2 months *versus* 31.2 months without trisomy 12; *P*=0.004) (Figure 1A). Deletion of *TP53* was associated with shorter progression-free survival in univariate analysis (median 18.7 months *versus* 30 months without *TP53* deletion; *P*=0.05) (Figure 1B), and with shorter disease-free survival (median 7.8 months *versus* 28.8 months without *TP53* deletion; *P*=0.001) (Figure 1C). There was no significant interaction between the effect of trisomy 12 or *TP53* deletion and treatment arm. The other abnormalities (abnormal cytogenetic findings, complex karyotype, translocation, deletions of 6q, 13q14 and *ATM*, trisomy 18 and *IGH* translocation) had no impact on the response rate, progression-free survival, disease-free survival or overall survival.

TP53 *gene sequencing*

Finally, we analyzed *TP53* sequences (exons 4-10) in three patients with 17p deletion in 13%, 17% and 52% of interphase nuclei (FISH analysis). No mutations were detected.

Discussion

The World Health Organization classification defines WM as a lymphoplasmacytic lymphoma with bone marrow involvement and an IgM monoclonal gammopathy of any concentration.[13] Until now, no specific chromosomal or genetic abnormalities have been recognized in this malignancy. However, the frequency of individual chromosomal abnormalities observed in WM differs from that in other B-cell neoplasias such as MZL, chronic lymphocytic leukemia (CLL) and multiple myeloma (MM). In particular, 6q deletion and trisomy 4 are more frequent in WM, while 13q14 deletion is less frequent in WM (13% in

Table 2. WM patients with 17p13 (*TP53*) deletion.

Patients	Age/ gender	BM involvement %	Culture	Karyotype	del17p (interphase FISH) (%)	*TP53* mutation	Other interphase FISH abnormalities
1*	72/M	82	TPA	49,XY,-3,+21,+add(21)(q),+mar1,+mar2[cp5]/46,XY[15]	7	nd	
2	77/F	47	CpG-ODN+IL-2	45,X,-X,add(17)(p12)[5]/46,XX[15]	50	no	
3	74/M	77	TPA	46,XY[20]	8	nd	del13q (40%)
4	72/M	un	TPA	44,X,-Y,del(6)(q11),der(10)t(10;13)(q22;q12),der(12)t(12;17)(q24;q10-11),-13,-17,+mar[6]/44,X,-Y,idem,add(5)(q35)[4]/46,XY[10]	13	no	del6q (25%)
5	58/M	59	TPA	46,XY,i(17)(q10)[1]/46,XY[29]	10	nd	
6	66/M	87	TPA	45,XY,-17[3]/46,XY[27]	10	nd	
7	63/M	un	TPA	45,X,-Y,del(6)(q22)[4]/46,XY[16]	15	nd	del6q (42%)
8	78/F	86	TPA	46,XX,del(8)(p?11p?21),i(17)(q10)[12]/46,XX[12]	nd	nd	
9	65/M	76	TPA	46,XY,del(6)(q?),add(10)(qter)[6]/45,idem,der(9)t(9;17)(p11;q11),-17[2]/46,XY[12]	13	nd	del6q (26%)
10	62/M	un	TPA	46,XY[30]	17	no	del6q (17%)
11	72/M	un	TPA	46,XY[20]	16	nd	del6q (16%)

Un: unknown. Nd: not determined. No: no TP53 *mutation detected. BM: bone marrow. TPA: 12-O-tetradecanoyl-phorbol-13-acetate. CpG-ODN, CpG-oligonucleotide. IL2: interleukin 2; *previously reported in Terré* et al., *Leukemia 2006 (Patient 29).*

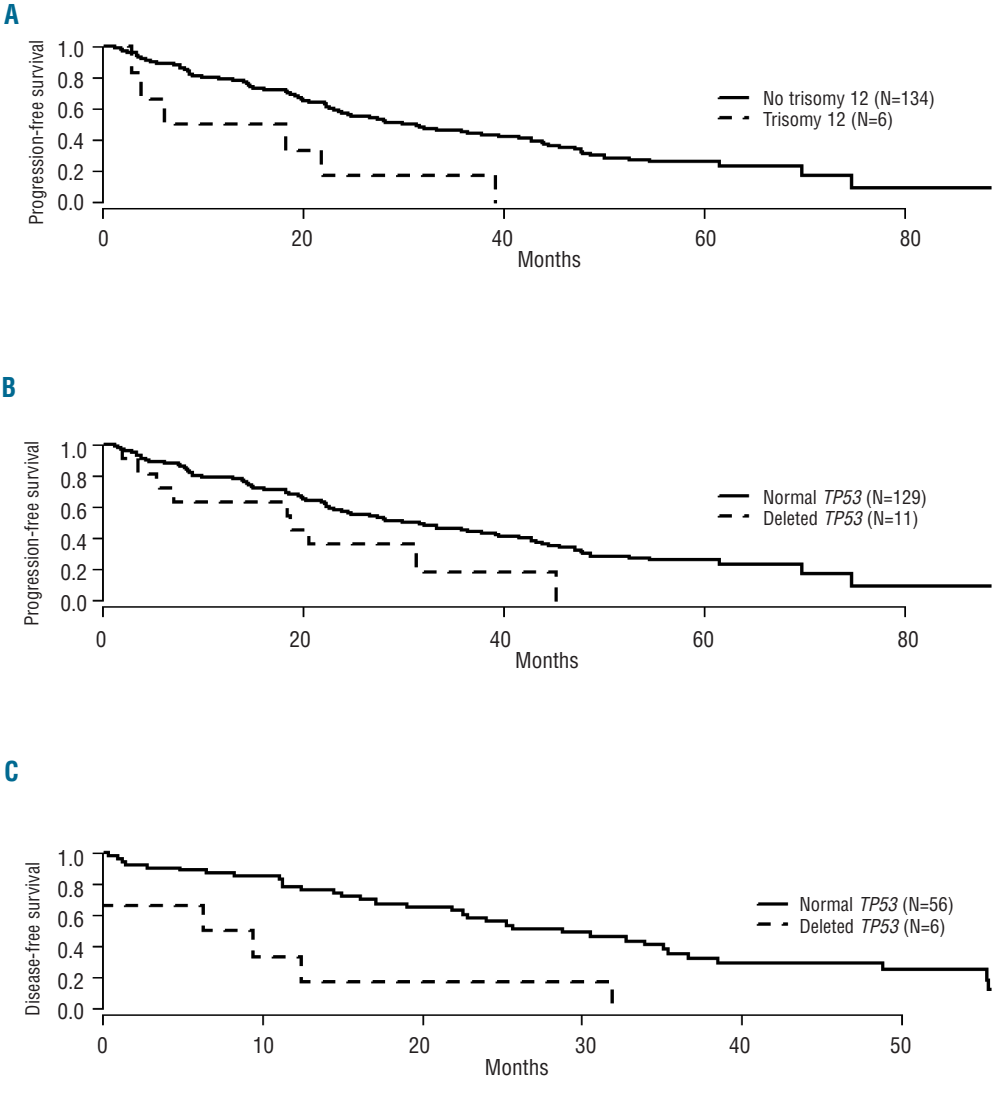

Figure 1. Progression-free survival according to trisomy 12 (A) and *TP53* deletion (B); and disease-free survival according to *TP53* deletion (C).

our series) than in CLL and MM (both ~50%).[14-16] Interestingly, trisomy 18, which is reported to be common in MZL (10-28%), was also frequent in our series of WM (15%), and was significantly associated with trisomy 4.[17,18] Finally, we confirm that translocations involving the *IGH* gene are very rare in WM (3%), whereas they are reported to be frequent in MM (~40%) and also, although less so, in MZL (~10%).[16,18,19]

Cytogenetic studies in WM patients have been hampered by the low mitotic index of tumor cells.[1] In the literature, chromosomal abnormalities are reported in only 15% to 32% of patients. The immunostimulatory factor CpG-oligonucleotide, used in combination with interleukin-2, has been reported to induce cell-cycle progression of CLL cells, and has been commonly used as a mitogen for CLL cell culture by cytogeneticists since 2007.[20,21] Karyotyping was successful in 82% of WM patients and detection of chromosomal abnormalities in 47% of cases. These high percentages could be explained by the fact that patients were symptomatic in our series and had progressive disease, as well as by the addition of CpG-oligonucleotide and interleukin-2.

Deletion of 6q is the most common cytogenetic abnormality reported in WM, and its frequency (38-54%) is much higher than in other B-cell diseases such as CLL (~6%) and MZL (<20%).[1,2,6,17,22] Two tumor-suppressor genes could be involved in the pathogenesis of WM: *BLIMP-1* on 6q21 and *TNFAIP3* on 6q23.[6,23] In our series, we detected 6q deletions in 30% of patients, a rate slightly lower than that reported in the literature. One possible explanation is that the FISH probe we used did not cover all deleted regions: several areas of minimal deletion have been reported on the long arm of chromosome 6.[6,24] In addition, some authors used different approaches, including cell sorting and cytoplasmic immunoglobulin m-FISH, which might be more sensitive.[6,24] Finally, none of our patients had received treatment for WM prior to the cytogenetic analysis, contrary to previous studies. Although 6q deletion was significantly associated with complex karyotypes in our large series, and with adverse characteristics such as hypoalbuminemia and high β2-microglobulin levels, 6q deletion had no adverse impact on patients' outcome, thus confirming the results of smaller studies.[1,2]

The minimal deleted region on 13q14 seems to be similar in WM and CLL, and includes the microRNA genes miRNA-15a and miRNA-16-1.[6,25] Deletion of 13q14, when isolated, is associated with a good prognosis in patients with CLL but seems to be associated with disease progression in MM.[16,22] In contrast, this abnormality had no influence on outcome and was not associated with adverse characteristics in our series of WM. It has been suggested that 13q14 deletions usually arise at the time of disease progression in WM,[4] but we found some 13q deletions shortly after diagnosis.

We confirmed our previous results regarding the occurrence of trisomy 4 in WM (8%), and found that trisomy 4 was significantly associated with trisomy 18 in our series.[3,6] As trisomy 4 has not yet been reported in other B-cell malignancies, it might help to distinguish WM from other indolent B-cell disorders. Trisomy 4 was significantly associated with high levels of β2-microglobulin in our series but did not influence clinical outcome. Surprisingly, it seemed to be significantly more frequent in males. This might explain the high proportion of males (28/39, 72%) in the first report of trisomy 4 in WM.[3]

The frequency of 11q22 (*ATM*) deletions is 7-24% in CLL, and this abnormality is often associated with lymphadenopathy and a poor prognosis.[14,22,26] In our series of WM, 11q deletion was associated with advanced age and hypoalbuminemia but had no impact on clinical outcome.

Deletion of 17p13, including the *TP53* gene, is associated with poor prognosis in many B-cell disorders, including CLL, MZL and MM,[16,18,22] and its frequency is reported to increase during the course of WM.[4] In our series, in which the frequency of 17p deletion was 8%, some cases were observed early in the disease course. Like Schop *et al.*, we found that patients with 17p deletion had a higher percentage of tumor cells in their bone marrow.[4] Unlike other chromosomal abnormalities, which were associated with adverse characteristics but did not affect clinical outcome in our series, *TP53* deletion did not correlate with biological variables. However, *TP53* deletion was associated with a short progression-free survival and short disease-free survival in univariable analyses. Of note, when adjusting for treatment arms and ISSWM risk groups, *TP53* deletion was still associated with shorter progression-free survival and shorter disease-free survival. Our survival analysis showed no difference between WM patients with and without *TP53* deletion, after a median follow-up of 39.7 months, although this needs to be confirmed in the longer term. WM patients with 17p deletion may benefit from adapted treatment, as do CLL patients.

Deletion of 17p is associated with *TP53* mutation in about 80% of CLL patients. Although CLL with 17p deletion but without *TP53* mutation is rare, it has a poor prognosis.[27] In MM, 63% of 17p deletions were not associated with *TP53* mutation.[28] Few data on *TP53* mutations in WM have been reported. The recently established WM cell line MWCL-1 is reported to carry a monoallelic 17p deletion and a missense mutation in exon 5 of the remaining copy of *TP53*, both anomalies being present in the original patient's biopsy.[29] We found no *TP53* mutations in the three WM patients we analyzed, possibly because of the low sensitivity of the sequencing approach and the relatively low percentage of cells with *TP53* deletion (13% and 17%). Alternatively, an inactivating mutation of the second copy of *TP53* might occur later in the disease. Longitudinal studies would allow us to answer this question and to detect the possible appearance of *TP53* abnormalities in *TP53* wild-type patients.

The reported frequencies of trisomy 12 are 11-15% in CLL and 8-15% in MZL.[18,22] This abnormality is often associated with an atypical morphology and has a controversial prognostic influence in CLL.[22,30] Trisomy 12 was very rare in our series of WM patients (<5%), and was significantly associated with short progression-free survival. When adjusting for treatment arms and ISSWM risk groups, trisomy 12 was still associated with shorter progression-free survival. In contrast, it did not influence overall survival. Trisomy 12 may reflect a particular aspect of sub-types of B-cell disorders that warrants further investigations.

In conclusion, this study provides the first comprehensive overview of the cytogenetic profile of WM in untreated patients enrolled in a prospective trial. It identifies a pattern of chromosomal abnormalities that may help with diagnosis and prognostication. Treatment strategies in WM have evolved considerably since the inception of this trial and the reported data should be interpreted in the light of these developments. The prognostic values of

TP53 deletion and trisomy 12 need to be confirmed in patients treated with new combinations of treatments with monoclonal antibodies.

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