"Double-hit" chronic lymphocytic leukemia: An aggressive subgroup with 17p deletion and 8q24 gain

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Abstract

Chronic lymphocytic leukemia (CLL) with 17p deletion (17p-) is associated with a lack of response to standard treatment and thus the worst possible clinical outcome. Various chromosomal abnormalities (including unbalanced translocations, deletions, ring chromosomes and isochromosomes) result in the loss of 17p and one copy of the *TP53* gene. The objective of the present study was to determine whether the type of chromosomal abnormality leading to 17p- and the additional aberrations influenced the prognosis in a series of 195 patients with 17p-CLL. Loss of 17p resulted primarily from an unbalanced translocation (70%) with several chromosome partners (the most frequent being chromosome 18q), followed by deletion 17p (23%), monosomy 17 (8%), isochromosome 17q [i(17q)] (5%) and a ring chromosome 17 (2%). In a univariate analysis, monosomy 17, a highly complex karyotype ($\geq$5 abnormalities), and 8q24 gain were associated with poor treatment-free survival, and i(17q) (p=0.04), unbalanced translocations (p=0.03) and 8q24 gain (p=0.001) were significantly associated with poor overall survival. In a multivariate analysis, 8q24 gain remained a significant predictor of poor overall survival. We conclude that 17p deletion and 8q24 gain have a synergistic impact on outcome, and so patients with this "double-hit" CLL have a particularly poor prognosis. Systematic, targeting screening for 8q24 gain should therefore be considered in cases of 17p- CLL.

Keywords
chronic lymphocytic leukemia
17p deletion
prognosis
8q24 gain
isochromosome 17q

Introduction

Chronic lymphocytic leukemia (CLL), the most common leukemia in adults, has a highly variable course, reflecting its biological heterogeneity. Many patients do not require treatment for years, whereas others exhibit aggressive disease, do not respond to treatment, and thus have a poor prognosis. Chromosomal abnormalities (including deletions of 11q, 13q or 17p (17p-), and trisomy 12) are present in about 80% of cases of CLL. Fluorescence *in situ* hybridization (FISH) is used to routinely screen for these chromosomal abnormalities [1]. Patients with 17p- CLL have the worst clinical outcomes, and the shortest progression-free and overall survival (OS) times [2]. The 17p deletion is found in 10% or less of patients with CLL but in up to 40% of relapsed or treatment-refractory patients. Loss of the short arm of the chromosome 17 results from various chromosomal abnormalities, including unbalanced translocations, deletions, ring chromosomes and isochromosomes [3]. All these aberrations lead to the loss of one copy of the *TP53* gene, and the remaining allele is mutated in most instances. Furthermore, 17p- is often associated with genomic complexity as detected with conventional karyotyping or genomic microarrays [4, 5].

The dismal prognosis for patients with 17p- CLL is mainly due to a lack of response to conventional treatments. In keeping with the role of *TP53* as a pivotal regulator of the DNA response pathway, loss of this gene is associated with resistance to DNA-damaging agents (e.g. fludarabine and cyclophosphamide). Recently, new drugs targeting the B-cell receptor signaling pathway or the apoptosis machinery have shown efficacy in 17p- CLL, with a better treatment response and longer progression-free survival [6-8]. Despite good initial responses to these new targeted therapies, relapse can still occur - particularly in patients bearing a complex karyotype (K) [9]. In contrast, some patients with *de novo* 17p- CLL have stable disease and remain asymptomatic for prolonged periods of time [10, 11]. All these observations underline the clinical heterogeneity of 17p- CLL. It has been shown that a number of clinical and biological markers (such as *IGHV* mutational status, the size of the 17p- clone, and genomic complexity) are significantly linked to the clinical outcome in the subgroup of patients with 17p- CLL [12, 13].

The objectives of the present study of a series of 195 patients with 17p- CLL were to determine whether (i) the type of chromosomal abnormality leading to 17p loss (i.e. translocations, deletions, rings and isochromosomes, as identified by K analysis) was associated with the prognosis, and (ii) additional aberrations had an impact on the clinical outcome.

Methods

Patient selection

Databases from 20 French and Belgian institutions were retrospectively screened for cases with a morphological and immunological diagnosis of CLL according to the iwCLL criteria [14], in which informative K analysis showed a loss of 17p and FISH confirmed the loss of the *TP53 gene*. A total of 195 patients were identified. The patients' clinical and biological characteristics were extracted retrospectively from medical records. The study was performed in accordance with the Declaration of Helsinki, and was approved by the local investigational review board (*CPP-Ile-de-France VI*, Paris, France; date: 09/15/2011).

Karyotype and FISH analyses

All the K results were reviewed by the members of the *Groupe Francophone de Cytogénétique Hématologique* and then classified according to the International System for Human Cytogenetic Nomenclature (ISCN 2016). Complex Ks were defined as the presence of three or more numerical or structural chromosomal abnormalities (CK$\geq$3), and highly complex Ks were defined as the presence of five or more abnormalities (CK$\geq$5). Monosomal Ks were defined as the presence of 2 autosomal monosomies or 1 monosomy with at least 1 structural abnormality. Data from routine FISH analyses were available for some of patients: 11q22 (*ATM*) (n=158), 13q14 (n=118), centromere of chromosome 12 (n=102), and 8q24 (*MYC*) (n=12).

TP53 mutations and functional assays

TP53 mutations were analyzed by (i) Sanger sequencing of exons 2 to 11 (n=17), exons 4 to 10 (n=29) or exons 4 to 9 (n=12), or (ii) next-generation sequencing (NGS) on a MiSeq® platform (Illumina, San Diego, CA) using the CLL MASTR PLUS kit (Multiplicom, Niel, Belgium) (n=9) or on a Ion Torrent platform (Life Technologies, Carlsbad, CA) using the Ion AmpliSeq™ TP53 Panel (Life Technologies) (n=8). A negative result with Sanger sequencing was not considered to be informative because this technique does not cover all the exons and is not sensitive (10%). A functional assay of p53 was carried out as described previously [15].

Statistical analysis

Treatment-free survival (TFS) and OS were defined as the time interval between diagnosis and first-line treatment or death, respectively, or (in the absence of these events) last follow-up.

Categorical variables were compared using the chi-squared test or Fisher's exact test, while continuous variables were compared using the Mann-Whitney test. Survival analyses were performed using the Kaplan-Meier method. The log-rank test was used for intergroup comparisons of OS or TFS curves. The variables analyzed were age (<65 vs. $\geq$65), Binet stage (A vs. B/C), *IGHV* mutation status, the percentage of cells bearing the *TP53* deletion ($\leq$20% vs. >20%, $\leq$ 35% vs. >35%, $\leq$

80% vs. >80%), occurrence of 17p- after treatment, the number of 17p abnormalities (1 vs ≥2), a 17p abnormality alone (according to the K), an unbalanced translocation involving 17p (U-translocation-17p), a deletion 17p, a monosomy 17, an isochromosome 17q [i(17q)], a der/dic(17;18)(q10;q10), a CK with ≥3 or ≥5 abnormalities, a monosomal K, U-translocations (all: 17p and not 17p), the presence of additional U-translocations (U-translocation-not 17p), 8q24 gain, 8p deletion, trisomy 12, and 11q deletion. Multivariate analysis (with Cox proportional hazards regression models) was subsequently performed to assess the independent prognostic value of covariates that were significant in the univariate analysis. There were too few data on *IGHV* mutations for inclusion in the multivariate analysis. The quality of the multivariate models for TFS and OS was confirmed in a log-likelihood-ratio test ($p<0.0001$). The prognostic significance of each variable was assessed with the Wald test.

To compare survival in patients in whom 17p- occurred before *vs.* after treatment, the time interval between the first documentation of 17p- and death or last follow-up was also evaluated.

All tests were two-sided, and the threshold for statistical significance was set to $p\leq0.05$. All statistical analyses were performed using MedCalc software (version 17.8.6, MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2017).

Results

Characteristics of the study population

The characteristics of the 195 included patients (median [range] age at diagnosis: 63 [33-88] years; males: 66%) are summarized in Table I. The Binet stage at diagnosis was A in 100 patients (59%), B in 48 (29%), and C in 21 (12%). Data on *IGHV* mutational status was available for 47 patients, of whom 38 (81%) did not bear mutations. The median [range] time since after diagnosis was 70 months [0-401]. Most of the patients with informative data had been treated (158 out of 182, 87%), and the median number of treatments was 2.5 [0-10]. In 71 patients, the time of occurrence of the 17p- could not be determined because (i) cytogenetic analyses were not performed before treatment (n=58), or (ii) data on treatment were missing (n=13). When considering the remaining 124 patients, 28 (23%) did not present with 17p- at diagnosis; hence, the deletion had occurred after treatment (a median of 77.5 months [22-291] after diagnosis). In 96 of the 124 patients (77%, including 24 patients not having been treated at last follow-up), the 17p- was present before treatment; the median time interval between diagnosis and first detection of 17p- was 1 month [0-291]. The *TP53* gene was mutated in 55 of the 60 (92%) patients with informative data. The protein p53 was dysfunctional in all 42 cases tested, including 2 patients in whom a *TP53* mutation was not detected by NGS.

Karyotyping and FISH data

Karyotyping and FISH results are summarized in Table II. According to K, the median [range] number of chromosomal abnormalities (including 17p-) was 4 ([1-26]; a CK≥3 was found in 141 of the 195 (72%) patients, including 89 (46%) with a highly CK≥5. The K was monosomal in 52 of the 195 patients (27%). A large majority of patients (181 out of 195, 93%) displayed one or more U-translocation(s); some involved 17p, and others did not. The presence of U-translocations was strongly associated with CK≥3 (78% vs. 0% in patients with and without U-translocations, respectively; p<0.0001) and CK≥5 (49% vs. 0%, respectively; p=0.0001). A total of 240 17p abnormalities were found in the 195 patients: 162 (83%) of these patients had one 17p abnormality, 25 (13%) had two independent 17p abnormalities, and 8 (4%) had three or more 17p abnormalities. The 17p- was the sole abnormality detected by K in 28 of the 195 (14%) cases. When other abnormalities were also present, the 17p- was in the primary clone in 43 of the 195 (26%) patients, in a sub-clone in 42 (25%), in the same clone as the other aberrations in 64 (38%), or in an independent clone in 18 (11%). In the majority of cases, loss of 17p resulted from a U-translocation involving 17p (-17p) and various chromosome partners (Supplemental Figure 1). A total of 167 U-translocations(-17p) were found in 158 of the 195 patients (81%). The partner was not identified in 35 cases [add(17p)]. Of the 132 translocations with an identified partner, 68 were arm-to-arm translocations; 32 of the latter involving the long arm of the chromosome 18. In 7 of these 32 cases, the dicentric nature of the derivative chromosome dic(17;18)(p11;p11) was proven by FISH with centromere probes. According to the ISCN nomenclature, the other cases were der(17;18)(q10;q10). Overall, der/dic(17;18) was the most frequent U-translocation(-17p) because it was present in 32 of the 195 patients (16%). The other frequent arm-to-arm translocations involved chromosomes 8q (n=11, resulting in 8p deletion), 14q (n=6), 4q (n=4), 13q (n=4), and 21q (n=4). The remaining 64 U-translocations(-17p) (not arm-to-arm) involved a wider variety of partners - most frequently chromosome 8 (n=15); this led to del8p (n=6), gain8q (n=6), or del8q (n=3) (Supplemental Figure 1B). The other 17p abnormalities were 17p deletion (n=45 out of 195, 23%), monosomy 17 (n=15, 8%), i(17q) (n=9, 5%) and ring chromosome 17 (n=4, 2%) (Table II and Supplemental Figure 1A). Lastly, FISH showed that the percentage of cells with a *TP53* deletion ranged from 3% to 100% (median: 70%).

When considering the additional abnormalities associated with 17p-, U-translocations(-not 17p) were found in 121 of the 195 patients (63%). The presence of U-translocations(-not 17p) was associated with CK≥3 (95% vs. 35% in patients with and without U-translocations(-not 17p), respectively; p<0.0001) and CK≥5 (68% vs. 9%, respectively; p<0.0001). After combination of the K and available FISH findings, 13q14 deletion was detected in 71 of the 118 documented cases (60%), 8p deletion in

40 out of 189 cases (21%), trisomy 12 in 30 out of 195 cases (15%), 8q24 gain in 14 out of 112 cases (12.5%), and 11q deletion (*ATM* gene) in 20 out of 161 cases (12%).

Treatment-free and overall survival

Treatment-free survival was studied for the 96 patients in whom 17p was present before treatment. The median TFS time was 18 months. In a univariate analysis, the parameters associated with significantly shorter TFS were Binet stage B/C (hazard ratio (HR)=6.24, p<0.0001), monosomy 17 (HR=2.07, p=0.04), a high CK (HR=1.81, p=0.009) and 8q24 gain (HR=6.46, p<0.0001). In a multivariate analysis, stage B/C and 8q24 gain remained significantly associated with shorter TFS (Table III).

For the study population as a whole, the median OS time from diagnosis was 179 months. In a univariate analysis, we found that age ≥65, Binet stage B/C, or unmutated *IGHV* status were associated with a significantly shorter median OS time (Table III, Supplemental Figure 2). There were no significant statistical differences in the impact on OS between the different abnormalities leading to loss of 17p, i.e. U-translocations(-17p), der/dic(17;18), monosomy 17 or deletion 17p. Likewise, the number of 17p- abnormalities and the percentage of cells harboring the del*TP53* (whether dichotomized or not) were not significant factors. The presence of an i(17q) was correlated with trisomy 12 (44% vs. 14% in patients with or without i(17q), respectively; p=0.03), a higher percentage of tumor cells with del*TP53* (a median of 88% vs. 60% in patients with or without i(17q), respectively; p=0.003), and a shorter OS (69 vs. 179 months in patients with or without i(17q), respectively; p=0,04) (Figure 1). Analysis of the impact of the other cytogenetic aberrations revealed that the presence of U-translocations(-not 17p) was associated with a shorter OS time (153 vs. 223 months in patients with or without U-translocations(-not 17p), respectively; p=0.03). When taking account of all the U-translocations (i.e. 17p and not 17p), a shorter OS was also observed in the group with U-translocations (171 months vs. median not reached, p=0.04). Lastly, the presence of an 8q24 gain strongly impacted the OS (HR=3.48, p=0.001) (Figure 1). In a multivariate analysis, age ≥ 65 (HR=2.65, p=0.03), Binet stage B/C (HR=10.01, p<0.0001) and 8q24 gain (HR=3.73, p=0.01) were independently and significantly associated with poor OS (Table III).

When comparing the survival time after the first documentation of 17p-, the patients who acquired this abnormality after treatment had a shorter OS time than those presenting with 17p- prior to treatment (HR=2.02, p=0.03) (Supplemental Figure 3).

Discussion

To the best of our knowledge, the present study of 195 CLL patients with 17p- is the largest cohort in which the clinical significance of chromosome 17p abnormalities has been assessed in detail. In line

with the literature data, we found that loss of the short arm of chromosome 17 is mainly due to unbalanced translocations (in 70% of cases), rather than 17p deletion, an isochromosome 17q or monosomy 17 [3, 16]. Most of the U-translocations(-17p) (52%) were arm-to-arm events. In the remaining U-translocations(-17p), the breakpoints were mainly located in 17p11 or p12. In general (and regardless of the type of 17p abnormality), all or almost all of the 17p arm was lost. This supports the recent hypothesis whereby the effects of 17p deletion on tumor progression and treatment resistance might involve several genes and might not be solely due to *TP53* loss [17].

The most frequent U-translocation(-17p) was dic/der(17;18), since it accounted for 16% of the study population. Woyach et al. suggested that dic(17;18) may correspond to the most aggressive subset of 17p- CLL cases [18]. We could not confirm this hypothesis; when compared with the other chromosomal abnormalities involving 17p in our large series, dic(17;18) did not have a significant impact on outcome.

In a univariate analysis, we found that monosomy 17 was significantly associated with a shorter TFS (relative to the other 17p abnormalities), and that isochromosome 17q was significantly associated with a shorter OS. Isochromosome 17q has been described as a frequent aberration (up to 33%) in small series of CLL cases with a *TP53* deletion [3, 19]. A much lower incidence (4%) was observed in the present study. Moreover, i(17q) was significantly associated with trisomy 12 (44%, p=0.03) and a larger clone 17p- size. In a recent study, CLL patients with i(17q) tended to have poorer OS than patients with other anomalies affecting 17p13 – even though all the tested individuals were *IGHV* mutated [20]. In the present series, the *IGHV* genes were sequenced in only 2 of the 9 patients with i(17q); neither carried *IGHV* mutations. This finding shows that mutated *IGHV* status is not a constant feature in cases of i(17q). The present results corroborated the association between i(17q) status and a poor outcome because this defect was significantly correlated with shorter OS (p=0.04). However, further studies are needed to establish whether i(17q) is an independent prognostic marker in 17p- CLL.

In accordance with previous reports [12, 21, 22], we observed a shorter OS (calculated following the first documentation of 17p-) in patients having acquired the 17p- after treatment than in those with 17p- present before treatment. This finding might simply reflect the fact that patients with acquired 17p- have more advanced disease but may also suggest that treatment selected a more aggressive clone with a growth advantage.

In contrast to other series, we did not observe a significant association between the percentage of cells with *TP53* deletion and the clinical outcomes. In the literature, it has been reported that a low percentage of 17p- cells is associated with a more favorable outcome. However, the optimal cut-off varied markedly from one study to another (from 10% to 80%) [10, 12, 23, 24] - indicating that this parameter is cohort-dependent and should not be taken into account when evaluating the prognosis

of patients with CLL. Furthermore, several reports have documented the impact of small clones with disrupted *TP53* genes on disease progression [25, 26].

Although only a small proportion of 17p- patients were analyzed, we found that all the tested individuals had a dysfunctional p53 protein (including 2 cases without a *TP53* mutation). These results highlight the importance of continuing to screen for 17p loss on a routine basis. FISH is still the best technique for detecting *TP53* deletion; its sensitivity threshold of about 5% is much lower than that obtained with innovative tools such as single nucleotide polymorphism arrays, multiplex ligation-dependent probe amplification and massively parallel sequencing.

We also looked at whether additional chromosomal abnormalities could influence the outcome. We confirm that a complex K is very frequent in 17p- CLL, since it was detected in 72% of our patients [12, 24, 27]. Karyotype complexity is a recently discovered factor for a poor prognosis in CLL in general [4, 28, 29] and in the 17p- subset [24, 30]. In the literature, the cut-off used to define a complex K varies from 3 to 5 abnormalities [4, 31]. In our series, a highly CK ($\geq$5 abnormalities) was associated with worse TFS, whereas a CK ($\geq$3 abnormalities) had no clinical impact. This finding suggests that a cut-off of 5 abnormalities is more suitable for identifying 17p- CLL patients with the most aggressive disease. Previous studies of unselected CLL patients have described the poor prognosis of unbalanced translocations [4, 27, 30, 32, 33]. Our present results showed that U-translocations are also associated with shorter OS in the 17p- CLL subset. Regarding the classical abnormalities detected by FISH in CLL (such as tri12, del13q and del11q), we were surprised to find the same frequency of these aberrations in the 17p- subset as in the whole population of patients with CLL [1]. This suggests that 17p- appears independently, and that none of the other abnormalities promotes its occurrence. However, i(17q) - correlated with trisomy 12 - appears to be an exception.

The 8q24 gain (encompassing the *MYC* gene) is detected with microarrays in 3-4% of the overall population of patients with CLL, and is independently associated with shorter OS and/or shorter time to first treatment [34-36]. In the literature, the frequency of 8q24 gain is higher in the 17p- CLL subset and ranges from 9% to 44% [13, 24, 35-37]. In the present large series, we found an 8q24 gain in 12.5% of patients. Furthermore, we demonstrated that 8q24 gain is a strong, predictive marker of poor outcome within the 17p- CLL subgroup, and has independent prognostic value for OS. This observation suggests that *TP53* deletion and *MYC* gain act in synergy; the outcome is particularly dismal when both are present. Indeed, the occurrence of both alterations may represent a "double-hit" CLL that is reminiscent of the aggressive, "double-hit", high-grade B-cell lymphomas harboring rearrangements of *MYC* and *BCL2* or *BCL6* [38]. It is noteworthy that we did not observe any translocation involving *MYC* in the present series. Given the high frequency of genomic complexity in these patients, 8q24 gain can be missed by karyotyping. In order to validate the clinical significance

of this aberration, 17p- CLL patients enrolled in prospective clinical trials should be systematically screened by FISH analysis using a *MYC* probe.

In conclusion, our data show that patients with 17p- CLL often present CK (≥3 abnormalities) and highly CK (≥5 abnormalities), unbalanced translocations, 8q24 gain, and unmutated *IGHV*. The various abnormalities leading to loss of 17p do not have all the same clinical significance: i(17q) is associated with a shorter OS than the other 17p aberrations. Furthermore, the presence of additional unbalanced translocations aggravates the outcome. Our results highlight the value of conventional karyotyping for identifying alterations that modulate the prognosis in this aggressive subset of patients. Lastly, 8q24 gain is a strong, independent factor for poor survival; systematic, targeted screening for this abnormality should be considered with a view to better defining the prognosis of patients with 17p- CLL and identifying the very high-risk "double-hit" subgroup.

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Legends to figures

Figure 1

Overall survival in the whole study population, according to the presence or absence of isochromosome 17q [i(17q)] (A), unbalanced translocations (U-translocations) (all, including 17p) (B), additional U-translocations to 17p- (C), and 8q24 gain (D).

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