Abstract

To analyze the impact of the 11q deleted (11q-) cells in CLL patients on the time to first therapy (TFT) and overall survival (OS), 2,493 patients with CLL were studied. 242 patients (9.7%) had 11q-. Fluorescence in situ hybridization (FISH) studies showed a threshold of 40% of deleted cells to be optimal for showing that clinical differences in terms of TFT and OS within 11q- CLLs. In patients with /C21 40% of losses in 11q (11q-H) (74%), the median TFT was 19 months compared with 44 months in CLL patients with /C21 40% del(11q) (11q-L) (P<0.0001). In the multivariate analysis, only the presence of 11q-L, mutated IGHV status, early Binet stage and absence of extended lymphadenopathy were associated with longer
Introduction

In chronic lymphocytic leukemia (CLL) the presence of cytogenetic aberrations assessed by fluorescence in situ hybridization (FISH) influences the prognosis, in terms of time to first therapy (TFT), response to treatment, and overall survival (OS) [1]. Deletions on 11q are observed in 9–18% of CLL patients [1,2]. These patients are younger, have abdominal bulky lymph node involvement [3,4], and often show ZAP-70 expression, unmutated status of \( IGHV \) and shorter survival, although with a highly variable clinical outcome. The use of chemoimmunotherapy, including rituximab and cyclophosphamide to fludarabine schedules, can improve the response in patients with 11q deletion [5].

Deletions of 11q almost invariably include the ataxia telangiectasia mutated (\( ATM \)) gene [6]. This important tumor suppressor gene plays a crucial role in DNA repair and recombination, and regulates cell cycle progression [7]. Although mutations of this gene have been linked to poor prognosis and are associated with 11q deletions in CLL patients, due to its extreme size (62 coding exons) with lack of well characterized (hot-spot) mutations, \( ATM \) sequencing studies in CLL have been challenging, leaving several issues unresolved [8–10].

The application of next-generation sequencing (NGS) allows the detection of new candidate genes with frequent mutations in CLL patients as detected by whole-exome and whole-genome sequencing [11–14]. Besides \( TP53 \) mutations [15,16], \( NOTCH1 \) and \( SF3B1 \), found in around 10% of newly diagnosed CLL patients, are the most frequently mutated genes [17,18]. Patients with mutations in some of these genes have been associated with shorter TFT and OS [19,20]. Other recurrent mutations in \( MYD88, FBXW7, XPO1 \) and \( BIRC3 \) genes have been reported at frequencies below 10% [19,21]. Moreover, \( BIRC3 \), a negative regulator of NF\( \kappa \)B signaling pathway, is located near to \( ATM \) gene, at 11q22 [9].

In the last few years, it has been reported that patients with CLL and 13q deletion may differ in their outcomes depending on the percentage of cells displaying this aberration [2,22–24]. To assess the potential prognostic value of the number of cells with deletion on 11q and to gain insight into the molecular basis of this abnormality in CLL, we have performed a multicenter study of a large series of patients diagnosed with 11q- CLL to determine whether the frequency of losses in 11q has an influence on OS and TFT. Furthermore, NGS studies were carried out, in a subset of patients, to analyze the mutational status of \( ATM, TP53, NOTCH1, SF3B1, MYD88, FBXW7, XPO1 \) and \( BIRC3 \) in this group of patients.

Methods

Patients

A total of 2,493 patients registered in the DataBase of CLL of the Spanish Group of Cytogenetics (GCECGH) and the Spanish Group of CLL (GELLC) were included. The diagnosis of CLL...
was made according to the International Workshop on CLL (IWCLL) criteria [25]. In all cases, an immunophenotypic analysis was performed by flow cytometry. FISH studies, including specific probes for at least the 11q22.3–23.1, 12p11.1-q11, 13q14, and 17p13 regions were carried out.

A total of 242 patients (9.7%) had an 11q deletion. The final analysis was limited to 197 cases, including 11q deletion performed at diagnosis of CLL, after excluding cases with monoclonal B-cell lymphocytosis, clonal evolution or inappropriate follow-up (Table A in S1 File). Basic clinical and biological data were recorded by reviewing the GCGCGH and GELLC Data-Base. The study was approved by the local ethical committees “Comité Ético de Investigación Clínica, Hospital Universitario de Salamanca”. Written informed consent was obtained from each patient before they entered the study.

Fluorescence in situ hybridization (FISH)

Interphase FISH was performed in peripheral blood samples using commercially available probes for the 13q14, 12p11.1-q11 (alpha satellite), 11q22/ATM and 17p13/P53 regions (Vysis/Abbott Co, Abbott Park, IL, USA). Dual-color FISH using differently labeled control probes and test probes were performed. The methods used for FISH analysis have been described elsewhere [26]. Signal screening was carried out in at least 200 cells with well-delineated fluorescent spots. In cases with 11q deletion a score of ≥10% was considered positive, according to the cut-off of our laboratories.

Next-generation sequencing analysis

A total of 25 11q- CLL patients were included in sequencing studies. Samples were obtained at diagnosis in all cases. NGS was performed using 454 Titanium Amplicon chemistry (Roche Applied Science, Penzberg, Germany) [27] to investigate the ATM, TP53, NOTCH1, SF3B1, MYD88, FBXW7, XPO1 and BIRC3 mutations in 11q- CLL patients. Information about primer sequences is shown in Table E in S1 File and the PCR conditions are described in Table F in S1 File. The oligonucleotide design was performed as part of the IRON-II network.

All data were generated using the GS FLX and Junior Sequencer Instrument software version 2.7 (Roche Applied Science). To detect variants, filters were set to display sequence variants occurring in more than 2% of bidirectional reads per amplicon in at least one patient. Table G in S1 File shows the median number of reads generated for each gene, allowing variants to be identified down to a detection limit of 2% [28].

Detailed methods are described in the S1 File. The sequencing data are uploaded to the Sequence Read Archive (SRA) (http://trace.ncbi.nlm.nih.gov/Traces/sra/) under accession number PRJNA297249. All the information is accessible with the following link http://www.ncbi.nlm.nih.gov/bioproject/297249.

Statistical analysis

Statistical analysis were performed using SPSS 20.0 for Windows (SPSS, Chicago, IL, USA). TFT and OS were analyzed on the date of the initial FISH study, coinciding in all cases with CLL diagnosis. The number of losses in 11q-deleted nuclei was divided into deciles to better define the most significant cut-off point for TFT and OS. The chi-square test was used to assess associations between categorized variables, while continuous variables were analyzed with the Mann-Whitney U test. Statistically significant variables related to TFT and OS were estimated by the Kaplan-Meier method, using the log-rank test to compare the curves of each group. Univariate and multivariate analyses of the TFT and OS employed the Cox regression method. Results were considered statistically significant for values of P≤0.05.
Results

Clinical and biological characteristics of CLL patients carrying 11q deletion

One hundred ninety-seven patients with 11q deletion were selected for the analysis. There was a predominance of males (76.6%), and the median age was 65 years (range: 28–97 years). Most patients (61%) were in Binet stage A and only 14.9% had B symptoms. In 46.4% of patients the lymphocyte blood count was ≥20 x 10^9/L, while 31.8% and 28.9% patients, respectively, had high serum β2-microglobulin and high serum LDH levels. A total of 68.5% of patients had lymph node involvement, and splenomegaly was detected in 23.4% of cases. Regarding biological characteristics, IGHV unmutated cases were present in 66.1% of cases, while CD38/C21 ≥30% and ZAP-70/C21 ≥20% were detected in 55% and 55.7% of patients, respectively (Table A in S1 File).

Fifty-one patients (25.9%) had <40% of 11q-deleted cells, while 146 cases (74.1%) had ≥40% of such cells. Different cut-off points were analysed, and 40% 11q deleted nuclei was selected to better separate patients with different disease outcome. In 82 out of 197 patients (41.6%) 11q- was the sole cytogenetic aberration, while 115 patients (58.4%) had 11q deletion plus other cytogenetic abnormalities (108 cases had a 13q deletion, 14 had trisomy 12, and 6 patients showed a 17p deletion).

No significant differences in clinical or biological features were found between patients with low (<40%) and high (≥40%) frequencies of 11q- cells, except for the number of lymphocytes, Binet stage, IGHV mutational status, need for therapy, and death during follow-up (Table 1).

CLL patients with a low number of 11q- cells have a prolonged time to first therapy (TFT)

All 197 patients were evaluable for analysis of TFT, response to therapy and OS. By the time of analysis, 151/197 (76.6%) had received treatment, with a median TFT of 25 months (95% CI, 31–44 months) (Fig A in S1 File.). In terms of TFT, no differences in the group of 11q deletion as unique FISH cytogenetic aberration compared with the group of 11q deletion plus other FISH alterations were observed. A significantly longer TFT was detected in the cohort of patients with <40% of 11q deleted cells (median, 44 months; 95% CI, 33–55 months) vs those patients ≥40% losses in 11q (median, 19 months; 95% CI, 12–24 months) (P<0.0001) (. 1A). Of note, 52% of patients in the former group required treatment while 70.5% of patients with ≥40% of 11q-deleted nuclei were treated. Variables associated with a longer TFT were early clinical stage (P = 0.024), absence of extended lymphadenopathy (<2 node areas involved) (P<0.0001), absence of splenomegaly (P = 0.045), low serum LDH (P = 0.045), low serum β2M (P = 0.019), low CD38 expression (P = 0.023), low ZAP70 expression (P = 0.025), mutated IGHV status (P<0.0001) and del(11q) in <40% of cells (P<0.0001) (Table B in S1 File). In the multivariate analysis, only the presence of del(11q) in <40% of cells (Hazard Ratio, HR, 4.475; 95% CI, 1.813–7.171; P = 0.001), mutated IGHV status (HR, 3.659; P = 0.005), early Binet stage (HR 2.492; P = 0.023) and absence of extended lymphadenopathy (HR 1.854; P = 0.016) identified independent risk factors associated with longer TFT (Table 2).

In addition, in patients with del(11q) as the sole cytogenetic aberration, a longer TFT was observed in patients with 11q-L (median 45 months vs 15 months, P < 0.0001).

CLL patients with a low number of 11q- cells have longer overall survival (OS)

By the time of analysis, 60/197 patients (30.5%) had died. The median OS of the global series was 106 months (95% CI, 97–128 months) (Fig A in S1 File). Significantly longer survival was
| Characteristic | Category | del(11q) \(<40\%\) N = 51 (26\%) | del(11q) \(\geq 40\%\) N = 146 (74\%) | \(P\) |
|---------------|----------|-------------------------------|---------------------------------|------|
| Age, years    |          | 62 (28–84)                    | 65 (33–91)                      | 0.13 |
| White blood cells, range /μL |          | 17,900 (7,800–98,100)         | 28,000 (6,600–365,000)          | 0.008|
| Lymphocytes, range /μL |          | 12,600 (5,100–84,8500)        | 21,100 (5,200–364,000)          | 0.007|
| Lymphocytes > 20 x 10^9/L | Yes | 14                            | 75                              | 0.005|
| Hemoglobin, range g/dL |          | 14 (6–17)                     | 14 (5–17)                       | 0.91 |
| Platelet count, range /μL |          | 195,000 (63,000–352,000)      | 182,000 (2,000–412,000)         | 0.44 |
| IGHV (n = 56)* | Mutated | 9                             | 10                             | 0.024|
|                | Unmutated | 6                           | 31                             |      |
| ZAP-70 (n = 79)* | +      | 12                           | 32                             | 0.44 |
|                | -       | 11                           | 24                             |      |
| CD38 (n = 130)* | +      | 16                           | 40                             | 0.139|
|                | -       | 19                           | 56                             |      |
| del(11q) as sole cytogenetic aberration | Yes | 20                           | 62                             | 0.74 |
|                | No      | 31                           | 84                             |      |
| del(11q) + del(13q) | Yes | 29                           | 77                             | 0.63 |
|                | No      | 22                           | 69                             |      |
| Sex | Male | 40                           | 111                            | 0.84 |
|                | Female | 11                           | 35                             |      |
| LDH (n = 187)* | Normal | 34                           | 99                             | 0.49 |
|                | High    | 13                           | 41                             |      |
| β microglobulin (n = 170) * | Normal | 33                           | 80                             | 0.19 |
|                | High    | 11                           | 43                             |      |
| Binet stage (n = 195)* | A | 36                           | 83                             | 0.17 |
|                | B       | 10                           | 44                             |      |
|                | C       | 4                            | 14                             |      |
| Lymphadenopathy (n = 193)* | No | 20                           | 41                             | 0.12 |
|                | \(\leq 2\) nodal areas | 16                         | 43                             |      |
|                | \(> 2\) nodal areas | 13                         | 60                             |      |
| Hepatomegaly (n = 193)* | Yes | 3                            | 15                             | 0.57 |
|                | No      | 46                           | 129                            |      |
| Splenomegaly (n = 193)* | Yes | 10                           | 36                             | 0.43 |
|                | No      | 39                           | 108                            |      |
| B symptoms (n = 195)* |          |                               |                                |      |

(Continued)
observed in patients with a low frequency of losses in 11q-. Thus, in patients with loss of 11q in ≥40% of cells, the OS was 90 months (95% CI, 57–123 months), while in the group with <40% of losses in 11q, the median OS had not been reached (95% CI, 114–157 months) (P = 0.006) (Fig 1B). In the univariate analysis, early clinical Binet stage (P = 0.001), asymptomatic disease (P = 0.034), absence of hepatomegaly (P = 0.025) or splenomegaly (P<0.0001), lymphocyte count <20 x 10^9/L (P = 0.032), low serum of either LDH level (P<0.0001) or β2M (P<0.0001), the presence of an association of 11q deletion and 13q deletion (P = 0.045), and a low number (<40%) of cells with 11q- (P = 0.006) were associated with longer OS (Table C in S1 File). In the multivariate analysis, the variables independently related to longer OS were the absence of splenomegaly (HR, 1.786; P = 0.023), low serum LDH (HR, 2.076; P = 0.018), low serum β2M (HR, 2.448; P = 0.006) and the presence of del(11q) in <40% of cells (HR, 3.145; 95% CI, 1.474–6.691; P = 0.003) (Table 3).

Regarding the patients with del(11q) as the unique cytogenetic aberration, a longer OS was observed in patients with 11q-L (median not reached vs 70 months, P = 0.007)

The analyses of other cut-offs for the number of 11q- cells (<40% vs 40–59% vs ≥60%) showed similar results for TFT and OS (Fig B in S1 File).

| Table 1. (Continued) |
|----------------------|
| Characteristic       | Category | del(11q) <40%, N = 51 (26%) | del(11q) ≥40%, N = 146 (74%) | P       |
| Second Cancer (n = 172)* | Yes | 6 | 23 | 0.34 |
| Died during follow-up | Yes | 10 | 50 | 0.04 |
| Therapy during follow-up | Yes | 27 | 104 | 0.025 |
|                     | No | 44 | 122 |     |
|                     | No | 42 | 104 |     |
|                     | No | 41 | 96  |     |
|                     | No | 42 |     |     |

*Number of cases.

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Table 2. Multivariate Cox regression analysis of time to first therapy in 11q- CLL patients with respect to the number of losses detected by FISH: <40% (n = 51) or ≥40% (n = 146). *

| Variable                        | Hazard Ratio | 95% CI       | P (log-rank test) |
|---------------------------------|--------------|--------------|-------------------|
| del(11q) <40%                   | 4.475        | 1.813–7.171  | 0.001             |
| Mutated IGHV                     | 3.659        | 1.478–9.057  | 0.005             |
| Early Binet stage                | 2.492        | 1.137–5.463  | 0.023             |
| Non-extended lymphadenopathy (≤ 2 nodal areas) | 1.854 | 1.121–3.065 | 0.016 |

*The following covariates were included in the final model: age, sex, Binet stage, splenomegaly, extended lymphadenopathies, LDH, β2 microglobulin, CD38, ZAP70, IGHV mutation status and percentage 11q deleted nuclei.

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Biallelic inactivation of the *ATM* gene is observed in one-third of 11q- CLL patients

*ATM* mutations were found in eight (32%) of 25 patients with 11q-. In total, 14 different mutations were detected by *ATM* molecular mutation screening: 11 point mutations (7 missense and 4 nonsense; 78.6%) and three frameshift mutations (2 deletions and 1 insertion; 21.4%). These mutations are shown in Fig 2 and listed in Table 4. All patients with mutated *ATM* had at least one truncating or damaging mutation. Interestingly, four of the eight patients with *ATM* mutations carried more than one type of mutation. Thus, two patients with *ATM* mutations carried two different mutations while two other patients with *ATM* mutations carried three mutations. It is of note that the patients with several *ATM* mutations had different mutational loads, suggesting the presence of independent clones or clonal evolution with the

![Diagram](https://example.com/diagram.png)

**Fig 1.** (A) Time to first therapy (TFT) and (B) overall survival (OS) of 197 patients with 11q deletion CLL and <40% or ≥40% FISH losses.

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| Variable                  | Hazard Ratio | 95% CI          | P (log-rank test) |
|---------------------------|--------------|-----------------|-------------------|
| Del 11q <40%              | 3.145        | 1.474–6.691     | 0.003             |
| Low serum β₂ microglobulin| 2.448        | 1.260–4.753     | 0.006             |
| Low serum LDH             | 2.076        | 1.061–4.064     | 0.018             |
| Absence of splenomegaly   | 1.786        | 1.044–4.481     | 0.023             |

*The following covariates were included in the final model: age, sex, Binet stage, splenomegaly, extended lymphadenopathies, LDH, β₂ microglobulin, CD38, ZAP70, IGHV mutation status and percentage 11q deleted nuclei.*

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acquisition of a second mutation. However, it could not be confirmed whether the mutations from one patient belonged to different clones, since they were located on distinct sequencing reads in different amplicons.

All the mutations were located in exons 3–60, between amino acids 29 and 2913, and involved the FAT and PI3K domains of the ATM protein. Five mutations resulted in a truncated form of the ATM protein (termed ‘truncating’) while the remaining mutations resulted in the expression of the full-length, but mutated form of the ATM protein (termed ‘non-truncating’). These missense mutations were analyzed with SIFT algorithms, which predicted six of them to be damaging. The median mutational burden was 14.3% (range, 2.0–92.5%). Eight of 14 (57.1%) variants had a mutational load of ≤15% and thus would not have been detected by capillary Sanger sequencing.
The TFT and OS were assessed in all patients and related to the mutational status of the ATM gene. There was no difference in TFT or OS between 11q- patients with and without ATM mutations (\(P<0.970\) and \(P<0.623\), respectively). Interestingly, bivariate analyses indicated that the presence of ATM mutations was a useful characteristic for identifying CLL patients with a different TFT in the subgroup of CLL patients with low frequencies of 11q-.

Thus, CLL with ATM mutations had a shorter TFT than patients without ATM mutations (36 vs 46 months; \(P=0.046\)). By contrast, 11q patients harboring /C21 40% of 11q- had shorter TFT independently of ATM mutational status. Moreover, there was no significant association between the presence of an ATM mutation and other clinical or biological prognostic factors (Table D in S1 File).

SF3B1 is a frequently mutated gene in CLL patients with 11q-

Mutations in TP53, NOTCH1, SF3B1, MYD88, FBXW7, XPO1 and BIRC3 were analyzed in the entire cohort of 25 CLL patients. In total, 20 mutations were detected: eight patients had SF3B1 mutations, five cases had mutations in NOTCH1, four in TP53, two in XPO1, while one CLL had a mutation in BIRC3. Most of them had previously been described as mutations in the COSMIC database. The median mutational burden was 27% (range, 3–81%). In 7/20 (35%) variants the mutation load was \( \leq 15\%\). The frequency of 11q- CLL with associated mutations
was 8/25 (32%) for SF3B1, 5/25 (20%) for NOTCH1, 4/25 (16%) for TP53, 2/25 (8%) for XPO1 and 1/25 (4%) for BIRC3. These mutations are shown in Fig 2 and listed in Table 5. Confirming previously published sequencing data, the most frequent SF3B1 mutation was p. Lys700Glu (4/8, 50%) while the presence of p.Pro2514ArgfsX4 was the most frequent NOTCH1 mutation (5/5, 100%). In addition, 50% of the 11q− CLL patients with TP53 mutations also showed 17p−.

In terms of prognostic relevance, significant differences were observed only in TFT between 11q− patients with and without NOTCH1 mutations (5 vs 36 months; \(P = 0.031\)) and in OS between patients with and without TP53 mutations (1 vs 197 months; \(P < 0.003\)) (Fig C in S1 File).

Genetic mutations are associated with a higher percentage of 11q− cells

As a next step towards understanding the clinical differences within the 11q− subgroup, the association between the presence of genetic mutations and the percentage of 11q− cells in CLL patients was examined. The incidence of ATM mutations was similar in the two groups. Thus, 29.4% of patients with a high frequency of 11q− exhibited ATM mutations while 37.5% of patients with a low frequency of 11q losses had ATM mutations (\(P = 0.513\)). However, considering the mutations of all the genes analyzed, fewer patients with low frequencies of 11q− had mutations among genes examined compared with the subgroup of a high number of losses.

Table 5. Mutations in other genes in 25 CLL patients with del(11q).

| Patient ID | FISH | % 11q- | 11q- group | Other abnormalities | Mutations | Gene | Mutational load (%) | Sequence change | Exon | Protein change | COSMIC database |
|-----------|------|--------|------------|-------------------|-----------|------|---------------------|----------------|-------|----------------|-----------------|
| 10        | 26   | 11q-L  | -          |                   | SF3B1     | 17   | c.2225G>A           | 16             | p.Gly742Asp | COSM145923    |
| 14        | 27   | 11q-L  | 30% 13q-  |                   | NOTCH1    | 6    | c.7541_7542delCT    | 34             | p.Pro2514ArgfsX4 | COSM12774 |
| 22        | 48   | 11q-H  | -          |                   | SF3B1     | 4    | c.2098A>G           | 15             | p.Lys700Glu | COSM84677    |
| 22        | 48   | 11q-H  | -          |                   | TP53      | 28   | c.824G>A            | 8              | p.Cys275Tyr | COSM10893    |
| 21        | 48.5 | 11q-H  | 81.5% 13q- |                   | SF3B1     | 25   | c.1645C>T           | 12             | p.Arg549Cys | COSM1014502  |
| 21        | 48.5 | 11q-H  | 81.5% 13q- |                   | TP53      | 16   | c.1024C>T           | 10             | p.Arg342X   | COSM11073    |
| 25        | 62   | 11q-H  | -          |                   | NOTCH1    | 68.5 | c.7541_7542delCT    | 34             | p.Pro2514ArgfsX4 | COSM12774 |
| 18        | 70   | 11q-H  | 37.5% 13q- |                   | SF3B1     | 34   | c.2098A>G           | 15             | p.Lys700Glu | COSM84677    |
| 6         | 73   | 11q-H  | -          |                   | SF3B1     | 39   | c.2098A>G           | 15             | p.Lys700Glu | COSM84677    |
| 6         | 73   | 11q-H  | -          |                   | NOTCH1    | 4    | c.7541_7542delCT    | 14             | p.Pro2514ArgfsX4 | COSM12774 |
| 20        | 78   | 11q-H  | 63% 13q-  |                   | SF3B1     | 46.5 | c.1988C>T           | 14             | p.Thr663Ile | COSM145921   |
| 16        | 83   | 11q-H  | 56% 13q-  |                   | SF3B1     | 4    | c.2098A>G           | 15             | p.Lys700Glu | COSM84677    |
| 13        | 83   | 11q-H  | 86% 13q-  |                   | TP53      | 7.5  | c.613T>G            | 6              | p.Tyr205Asp | COSM43844    |
| 9         | 83.5 | 11q-H  | 61% 13q-  |                   | NOTCH1    | 3    | c.7541_7542delCT    | 34             | p.Pro2514ArgfsX4 | COSM12774 |
| 5         | 84.5 | 11q-H  | 87% 17p-  |                   | NOTCH1    | 45   | c.7541_7542delCT    | 34             | p.Pro2514ArgfsX4 | COSM12774 |
| 19        | 84.5 | 11q-H  | 85% 13q-  |                   | XPO1      | 47.5 | c.1711G>A           | 15             | p.Glu571Lys | COSM96797    |
| 4         | 89   | 11q-H  | 87% 17p-  |                   | TP53      | 81   | c.833C>G            | 8              | p.Pro278Arg | COSM10887    |
| 4         | 89   | 11q-H  | 87% 17p-  |                   | BIRC3     | 12   | c.1813T>C           | 9              | p.X605ArgfsX11 | -           |
| 1         | 90   | 11q-H  | 87% 13q-  |                   | SF3B1     | 50.5 | c.1986C>G           | 14             | p.His662Gln | COSM110692   |
| 17        | 97   | 11q-H  | 97% 13q-  |                   | XPO1      | 51   | c.1712A>C           | 15             | p.Glu571Ala | COSM1291526  |

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11q- (4/8, 50% vs 16/17, 94.1%; \( P = 0.023 \)) (Fig 3). Interestingly, among the CLL patients without a gene mutation, the median proportion of the CLL tumor population with an 11q deletion was significantly lower than that of CLL patients with mutated genes (20.5%, range, 12–71.5% vs 80.3%, range, 26–97%; \( P = 0.007 \)). TP53 mutations were present only in patients with a high frequency of 11q- cells.

**Discussion**

In this study, we analyzed the outcome of 11q-deleted CLL patients at diagnosis with respect to TFT and OS, and the presence of mutations in the most relevant genes to date in CLL. The importance of the percentage of cells displaying a genetic aberration determined by FISH, as an independent prognostic factor in CLL, has recently been recognized in 17p-, 13q- and +12 [2,22–24,29,30], whereby a high number of cells with 13q deletion has been associated with a worse outcome [2,22–24]. In fact, patients displaying a high degree of infiltration of 13q- had an intermediate prognosis, with a shorter time to first therapy and survival than those with normal cytogenetics or trisomy 12 [2,22–24]. In addition, patients with fewer losses in 17p or patients with a low number of trisomy 12 have a better outcome [29,31]. Several reports have shown the 11q deletion to be a factor predicting poor prognosis in CLL [1,25,32]. In the present study, we have confirmed these previous observations, whereby CLL patients with 11q- had a median time of 2 years to receipt of first therapy and an overall survival of 8 years. Although the present study is a retrospective and multicenter analysis of a series of CLLs, the characteristics of the patients agree real-world basis. Thus, a median age of 65 years, predominance of male sex (76%) and frequent lymphadenopathy (69%) were observed, as previously described.
In addition, 66% of patients had unmutated IGHV status with more than 50% of cases expressing CD38 and/or ZAP-70. Of note, we have observed that the number of cells carrying 11q- influences the disease outcome in terms of time to first therapy and overall survival. These results are consistent with those of two recently published series that reported a longer TFT in patients with 11q deletion and a low number of losses [33,34]. However, one of these studies found no improvement in the overall survival in this group of patients [34]. Therefore, the present study confirms that patients with 11q deletion do not comprise a homogeneous subgroup. We identified a threshold of 40% of deleted cells to be optimal for showing that a low number of losses in 11q is associated with a better outcome in terms of TFT and OS. However, the use of other cut-off points (i.e., <40% vs 40–59% vs ≥60%) yielded similar results.

Interestingly, this study showed that CLL with losses in 11q with early clinical stage, mutated IGHV status and/or a low number of losses in the 11q chromosome had a TFT of more than two years compared with cases with >40% of cells with 11q deletion (median, 44 vs 19 months), similar to previously reported results [34]. Therefore, we identified the presence of advanced clinical stages, unmutated IGHV, and a high number of losses in 11q as the main predictors indicating therapy in the group of CLL displaying 11q-.

Moreover, CLL patients with a lower percentage of 11q deletion had a better OS than those with ≥40% of 11q-deleted nuclei (median, not reached vs 90 months), with an estimated 3 years longer survival in patients with less than 40% of 11q deletions. In addition, clinical (absence of splenomegaly) or biological variables (low 11q-, low LDH and β2M) were included in the final multivariate model of OS. Therefore, the assessment of the number of 11q- cells should be included to better define survival in patients with CLL displaying this genetic abnormality and could be incorporated into the design of clinical trials to define their influence on the response to new therapies and on survival.

To better define the molecular characteristics of the CLL patients displaying losses in 11q, a mutational study performed by next-generation sequencing, including the most frequently mutated genes in CLL, was carried out. The results showed that patients with a low number of 11q losses displayed fewer mutations. Interestingly, ATM mutations were present in both cohorts of 11q- patients at a similar frequency to those previously reported [9,35–37]. We found no significant impact of ATM mutations on prognosis for all the 11q- patients, as described by other authors [9,37]. However, focusing on the group of patients with a low number of 11q losses, ATM mutations were useful for identifying CLL patients with a shorter TFT. Thus, our results suggest that the integration of molecular markers, such as ATM mutations, and the FISH analysis, in patients showing loss of 11q could provide a better prognostic stratification than has been recently demonstrated in other CLL patients [20].

The presence of gene mutations has been widely demonstrated in CLL [11–14]. Mutations of TP53, NOTCH1, SF3B1 and BIRC3 are known to be associated with a worse prognosis, while mutations in MYD88 are related to a better outcome [18,19,21,38,39]. However, some of the results concerning the incidence and independent prognostic value of these mutations are controversial [12,14]. We observed a higher percentage of CLL patients with XPO1 mutations in our cohort of patients than in those described by others [12,13,19,40]. However, it should be taken in account the limitation of the size of our sequencing samples cohort. Consistent with previous studies, mutations of NOTCH1 and TP53 occurred as mutually exclusive events [13,15]. Of note, TP53 mutations were only detected in patients with a high frequency of 11q losses. Furthermore, NOTCH1 and SF3B1 mutations were more frequent in this group of 11q- CLLs (Fig 3). Therefore, our study provides new insights into the molecular basis of the worse outcome of CLL patients who have losses in 11q. It should be noted that the high frequency of gene mutations did not involve the ATM gene, suggesting that the clonal evolution of these patients was not driven by ATM mutations.
(heterogeneity) affecting any CLL-related gene could be the basis of the dismal prognosis of patients with a high frequency of 11q- [12,40,41].

In summary, our results suggest that in patients with CLL, the frequency of 11q-deleted cells influences the clinical outcome, and a low number of 11q- is associated with a longer time to progression and overall survival. In addition, this study shows that fewer CLL patients with low frequencies of 11q- had mutations among genes examined. Our findings, derived from a large retrospective cohort of CLL patients from several Spanish institutions, need to be validated in prospective clinical trials.

**Supporting Information**

S1 File. Supplementary methods data. Characteristics of the series of 197 CLL patients with 11q deletion (Table A). Univariate analysis of time to first therapy in 11q- CLL patients with respect to the number of losses detected by FISH: <40% (n = 51) or ≥40% (n = 146) (Table B). Univariate analysis of overall survival in 11q- CLL patients with respect to the number of losses detected by FISH: <40% (n = 51) or ≥40% (n = 146) (Table C). Main clinical and biological characteristics of 25 CLL patients with 11q- with respect to ATM mutational status (Table D). PCR primers used for next-generation sequencing studies (Table E). A: PCR amplification protocol for ATM. B: PCR amplification protocol for the remaining genes (Table F). Median frequency of reads generated by next-generation sequencing (NGS) (Table G). A. Time to first therapy (TFT) and B. Overall survival (OS) of the global series of 197 CLL patients with 11q deletion (Fig A). A. Time to first therapy (TFT) and B. Overall survival (OS) of patients with CLL and 11q deletion and a percentage of FISH losses <40%, 41–59% or ≥60% (Fig B). Kaplan-Meier plots of time to first therapy (TFT) (A) and overall survival (OS) (B) from diagnosis for 11q- CLL patients sequenced for NOTCH1 and TP53, respectively (Fig C) (DOC)

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**Author Contributions**

Conceived and designed the experiments: JAH MHS AERV JMHR. Performed the experiments: MHS AERV CR RB. Analyzed the data: JAH MHS AERV CR RB. Contributed reagents/materials/analysis tools: VG AK RC CH AP AAM NP JD TG JAQ JG IF GMN JMA PA EL IM IG MG. Wrote the paper: JAH MHS AERV CR RB VG FB BE MG JMHR.

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