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# Low prevalence and independent prognostic role of del(11q) in Chinese patients with chronic lymphocytic leukemia

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ABSTRACT

The 11q deletion (del(11q)) is a conventional cytogenetic aberration observed in chronic lymphocytic leukemia (CLL) patients. However, the prevalence and the prognostic value of del(11q) are still controversial. In this research, we retrospectively explored the prevalence, association, and prognostic significance of del(11q) in 352 untreated and 99 relapsed/refractory Chinese CLL patients. Totally 11.4% of untreated and 19.2% of relapsed/refractory patients harbored del(11q). Del(11q) was more common in patients with β2-microglobulin > 3.5 mg/L, positive CD38, positive zeta-chain associated protein kinase 70, unmutated *immunoglobulin heavy variable-region gene* and *ataxia telangiectasia mutated* mutation. Kaplan-Meier method and univariate Cox regression indicated that del(11q) was an independent prognostic factor for overall survival (OS). Based on the results of univariate Cox regression analysis, two nomograms that included del(11q) were established to predict survival. Desirable area under curve of receiver operating characteristic curves was obtained in the training and validation cohorts. In addition, the calibration curves for the probability of survival showed good agreement between the prediction by nomogram and actual observation. In summary, the prevalence of del(11q) is relatively low in our cohort and del(11q) is an unfavorable prognostic factor for untreated CLL patients. Besides, these two nomograms could be used to accurately predict the prognosis of untreated CLL patients.

## Introduction

Chronic lymphocytic leukemia (CLL) is a common type of mature B-cell malignancy in adults, with heterogeneous clinical courses. Some patients can "watch and wait" for a long time, while some patients need treatment immediately after diagnosis. Previous studies reported that many factors could indicate the clinical course of CLL, including clinical as well as laboratory (age, stage, β2-microglobulin (β2-MG), thymidine kinase 1 (TK-1), etc.), immunophenotypic (CD38, zeta-chain associated protein kinase 70 (ZAP-70), CD49d, etc.), cytogenetic (chromosome, fluorescence in situ hybridization (FISH), etc.) and molecular (immunoglobulin heavy variable-region gene (*IGHV*) status, gene mutations, etc.) biomarkers. 11q deletion (del(11q)), an important cytogenetic aberration, occurs in nearly 20% of previously untreated European CLL patients and is often accompanied by unmutated *IGHV* [1–3]. In Asian countries, the prevalence of del(11q) is relatively low, ranging from

*Abbreviations:* Del(11q), 11q deletion; CLL, chronic lymphocytic leukemia; β2-MG, β2-microglobulin; TK-1, thymidine kinase 1; ZAP-70, zeta-chain associated protein kinase 70; IGHV, immunoglobulin heavy variable-region gene; ATM, ataxia telangiectasia mutated; BIRC3, baculoviral IAP repeat containing 3; NF-κB, nuclear factor-κB; CLL-IPI, chronic lymphocytic leukemia-international prognostic index; TP53, tumor protein 53; ALC, absolute lymphocyte count; LDH, lactate dehydrogenase; ESR, erythrocyte sedimentation rate; CA-125, carbohydrate antigen 125; 25VitD, 25 hydroxyvitamin D; TFS, treatment-free survival; OS, overall survival; C-index, concordance index; AUC, area under curve; ROC, receiver operating characteristic.

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6.9% to 12.5% according to previous studies [4–6]. Del(11q) affects many genes, such as ataxia telangiectasia mutated (*ATM*) and baculoviral IAP repeat containing 3 (*BIRC3*) gene [7]. *ATM* gene is involved in the cellular response to DNA damage and *BIRC3* gene is a negative regulator of the non-canonical nuclear factor-κB (NF-κB) pathway [3].

To date, the exact role of del(11q) in CLL patients remains controversial. Dohner et al. [2] compared the survival of CLL patients with del (17p), del(11q), trisomy 12, normal karyotype, and sole del(13q) and found that del(17p) and del(11q) were independent adverse prognostic factors for CLL patients. While Huang et al. [4] failed to validate the value of del(11q) in their cohort. Many other studies also explored the role of del(11q) in different situations [7]. Most of the studies found that del(11q) was associated with shorter survival, while some studies failed to find the relationship between del(11q) and unfavorable prognosis. Tsimberidou et al. [8] even found that del(11q) was associated with high rates of response, survival, and relapse-free survival when treated with chemoimmunotherapy. Therefore, it is necessary to confirm the effects of del(11q) on survival.

Nomograms have been developed to estimate individual survival probability in many kinds of diseases in recent years. Most can be used to accurately predict prognosis and their efficacy is as good as that of traditional prognostic systems [9–11]. In CLL, the most classic model is chronic lymphocytic leukemia-international prognostic index (CLL-IPI) [12]. Previous researches also established several nomograms to predict survival in untreated CLL patients, but most of them excluded cytogenetic as well as molecular aberrations [13–18].

In this research, we retrospectively analyzed the prevalence of del (11q), the relationships between del(11q) status and clinical, immunophenotypic, cytogenetic as well as molecular characteristics, and the effects of del(11q) on survival in untreated CLL patients. In addition, we established prognostic nomograms including del(11q) to predict survival for untreated CLL patients and validated their performance.

## Materials and methods

### *Patients*

This was a single-center retrospective study. A total of 546 patients were diagnosed with CLL between January 2011 and December 2019 in the department of hematology, the First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital. Four hundred and fifty-one patients (451/546, 82.6%) who did del(11q) test were included in this study. Diagnostic criteria were referred to the International Workshop on CLL-National Cancer Institute criteria. This study was approved by the hospital ethics committee (2018-SRFA-087) and all patients provided informed consent according to the Declaration of Helsinki.

### *Detection of clinical, cytogenetic, molecular, and immunophenotypic aberrations*

Laboratory examination data such as absolute lymphocyte count (ALC), platelet count, hemoglobin concentration, lactate dehydrogenase (LDH) concentration, TK-1 concentration, β2-MG concentration, erythrocyte sedimentation rate (ESR), ferritin concentration, carbohydrate antigen 125 (CA125) concentration, and 25 hydroxyvitamin D (25VitD) concentration were collected in our study [19]. We used peripheral blood/bone marrow samples to detect cytogenetic, molecular, and immunophenotypic aberrations. FISH was conducted to detect del(17p), del(11q), del(13q), and trisomy 12 according to the procedures described previously [20]. FISH probes included: LSI D13S319 for detection of del(13q14), LSI ATM for detection of del(11q22.3), CEP12 (centromere 12) for detection of trisomy 12 and LSI p53 for detection of del(17p13). Gene mutations were detected by Sanger sequencing or next-generation sequencing. The primer sequences of Sanger sequencing for mutation detection (*TP53, NOTCH1, SF3B1*, and *MYD88*) were reported in previous papers [21]. *IGHV* mutational status, CD38, ZAP-70, and CD49d were detected according to corresponding protocols [21], and the cut-off values for mutation or positivity were 98%, 30%, 20%, and 30%, respectively. All data were collected at the same time or the same disease state.

### *Statistical analyses*

SPSS 23 (IBM Corporation, Armonk, NY, USA) was used to analyze data. Categorical variables were analyzed by $\chi 2$ test. Methods were selected according to the following principles: 1. Total count $\geq$ 40 and minimum expected count $\geq$ 5, we used Pearson Chi-Square. 2. Total count $\geq$ 40 and 1 $\leq$ minimum expected count $<$ 5, we used continuity correction. 3. Total count $<$ 40 or minimum expected count $<$1, we used Fisher's exact test. Continuous variables were analyzed by Mann–Whitney *U* test because of skewed distribution. Treatment-free survival (TFS) was calculated as the time from del(11q) detection to first-line treatment or indications appearance (if patients refused to receive the treatment). Overall survival (OS) was defined as the time from del (11q) detection to death or last follow-up. Survival curves were constructed by the Kaplan-Meier method, and the log-rank test was used to examine statistical associations. The Cox proportional hazards model was established to evaluate different factors affecting survival by univariate and multivariate analyses. For the multivariate analysis, we included variables whose P value was less than 0.2 during the univariate analysis.

Patients were randomly divided into training (70%) and validation (30%) cohorts by setting seed in R software version 4.0.1. Nomograms were formulated based on the results of univariate analysis ($P < 0.2$) and by using the rms package [10]. The performance of the nomograms was measured by concordance index (C-index), receiver operating characteristic (ROC) curves, and calibration plots. ROC curves and the corresponding area under the curve (AUC) were constructed to assess the predictive accuracy of each model by using survivalROC package. Calibration plots were drawn with predicted probability of survival as X axis and corresponding actual probability of survival as Y axis. The validation cohort was used to assess C-index, ROC curves, and calibration plots in order to validate the performance of the models generated depending on the data in the training cohort. $P < 0.05$ was defined as a statistically significant value. A Circos plot was drawn by using Circos Table Viewer (http://mkweb.bcgsc.ca/tableviewer/) to present the pairwise co-occurrence of del(11q) and other mutations as well as cytogenetic lesions in untreated CLL patients. Graphs were made by R software version 4.0.1 and GraphPad Prism 8.0 (GraphPad Software, Inc., La Jolla, CA, USA).

## Results

### *Patient characteristics and del(11q) frequency*

A total of 352 previously untreated and 99 relapsed/refractory CLL patients were enrolled in our study. The characteristics of untreated patients were presented in Table 1. The median age was 61 years (16–86 years), with a male/female ratio of 2.26:1. The percentage of *TP53* disruption, del(13q), trisomy 12, *SF3B1* mutation, *NOTCH1* mutation, *MYD88* mutation, *BIRC3* mutation, and *ATM* mutation were 14.7%, 46.1%, 16.1%, 4.9%, 8.8%, 10.0%, 2.1%, and 17.3%, respectively. Median follow-up time was 42 months (2–111 months), and 209 patients received treatment or had treatment indications during the follow-up period. Treatment regimens included: fludarabine/cyclophosphamide ± rituximab ($N$ = 53), bendamustine ± rituximab ($N$ = 30), chlorambucil ± rituximab ($N$ = 28), rituximab ($N$ = 18), high-dose methylprednisolone ± fresh frozen plasma + rituximab ($N$ = 5), hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (hyper-CVAD) ± rituximab ($N$ = 3), ibrutinib ($N$ = 19), ibrutinib + fludarabine/cyclophosphamide + rituximab (N=13), ibrutinib + rituximab ($N$ = 7), other treatment ($N$ = 2), and not available ($N$

**Table 1**
Baseline characteristics of 352 untreated chronic lymphocytic leukaemia patients.

| Variables ($N = 352$) | N (%) |
|---|---|
| Age > 65 years | 114 (32.4) |
| Male | 244 (69.3) |
| Rai I-IV or Binet B/C | 313 (88.9) |
| ALC > 50 × $10^9$/L | 89 (25.3) |
| Platelets < 100 × $10^9$/L | 93 (26.4) |
| Hemoglobin < 100 g/L | 61 (17.3) |
| LDH > ULN ($N = 343$) | 69 (20.1) |
| TK-1 > ULN ($N = 316$) | 38 (12.0) |
| β2-MG > 3.5 mg/L ($N = 332$) | 135 (40.7) |
| ESR ≥ 38 mm/h ($N = 303$) | 45 (14.9) |
| Ferritin > ULN ($N = 314$) | 19 (6.1) |
| CA125 > ULN ($N = 294$) | 19 (6.5) |
| 25VitD < LLN ($N = 150$) | 57 (38.0) |
| Light chain κ ($N = 292$) | 187 (64.0) |
| CD38 (≥30%) ($N = 298$) | 63 (21.1) |
| ZAP-70 (≥20%) ($N = 218$) | 91 (41.7) |
| CD49d (≥30%) ($N = 125$) | 27 (21.6) |
| *TP53* disruption ($N = 292$) | 43 (14.7) |
| Del(11q) | 40 (11.4) |
| Del(13q) ($N = 269$) | 124 (46.1) |
| Trisomy 12 ($N = 267$) | 43 (16.1) |
| Unmutated *IGHV* ($N = 291$) | 119 (40.9) |
| *SF3B1* mutation ($N = 265$) | 13 (4.9) |
| *NOTCH1* mutation ($N = 294$) | 26 (8.8) |
| *MYD88* mutation ($N = 259$) | 26 (10.0) |
| *BIRC3* mutation ($N = 236$) | 5 (2.1) |
| *ATM* mutation ($N = 220$) | 38 (17.3) |

Abbreviations: 25VitD: 25 hydroxyvitamin D; ALC: absolute lymphocyte count; ATM: ataxia telangiectasia mutated; β2-MG: β2-microglobulin; BIRC3: baculoviral IAP repeat containing 3; CA125: carbohydrate antigen 125; ESR: erythrocyte sedimentation rate; IGHV: immunoglobulin heavy variable-region gene; LDH: lactate dehydrogenase; LLN: lower limit of normal; M: mutated; MYD88: myeloid differentiation factor 88; SF3B1: splicing factor 3b subunit 1; TK-1: thymidine kinase 1; ZAP-70: zeta-chain associated protein kinase 70; TP53: tumor protein 53; ULN: upper limit of normal; UM: unmutated.

= 11). A total of 20 patients refused treatment when having indications.

Table 2 showed the prevalence of del(11q) in different disease states. In general, del(11q) was detected in 40 untreated (11.4%) and 19 relapsed/refractory (19.2%) patients. Specifically, a total of 7.1%, 13.9%, and 15.2% of patients harbored del(11q) when detected at diagnosis (no indication), between diagnosis and treatment, and before treatment, respectively.

*Clinical, immunophenotypic, cytogenetic, and molecular correlations*

Del(11q) was more common in untreated patients with β2-MG > 3.5 mg/L (17.8% vs 8.1%, $P = 0.008$), positive CD38 (23.8% vs 8.5%, $P = 0.001$), positive ZAP-70 (17.6% vs 7.9%, $P = 0.029$), unmutated *IGHV* (27.7% vs 2.3%, $P < 0.001$), and *ATM* mutation (31.6% vs 8.8%, $P < 0.001$) (Table 3). The pairwise co-occurrence of del(11q) and other mutations as well as cytogenetic lesions was shown in the Circos plot (Fig. S1). No preferable *IGHV* gene usage was observed in del(11q) subjects (Table S1). In addition, lower expression of CD19 (median: 206.1 vs 411.5, $P = 0.027$) and higher expression of CD38 (median: 18.9 vs 6.7, $P < 0.001$) were found in del(11q) individuals (Table 4).

**Table 2**
Del(11q) frequency by disease state.

| Disease state | Patients number | Del(11q) number (%) |
|---|---|---|
| At diagnosis (no indication) | 155 | 11 (7.1) |
| Between diagnosis and treatment | 65 | 9 (13.9) |
| Before treatment | 132 | 20 (15.2) |
| Relapsed/refractory | 99 | 19 (19.2) |

*Prognostic impact of del(11q)*

For untreated patients, by the Kaplan-Meier method and log-rank test, we found that patients with del(11q) had shorter TFS and OS ($P = 0.053$ and 0.024, Fig. 1). Median TFS for patients with and without del (11q) were 2 and 17 months, respectively. Median OS for patients with and without del(11q) were 75 months and not reached, respectively.

Untreated patients were randomly divided into training ($N = 248$) and validation cohorts ($N = 104$) for further research. The baseline patient characteristics of these two cohorts were shown in Table S2, and no significant difference was seen between these two groups. Cox regression analyses were carried out in the training cohort. Univariate Cox regression analysis showed that Rai I-IV or Binet B/C stage ($P < 0.001$), β2-MG > 3.5 mg/L ($P < 0.001$), unmutated *IGHV* ($P < 0.001$), and *TP53* disruption ($P = 0.014$) had adverse effects on TFS. Age > 65 years ($P < 0.001$), Rai I-IV or Binet B/C stage ($P = 0.048$), β2-MG > 3.5 mg/L ($P < 0.001$), unmutated *IGHV* ($P = 0.001$), *NOTCH1* mutation ($P = 0.029$), *TP53* disruption ($P = 0.002$), and del(11q) ($P = 0.002$) had unfavorable effects on OS. Factors with p value less than 0.2 were included in multivariate Cox regression analysis. Multivariate Cox regression analysis revealed that Rai I-IV or Binet B/C stage ($P = 0.004$) and β2-MG > 3.5 mg/L ($P < 0.001$) were independent adverse prognostic factors for TFS, while β2-MG > 3.5 mg/L ($P = 0.006$), *TP53* disruption ($P = 0.021$) and del(11q) ($P = 0.025$) were independent adverse prognostic factors for OS (Table 5).

*Prognostic nomogram*

The prognostic nomograms that integrated all factors with p value less than 0.2 in univariate Cox regression analysis in the training cohort were shown in Fig. 2. The prognostic nomogram for TFS consisted of six factors: stage, β2-MG concentration, *IGHV* status, *NOTCH1* status, *TP53* status, and del(11q) status (Fig. 2A). The prognostic nomogram for OS consisted of seven factors: age, stage, β2-MG concentration, *IGHV* status, *NOTCH1* status, *TP53* status, and del(11q) status (Fig. 2B). Three-year and five-year survival probabilities were presented in corresponding figures.

The C-index for TFS and OS prediction in the training cohort were 0.761 (95% CI, 0.753–0.769) and 0.791 (95% CI, 0.780–0.802), respectively. ROC curves were conducted to analyze the power of these two prognostic models in predicting TFS and OS of CLL patients. The AUCs were 0.889 and 0.838 for predicting 3-year and 5-year TFS (Fig. 3A), respectively. The AUCs were 0.782 and 0.922 for predicting 3-year and 5-year OS (Fig. 3B), respectively. In the validation cohort, the C-index for TFS was 0.691 (95% CI, 0.668–0.714), and that for OS was 0.789 (95% CI, 0.751–0.827). The associated AUCs were 0.795 and 0.735 for 3-year and 5-year TFS (Fig. 3C), respectively. The AUC was 0.732 for 3-year and 5-year OS (Fig. 3D). In addition, we compared our nomogram models with the golden standard CLL-IPI. No significant difference was seen between these two models (Fig. S2). Whether in the training (Fig. 4A) or validation (Fig. 4B) cohort, the calibration plots for the probability of survival at 3- and 5-year showed an optimal agreement between the prediction by the nomogram and actual observation.

We calculated the risk points of each patient according to the formula presented in the nomograms, and eventually divided patients into high risk (risk points > median) and low risk (risk points ≤ median) groups. In the training cohort, high risk patients had inferior TFS ($P < 0.001$) and OS ($P < 0.001$) than low risk patients (Fig. 5A). Similar results were seen in the validation cohort (Fig. 5B), although the p value for OS was not less than 0.05.

## Discussion

CLL, a heterogeneous B-cell chronic lymphoproliferative disorder, is often accompanied by cytogenetic aberrations, one of which is del(11q). In western countries, nearly 20% of untreated CLL patients harbor this

**Table 3**
The relationships between del(11q) status and patients' clinical, immunophenotypic, cytogenetic and molecular characteristics.

| Variables | Del(11q) | No del(11q) | P value | Variables | Del(11q) | No del(11q) | P value |
|---|---|---|---|---|---|---|---|
| Gender | | | | Light chain | | | |
| Male | 31 | 213 | 0.233 | κ | 19 | 168 | 0.200 |
| Female | 9 | 99 | | λ | 16 | 89 | |
| Age | | | | CD38 (≥ 30%) | | | |
| > 65 years | 11 | 103 | 0.483 | Positive | 15 | 48 | **0.001** |
| ≤ 65 years | 29 | 209 | | Negative | 20 | 215 | |
| Stage | | | | ZAP-70 (≥ 20%) | | | |
| Rai I-IV or Binet B/C | 39 | 274 | 0.117 | Positive | 16 | 75 | **0.029** |
| Rai 0 or Binet A | 1 | 38 | | Negative | 10 | 117 | |
| ALC | | | | CD49d (≥ 30%) | | | |
| > $50 \times 10^9$/L | 13 | 76 | 0.265 | Positive | 4 | 23 | 0.862 |
| ≤ $50 \times 10^9$/L | 27 | 236 | | Negative | 11 | 87 | |
| Platelets | | | | *TP53* disruption | | | |
| < $100 \times 10^9$/L | 12 | 81 | 0.585 | Yes | 4 | 39 | 0.557 |
| ≥ $100 \times 10^9$/L | 28 | 231 | | No | 31 | 218 | |
| Hemoglobin | | | | Del(13q) | | | |
| < 100 g/L | 9 | 52 | 0.359 | Positive | 11 | 113 | 0.208 |
| ≥ 100 g/L | 31 | 260 | | Negative | 20 | 125 | |
| LDH | | | | Trisomy 12 | | | |
| > ULN | 6 | 63 | 0.434 | Positive | 2 | 41 | 0.219 |
| ≤ ULN | 33 | 241 | | Negative | 28 | 196 | |
| TK-1 | | | | *IGHV* status | | | |
| > ULN | 3 | 35 | 0.610 | M | 4 | 168 | **<0.001** |
| ≤ ULN | 34 | 244 | | UM | 33 | 86 | |
| β2-MG | | | | *SF3B1* status | | | |
| > 3.5 mg/L | 24 | 111 | **0.008** | M | 3 | 10 | 0.417 |
| ≤ 3.5 mg/L | 16 | 181 | | UM | 29 | 223 | |
| ESR | | | | *NOTCH1* status | | | |
| ≥ 38 mm/h | 6 | 39 | 0.862 | M | 2 | 24 | 0.706 |
| < 38 mm/h | 32 | 226 | | UM | 33 | 235 | |
| Ferritin | | | | *MYD88* status | | | |
| > ULN | 1 | 18 | 0.562 | M | 0 | 26 | 0.088 |
| ≤ ULN | 37 | 258 | | UM | 32 | 201 | |
| CA125 | | | | *BIRC3* status | | | |
| > ULN | 5 | 14 | 0.132 | M | 0 | 5 | 1.000 |
| ≤ ULN | 32 | 243 | | UM | 28 | 203 | |
| 25VitD | | | | *ATM* status | | | |
| < LLN | 7 | 50 | 0.767 | M | 12 | 26 | **<0.001** |
| ≥ LLN | 13 | 80 | | UM | 16 | 166 | |

Abbreviations: 25VitD: 25 hydroxyvitamin D; ALC: absolute lymphocyte count; ATM: ataxia telangiectasia mutated; β2-MG: β2-microglobulin; BIRC3: baculoviral IAP repeat containing 3; CA125: carbohydrate antigen 125; ESR: erythrocyte sedimentation rate; IGHV: immunoglobulin heavy variable-region gene; LDH: lactate dehydrogenase; LLN: lower limit of normal; M: mutated; MYD88: myeloid differentiation factor 88; SF3B1: splicing factor 3b subunit 1; TK-1: thymidine kinase 1; ZAP-70: zeta-chain associated protein kinase 70; TP53: tumor protein 53; ULN: upper limit of normal; UM: unmutated.

**Table 4**
Baseline mean fluorescence intensity of untreated chronic lymphocytic leukaemia patients.

| Variables | Del(11q) (median, range, N) | No del(11q) (median, range, N) | P value |
|---|---|---|---|
| CD200 | 185.5 (72.7–429.0, 15) | 196.2 (7.0–1241.7, 126) | 0.570 |
| CD148 | 119.6 (34.6–338.3, 15) | 138.2 (32.4–1042.4, 126) | 0.460 |
| CD5 | 134.5 (9.4–1194.3, 15) | 160.3 (3.6–1804.7, 127) | 0.879 |
| CD19 | 206.1 (88.1–883.7, 15) | 411.5 (4.8–2278.2, 125) | **0.027** |
| CD20 | 539.3 (114.9–1964.3, 15) | 514.9 (2.9–3974.9, 125) | 0.342 |
| CD22 | 10.8 (5.3–33.3, 15) | 10.2 (1.3–327.0, 125) | 0.616 |
| CD23 | 206.3 (29.9–820.6, 15) | 167.3 (1.8–1322.9, 123) | 0.618 |
| FMC7 | 5.8 (2.8–14.9, 15) | 7.2 (1.3–264.9, 124) | 0.323 |
| CD38 | 18.9 (5.9–117.9, 15) | 6.7 (1.6–595.0, 124) | **<0.001** |

aberration and have an unfavorable prognosis. In retrospective studies, Dohner et al. [2] and Dickinson et al. [22] found that del(11q) was related to the progression of disease and patients with del(11q) had shorter TFS and OS. In phase II or III clinical trials, regardless of the kind of chemoimmunotherapy used, del(11q) was an independent adverse prognostic factor for progression-free survival and OS [1,23,24]. However, in Asian countries, the prevalence of del(11q) is relatively low, and its value for prognosis remains uncertain. Huang et al. [4] reported that only 6.9% of newly diagnosed CLL patients had del(11q), and *ATM* disruption was not a risk factor for their survival. Yoon et al. [6] considered del(11q)/del(17p) as an unfavorable factor. However, in this study, they took del(11q) and del(17p) into account at the same time, which would influence the judgment of sole del(11q), and thus, their results were unable to illustrate the real role of del(11q) in CLL.

We retrospectively analyzed the clinical data of 352 previously untreated and 99 relapsed/refractory CLL patients in order to explore the role of del(11q). Totally 11.4% of untreated and 19.2% of relapsed/refractory patients had del(11q). The prevalence of del(11q) in our study was lower than that reported by western countries. Besides, the frequency of del(11q) increased with the progression of the disease. Most of the patients with del(11q) harbored high concentrations of β2-MG, positive CD38 as well as ZAP-70, unmutated *IGHV*, and mutated *ATM*. Previous research reported that within patients with del(11q), 32% harbored *TP53* alterations [25]. However, no relationship between del (11q) and *TP53* alterations was shown in our cohort. Higher expression of CD38 and lower expression of CD19 were seen in del(11q) CLL cells, however, the specific mechanisms remained unclear. Kaplan-Meier method and univariate Cox regression analysis showed that del(11q) had unfavorable effects on TFS, while del(11q) failed to be an independent prognostic factor for TFS in multivariate Cox regression analysis. This may be because, in our cohort, patients with del(11q) also harbored other adverse prognostic factors such as unmutated *IGHV* and β2-MG > 3.5 mg/L. In addition, as reported by Huang et al. [4], no standard protocol or guideline was available in the early years in China, so some physicians recommended asymptomatic patients to receive

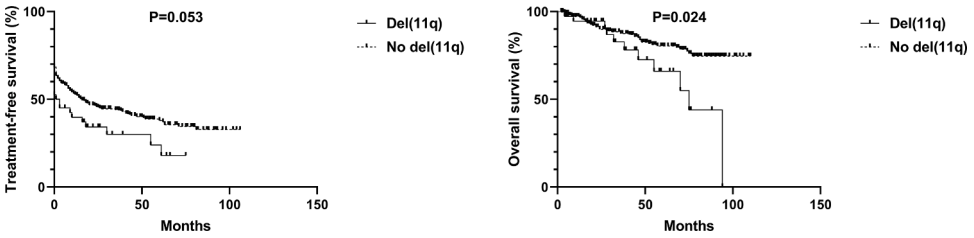


**Fig. 1.** Kaplan-Meier curves of treatment-free survival and overall survival for patients with and without del(11q).

**Table 5**
Univariable and multivariate Cox regression analysis of treatment-free survival and overall survival in training cohort.

| Characteristic | Treatment-free survival | | | | Overall survival | | | |
|---|---|---|---|---|---|---|---|---|
| | Univariate analysis | | Multivariate analysis | | Univariate analysis | | Multivariate analysis | |
| | HR (95% CI) | *P* value | HR (95% CI) | *P* value | HR (95% CI) | *P* value | HR (95% CI) | *P* value |
| Age > 65 years | 1.22 (0.87–1.71) | 0.247 | | | 3.08 (1.68-5.66) | <0.001 | – | – |
| Rai I-IV or Binet B/C | 6.15 (2.51–15.05) | <0.001 | 4.58 (1.64-12.81) | 0.004 | 7.40 (1.02-53.81) | 0.048 | – | – |
| TK-1 > ULN | 1.09 (0.67–1.77) | 0.731 | | | 0.87 (0.34-2.21) | 0.761 | | |
| β2-MG > 3.5 mg/L | 3.30 (2.32–4.69) | <0.001 | 3.16 (2.05-4.88) | <0.001 | 5.31 (2.68-10.53) | <0.001 | 4.02 (1.49-10.85) | 0.006 |
| Unmutated *IGHV* | 2.06 (1.44–2.95) | <0.001 | – | – | 3.57 (1.73-7.35) | 0.001 | – | – |
| *NOTCH1* mutation | 1.45 (0.83–2.52) | 0.194 | – | – | 2.69 (1.11-6.52) | 0.029 | – | – |
| *MYD88* mutation | 1.07 (0.54–2.11) | 0.851 | | | 0.59 (0.08-4.39) | 0.609 | | |
| *SF3B1* mutation | 1.54 (0.75–3.17) | 0.240 | | | 1.89 (0.45–8.00) | 0.385 | | |
| *TP53* disruption | 1.74 (1.12–2.70) | 0.014 | – | – | 3.09 (1.50–6.39) | 0.002 | 2.70 (1.16–6.28) | 0.021 |
| Del(11q) | 1.52 (0.97–2.38) | 0.065 | – | – | 2.98 (1.50–5.95) | 0.002 | 3.16 (1.16–8.59) | 0.025 |

Abbreviations: β2-MG: β2-microglobulin; CI: confidence interval; HR: hazard ratio; IGHV: immunoglobulin heavy variable-region gene; MYD88: myeloid differentiation factor 88; SF3B1: splicing factor 3b subunit 1; TK-1: thymidine kinase 1; TP53: tumor protein 53; ULN: upper limit of normal.

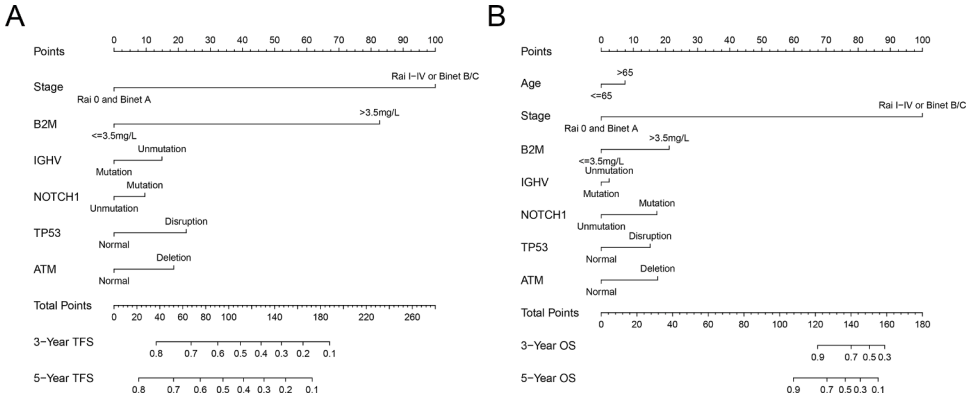


**Fig. 2.** Nomograms for treatment-free survival (A) and overall survival (B) of untreated CLL patients. Nomogram for treatment-free survival includes six factors: stage, β2-MG concentration, *IGHV* status, *NOTCH1* status, *TP53* status and del(11q) status. Nomogram for overall survival includes seven factors: age, stage, β2-MG concentration, *IGHV* status, *NOTCH1* status, *TP53* status and del(11q) status. The nomogram is used by totaling the points identified on the top scale for each independent covariate. This total is then identified on the total points scale to identify the probability of 3- and 5-year survival. Abbreviations: β2-MG: β2-microglobulin; IGHV: immunoglobulin heavy variable-region gene; TP53: tumor protein 53.

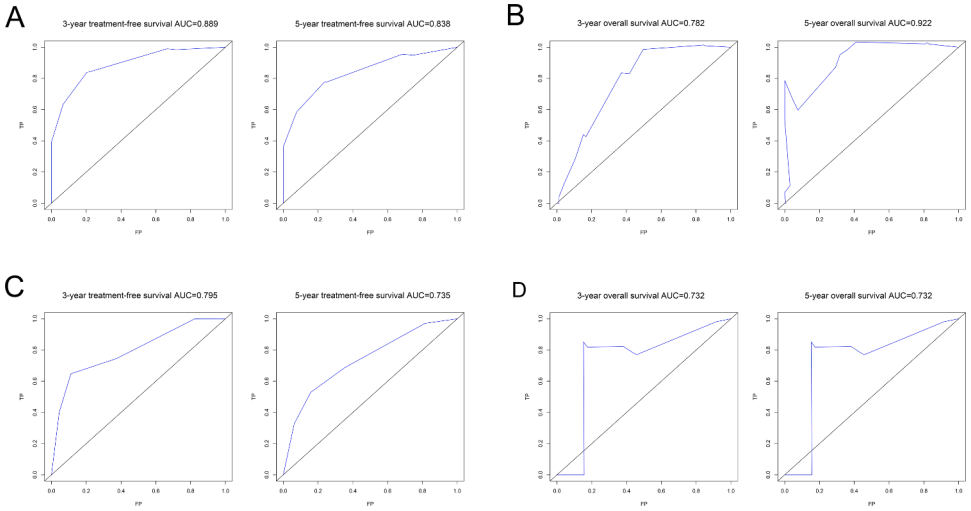


**Fig. 3.** The ROC curves of nomograms. (A) The AUC of 3- and 5-year treatment-free survival for treatment-free survival nomogram in training cohort. (B) The AUC of 3- and 5-year overall survival for overall survival nomogram in training cohort. (C) The AUC of 3- and 5-year treatment-free survival for treatment-free survival nomogram in validation cohort. (D) The AUC of 3- and 5-year overall survival for overall survival nomogram in validation cohort. Abbreviations: AUC: area under curve; ROC: receiver operating characteristic.

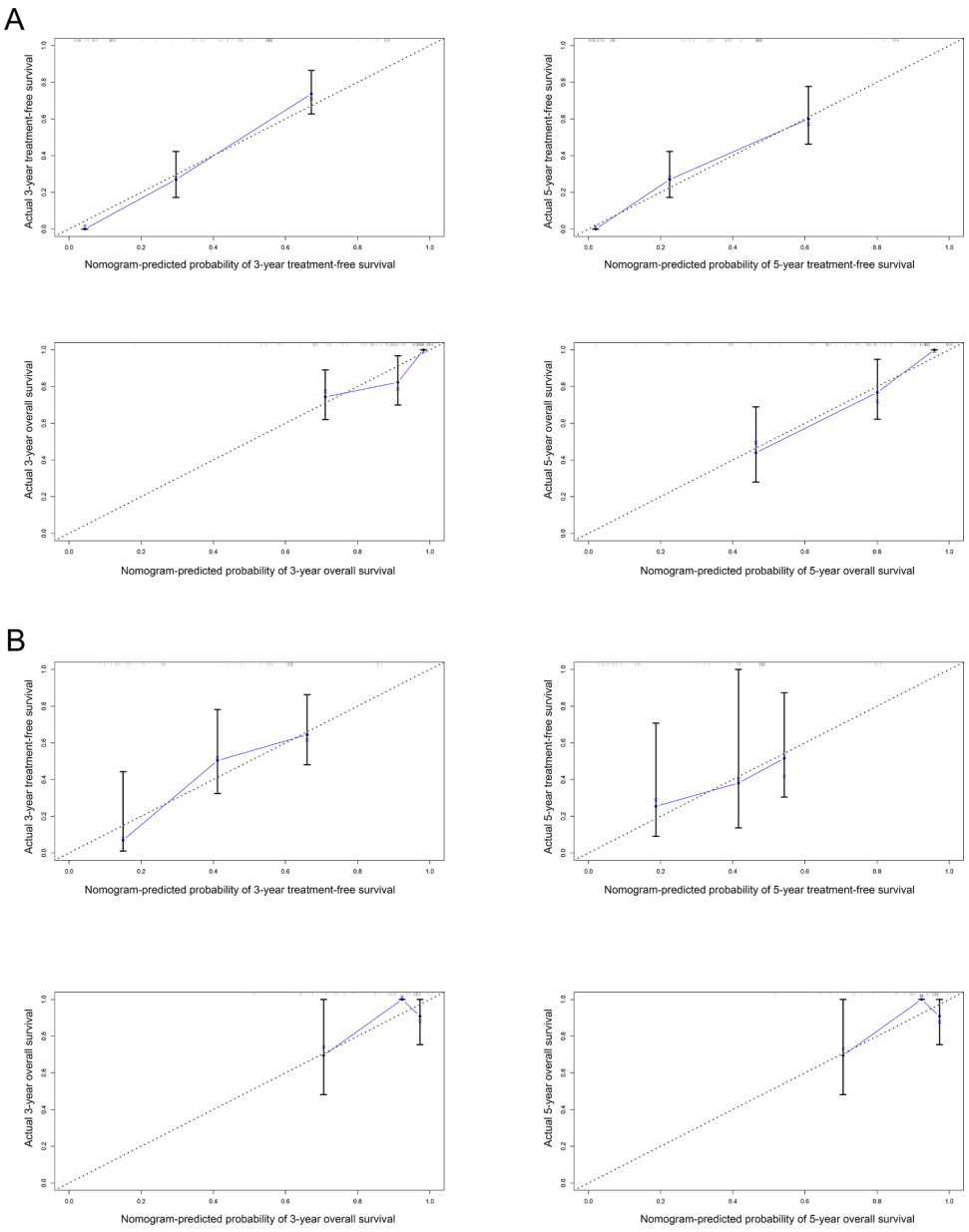


**Fig. 4.** The calibration curves for predicting patient treatment-free survival and overall survival at 3-year and 5-year in the training (A) and validation cohort (B). X axis was predicted probability of survival and *Y* axis was corresponding actual probability of survival.

therapy, which would shorten patients' TFS and interfere with the accuracy of the analysis. While for OS, del(11q) was an independent prognostic factor.

Several prognostic nomograms were constructed to predict the survival of CLL patients in previous researches. Wierda et al. [13] designed a prognostic nomogram consisting of age, sex, β2-MG concentration, ALC, Rai stage, and lymph nodes groups. This nomogram could effectively estimate the OS of untreated CLL patients according to the index score. In 2009, Wierda et al. [14] updated some prognostic factors and constructed two other prognostic nomograms. One included age, β2-MG concentration as well as treatment, and the other consisted of age, β2-MG concentration as well as alkaline phosphatase concentration. These two models also showed excellent performance for the prediction of OS. However, these three nomograms only took clinical and laboratory examination data into consideration and ignored the importance of cytogenetic as well as molecular aberrations. A new nomogram, which included the largest lymph node size in the neck, LDH concentration, number of lymph node sites in involved and FISH, was established again in 2011 to accurately predict TFS [16]. Nonetheless, this model excluded some classical prognostic factors such as age, β2-MG concentration, stage, and molecular aberrations. Therefore, we constructed two new nomograms to respectively predict the TFS and OS of untreated CLL patients in this research. The selection standards of factors involved in nomograms were based on the results of univariate Cox regression analysis. The good performance of these two nomograms was verified by calculating the C-index as well as the AUC of ROC curves, drawing calibration plots, and conducting internal validation. Besides, Kaplan-Meier curves showed that these two nomograms could stratify the prognosis of patients completely according to the prognostic points.

In summary, we retrospectively analyzed the prevalence, association, and outcomes of del(11q) in 352 previously untreated and 99 relapsed/refractory CLL patients in this study. Moreover, we established prognostic nomograms including del(11q) based on our data and validated their performance. Admittedly, there were some limitations in our study, such as small sample size, short follow-up time and lack of some patients' information. All these limitations would lead to bias and

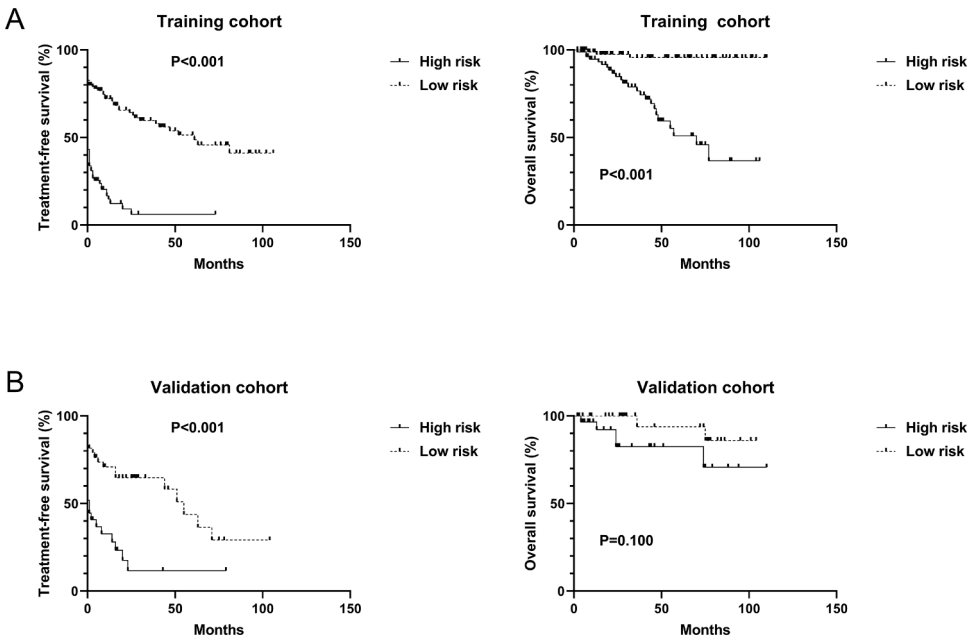


**Fig. 5.** Kaplan-Meier curves of survival for patients with different risk levels. (A) Kaplan–Meier curves of treatment-free survival and overall survival in training cohort. (B) Kaplan-Meier curves of treatment-free survival and overall survival in validation cohort.

should be taken into consideration in further studies. More researches are needed to further confirm the role of del(11q) in CLL patients.

## CRediT authorship contribution statement

**Yi-Xin Zou:** Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Han-Ning Tang:** Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Jing Zhang:** Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Xiao-Lu Tang:** Resources, Data curation. **Shu-Chao Qin:** Resources, Data curation. **Yi Xia:** Resources, Data curation. **Hua-Yuan Zhu:** Resources, Data curation. **Chun Qiao:** Formal analysis, Investigation. **Li Wang:** Formal analysis, Investigation. **Lei Fan:** Formal analysis, Investigation, Writing – review & editing. **Wei Xu:** Formal analysis, Investigation, Writing – review & editing. **Jian-Yong Li:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision. **Yi Miao:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision.

## Declaration of Competing Interest

The authors declare no conflict of interest.

### *Authors' contributions*

Y.M. and J.Y.L. designed the research study. Y.X.Z., H.N.T., J.Z., X.L. T, S.C.Q., Y.X. and H.Y.Z. performed the research. Y.X.Z., H.N.T., J.Z., C. Q., L.W., L.F. and W.X. analyzed the data. Y.X.Z., H.N.T. and J.Z. wrote the paper. L.F., W.X., J.Y.L. and Y.M. revised the manuscript and finalized the last version of the article. All authors checked and approved the submitted version.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2021.101176.

## Reference

[1] M. Hallek, K. Fischer, G. Fingerle-Rowson, et al., Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial, Lancet N. Am. Ed. 376 (9747) (2010) 1164–1174.
[2] H. Dohner, S. Stilgenbauer, A. Benner, et al., Genomic aberrations and survival in chronic lymphocytic leukemia, N. Engl. J. Med. 343 (26) (2000) 1910–1916.
[3] G. Fabbri, R. Dalla-Favera, The molecular pathogenesis of chronic lymphocytic leukaemia, Nat. Rev. Cancer 16 (3) (2016) 145–162.
[4] Y.J. Huang, M.C. Kuo, H. Chang, et al., Distinct immunoglobulin heavy chain variable region gene repertoire and lower frequency of del(11q) in Taiwanese patients with chronic lymphocytic leukaemia, Br. J. Haematol. 187 (1) (2019) 82–92.
[5] S.J. Wu, C.T. Lin, A. Agathangelidis, et al., Distinct molecular genetics of chronic lymphocytic leukemia in Taiwan: clinical and pathogenetic implications, Haematologica 102 (6) (2017) 1085–1090.
[6] J.H. Yoon, Y. Kim, S.A. Yahng, et al., Validation of Western common recurrent chromosomal aberrations in Korean chronic lymphocytic leukaemia patients with very low incidence, Hematol. Oncol. 32 (4) (2014) 169–177.
[7] T. Stankovic, A. Skowronska, The role of ATM mutations and 11q deletions in disease progression in chronic lymphocytic leukemia, Leuk. Lymphoma 55 (6) (2014) 1227–1239.
[8] A.M. Tsimberidou, C. Tam, L.V. Abruzzo, et al., Chemoimmunotherapy may overcome the adverse prognostic significance of 11q deletion in previously untreated patients with chronic lymphocytic leukemia, Cancer 115 (2) (2009) 373–380.
[9] S.Y. Kim, M.J. Yoon, Y.I. Park, M.J. Kim, B.H. Nam, S.R. Park, Nomograms predicting survival of patients with unresectable or metastatic gastric cancer who receive combination cytotoxic chemotherapy as first-line treatment, Gastr. Cancer 21 (3) (2018) 453–463.
[10] Y. Wang, J. Li, Y. Xia, et al., Prognostic nomogram for intrahepatic cholangiocarcinoma after partial hepatectomy, J. Clin. Oncol. 31 (9) (2013) 1188–1195.
[11] X. Hou, D. Wang, J. Zuo, et al., Development and validation of a prognostic nomogram for HIV/AIDS patients who underwent antiretroviral therapy: data from a China population-based cohort, EBioMedicine 48 (2019) 414–424.
[12] International CLLIPIwg, An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data, Lancet Oncol. 17 (6) (2016) 779–790.
[13] W.G. Wierda, S. O'Brien, X.M. Wang, et al., Prognostic nomogram and index for overall survival in previously untreated patients with chronic lymphocytic leukemia, Blood 109 (11) (2007) 4679–4685.

[14] W.G. Wierda, S. O'Brien, X. Wang, et al., Characteristics associated with important clinical end points in patients with chronic lymphocytic leukemia at initial treatment, J. Clin. Oncol. 27 (10) (2009) 1637–1643.
[15] P. Bulian, M. Tarnani, D. Rossi, et al., Multicentre validation of a prognostic index for overall survival in chronic lymphocytic leukaemia, Hematol. Oncol. 29 (2) (2011) 91–99.
[16] W.G. Wierda, S. O'Brien, X. Wang, et al., Multivariable model for time to first treatment in patients with chronic lymphocytic leukemia, J. Clin. Oncol. 29 (31) (2011) 4088–4095.
[17] M. Gentile, F.R. Mauro, D. Rossi, et al., Italian external and multicentric validation of the MD Anderson Cancer Center nomogram and prognostic index for chronic lymphocytic leukaemia patients: analysis of 1502 cases, Br. J. Haematol. 167 (2) (2014) 224–232.
[18] S. Molica, D. Giannarelli, M. Gentile, et al., External validation on a prospective basis of a nomogram for predicting the time to first treatment in patients with chronic lymphocytic leukemia, Cancer 119 (6) (2013) 1177–1185.
[19] Y. Miao, Y.X. Zou, D.L. Gu, et al., SF3B1 mutation predicts unfavorable treatment-free survival in Chinese chronic lymphocytic leukemia patients, Ann. Transl. Med. 7 (8) (2019) 176.
[20] Y. Miao, Y. Miao, K. Shi, et al., A higher percentage of cells with 13q deletion predicts worse outcome in Chinese patients with chronic lymphocytic leukemia carrying isolated 13q deletion, Ann. Hematol. 97 (9) (2018) 1663–1669.
[21] Y. Xia, L. Fan, L. Wang, et al., Frequencies of SF3B1, NOTCH1, MYD88, BIRC3 and IGHV mutations and TP53 disruptions in Chinese with chronic lymphocytic leukemia: disparities with Europeans, Oncotarget 6 (7) (2015) 5426–5434.
[22] J.D. Dickinson, L.M. Smith, W.G. Sanger, et al., Unique gene expression and clinical characteristics are associated with the 11q23 deletion in chronic lymphocytic leukaemia, Br. J. Haematol. 128 (4) (2005) 460–471.
[23] M.R. Grever, D.M. Lucas, G.W. Dewald, et al., Comprehensive assessment of genetic and molecular features predicting outcome in patients with chronic lymphocytic leukemia: results from the US Intergroup Phase III Trial E2997, J. Clin. Oncol. 25 (7) (2007) 799–804.
[24] D. Oscier, R. Wade, Z. Davis, et al., Prognostic factors identified three risk groups in the LRF CLL4 trial, independent of treatment allocation, Haematologica 95 (10) (2010) 1705–1712.
[25] C. Pérez Carretero, M. Quijada Álamo, M. Hernandez-Sanchez, et al., Clinical and Biological Impact of TP53 Alterations in Del(11q) Chronic Lymphocytic Leukemia, Blood 136 (Supplement 1) (2020) 6–7.