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RESEARCH ARTICLE

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TRAF3 alterations are frequent in del-3′IGH chronic lymphocytic leukemia patients and define a specific subgroup with adverse clinical features

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Abstract

Interstitial 14q32 deletions involving IGH gene are infrequent events in chronic lymphocytic leukemia (CLL), affecting less than 5% of patients. To date, little is known about their clinical impact and molecular underpinnings, and its mutational landscape is currently unknown. In this work, a total of 871 CLLs were tested for the IGH break-apart probe, and 54 (6.2%) had a 300 kb deletion of 3′IGH (del-3′IGH CLLs), which contributed to a shorter time to first treatment (TFT). The mutational analysis by next-generation sequencing of 317 untreated CLLs (54 del-3′IGH and 263 as the control group) showed high mutational frequencies of *NOTCH1* (30%), *ATM* (20%), genes involved in the RAS signaling pathway (*BRAF*, *KRAS*, *NRAS*, and *MAP2K1*) (15%), and *TRAF3* (13%) within del-3′IGH CLLs. Notably, the incidence of *TRAF3* mutations was significantly higher in del-3′IGH CLLs than in the control group

Claudia Pérez-Carretero and María Hernández-Sánchez contributed equally to this work.

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($p < .001$). Copy number analysis also revealed that *TRAF3* loss was highly enriched in CLLs with 14q deletion ($p < .001$), indicating a complete biallelic inactivation of this gene through deletion and mutation. Interestingly, the presence of mutations in the aforementioned genes negatively refined the prognosis of del-3′IGH CLLs in terms of overall survival (*NOTCH1*, *ATM*, and *RAS* signaling pathway genes) and TFT (*TRAF3*). Furthermore, *TRAF3* biallelic inactivation constituted an independent risk factor for TFT in the entire CLL cohort. Altogether, our work demonstrates the distinct genetic landscape of del-3′IGH CLL with multiple molecular pathways affected, characterized by a *TRAF3* biallelic inactivation that contributes to a marked poor outcome in this subgroup of patients.

1 | INTRODUCTION

Chromosome 14q abnormalities are infrequent events in chronic lymphocytic leukemia (CLL),[1–3] in comparison to other mature B-cell neoplasms such as multiple myeloma and B-cell lymphomas.[4–6] It is noteworthy that the 14q32.33 chromosomal band, which contains IGH locus, is clinically relevant in CLL, since the mutational status of its variable chain (IGHV) represents one of the most powerful prognostic markers.[7,8] Moreover, 14q32 rearrangements involving *IGH* gene have also a prognostic impact in CLL, showing these patients an intermediate-adverse outcome and a distinct mutational profile from other classic cytogenetic subgroups.[9–14] Furthermore, the long arm of chromosome 14 has been shown to be involved not only in reciprocal rearrangements in CLL, but also in deletions.[4,5,15]

Deletions on 14q (del(14q)) are present in 2%–5% of CLL patients.[5,16] This aberration is heterogenous in size, mapping between chromosomal bands 14q24 and 14q32, and the most frequent breakpoint at the telomeric site involving the *IGH* locus (14q32.3) is observed in 45%–65% of patients with the deletion.[4,5,17] Considering the IGH-deleted region, there is also some variability, as previous studies have identified a deletion in the constant 3′ flanking site of the gene, while others also described 5′IGH deletions in the variable region.[18,19]

Regarding its prognostic significance, few studies have shown that del(14q) CLL patients present an intermediate-adverse clinical outcome.[5,17] However, due to its low incidence, its clinical impact remains controversial in CLL. In addition, this deletion has been associated with trisomy 12, as well as IGHV-unmutated (IGHV-UM) or *NOTCH1* mutations,[4,17] although, so far, detailed molecular analyses of del(14q) CLL cohorts are scarce. It has been speculated that *IGH* rearrangements were suggestive for a transcriptional activation of an oncogene by juxtaposition to the $E\mu$ enhancer of IGH, but this molecular process still remains elusive.[4,5] Alternatively, the hypothesis that del(14q) might inactivate putative tumor suppressor genes has been raised. Concretely, *TRAF3* gene, a negative regulator of the NF-κB pathway located within the deleted region (14q32.32), can be encompassed within del(14q) and it is also rarely mutated in CLL patients.[20,21]

In the present study, we evaluate a large cohort of CLL patients with del(14q), integrating for the first time their clinical and molecular genetic characteristics. We show that CLL patients with 3′IGH deletion (del-3′IGH) exhibit an intermediate-adverse prognosis, with a distinct mutational profile characterized by *TRAF3*, *NOTCH1*, *ATM*, and RAS mutations that also refine the prognosis of this subgroup. Notably, we report for the first time a high frequency of *TRAF3* biallelic alterations (deletion and mutation) with negative clinical implications in a subset of CLL patients showing a loss in 14q32. This biallelic inactivation is also an independent prognostic factor for shortening the time to first treatment (TFT), with a potential role in disease progression within CLL patients with del(14q).

2 | METHODS

2.1 | Patients

The study was based on 871 CLL patients diagnosed according to the International Workshop on CLL (iwCLL) criteria.[22] All of them were screened for *IGH* deletion, and 54 patients exhibited a deletion of 300 kb at the centromeric side of the IGH constant region (del-3′IGH). Cases with this alteration were individually reviewed to rule out any different lymphoproliferative disorder (see Supplementary Methods). Samples and clinical data were collected between August 1995 and December 2020 from 16 Spanish institutions, and all centrally analyzed in the Molecular Cytogenetics Unit of the Center for Cancer Research in Salamanca, Spain. Clinical and biological data are summarized in Table S1. Median follow-up of patients was 60 months (range: 1–340). In total, 47.5% of CLL patients were treated according to iwCLL criteria. Most patients received chemotherapy or chemoimmunotherapy regimens (95.5%), and 5% received new targeted therapies (ibrutinib, venetoclax, or idelalisib).

Mutational analysis by next-generation sequencing (NGS) was performed in 317 CLL patients: 54 del-3′IGH and 263 as a control group. All samples were analyzed before the initiation of first-line treatment. Patients in the control group were selected according to

sample and clinical data availability and were representative of the disease in terms of demographic and clinical characteristics. Clinical and biological characteristics of 317 CLLs included in NGS analyses are shown in Table S2. Cytogenetic-risk classification criteria of patients are described in Supplementary Methods. CLL-IPI and IPS-E risk scores were calculated according to the original publications, and patients were classified into low-, intermediate-, and high-risk subgroups for both scoring systems[23,24] (Table S2).

The study was approved by the local ethical committee (*Comité Ético de Investigación Clínica, Hospital Universitario de Salamanca*). Written informed consent was obtained from all participants before they entered the study.

Additional CLL patient data for an external validation CLL cohort ($n = 450$) were extracted from the International Cancer Genome Consortium (ICGC) project in which 30 CLL patients showed 14q loss.[25]

2.2 | Fluorescence in situ hybridization

Interphase fluorescence in situ hybridization (FISH) was performed on peripheral blood or bone marrow untreated samples using the following commercially available probes: ATM, CEP12, D13S319, and TP53 (Vysis, Abbott Laboratories, IL, USA). LSI IGH Dual Color Break-apart FISH probe was performed for the identification of *IGH* cytogenetic alterations. 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. For the IGH probe, a score of ≥15% was considered positive, and for the rest of probes >10%, based on the cutoff value used by our laboratory. Median time from patients' diagnosis to FISH assessment was 2 months (range: 0–253).

2.3 | Next-generation sequencing

NGS studies were performed in 317 cases and in the same sample as the FISH test before the administration of any treatment, being 54 del-3′IGH and 263 non-del-3′IGH. Genomic DNA was extracted from CD19+ B-lymphocytes isolated from peripheral blood or bone marrow by magnetically activated cell sorting (MACS). B-cell purity was greater than 98% by flow cytometry, as previously described in our group.[27] The Agilent SureSelect^{QXT} Target Enrichment system for Illumina Multiplexed Sequencing (Agilent Technologies, Santa Clara, CA, USA) was used to produce custom-designed libraries of exonic regions from 54 CLL-related candidate driver genes as well as *BCL2*, *IGLL5* and *NOTCH1* UTR regions[13,28–30] (Supplementary Methods) (Table S3). Paired-end sequencing (151-bp reads) was run on the Illumina NextSeq instrument (Illumina, San Diego, CA, USA). Data analysis was performed using a previously validated in-house pipeline[13,28] (Supplementary Methods).

2.4 | Copy number variations analysis

Targeted-capture NGS data were also used to assess the deletion of *TRAF3* gene in CLL patients. The mean coverage depth of each individual target was first normalized in a set of 23 samples without deletion on 14q by FISH using the total read number of each sample. The mean coverage of all these samples was used as the reference. To detect *TRAF3* loss, the normalized coverage of targets of *TRAF3* gene from each study sample was compared with the mean coverage of the same target in the reference file generated above. Copy number variations (CNVs) were called using fixed thresholds representing the $\log_2$ ratio of mean coverage of testing to that of reference. A $\log_2$ ratio <-0.5 suggested a copy number loss/deletion of *TRAF3*. This method was based on a previously published analysis to detect deletions from targeted-capture NGS data.[31] It has been also used to determine CNVs in inherited platelet disorders[32] and, more specifically, in CLL patients using the same NGS approach.[33]

2.5 | Single-nucleotide polymorphism arrays

DNA was purified, amplified, labeled, and hybridized to the Affymetrix SNP6.0 platform (Affymetrix, Santa Clara, CA, USA) as previously described[34] for validation of *IGH* and *TRAF3* loss in CLL samples with available material.

2.6 | Statistical analysis

Statistical analyses were performed using IBM SPSS v23.0 for Windows (IBM Corp., Armonk, NY, USA) and R v4.0.2. Continuous variables were analyzed with the Mann–Whitney *U* test, while the chi-square and Fisher's exact tests were used to assess associations between categorical variables. Overall survival (OS) and time to first treatment (TFT) were calculated from the date FISH test was performed to the date of death, first treatment, or last follow-up (considering disease-unrelated deaths as competing events). Statistically significant variables related to OS and TFT 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 OS and TFT used the Cox regression method. Results were considered statistically significant for values of $p < .05$.

3 | RESULTS

3.1 | Del-3′IGH CLL patients display an intermediate prognosis with distinct clinical features

FISH analysis, including the IGH probe, was performed in a total of 871 CLLs, revealing a deletion of 300 kb at the centromeric side of the IGH constant region in 54 patients (54/871, 6.2%). The pattern observed in the FISH analyses in these patients was a single orange signal (3′IGH), and two green signals (5′IGH), corresponding to the two alleles (Figure 1A). According to this, the alteration detected was monoallelic, and the proportion of cells expressing the 3′IGH deletion (del-3′IGH) in each sample ranged from 15% to 98%, with a median of 65%. This deletion was confirmed by single-nucleotide polymorphism (SNP) arrays in del-3′IGH cases with available material (Figure S1, Table S4).

A detailed list of the clinical and biological characteristics of patients with del-3′IGH is shown in Table S1. Median age at diagnosis was 64.5 years (range: 43–89) and the gender ratio (M:F) was 2. In addition, 51.1% of del-3′IGH cases exhibited IGHV-UM and 23.5%, a Binet stage B or C. The presence of del-3′IGH was significantly associated with splenomegaly when compared with the control group (28.5% vs. 15.9%, $p = .028$) (Table S1). In addition, a total of 35 out of 54 del-3′IGH patients received therapy during follow-up, which was significantly more frequent than those patients in the control group (64.8% vs. 46.4%, $p = .011$) (Table S1). No other significant differences were observed between both groups with respect to clinical and biological parameters.

We observed that 77.8% (42/54) of del-3′IGH cases carried additional cytogenetic alterations, being the most common the del(13q) (19/54, 35.2%), found as the sole abnormality besides the del-3′IGH, followed by trisomy 12 (12/54, 22.2%), del(11q) (6/54, 11.1%), and del(17p) (5/54, 9.3%). It is noteworthy to mention that the incidence of trisomy 12 in the del-3′IGH subgroup tended to be higher than in the control group, although it did not reach the statistical significance (22.2% vs. 15.7%, $p = .25$) (Table S1).

In univariate analyses, the presence of del-3′IGH was associated with reduced TFT (hazard ratio [HR] = 0.851, 95% confidence interval [CI] = 0.76–0.95, $p = .005$) (Figure 1B), although it was not an independent prognostic marker in multivariate analyses (data not shown). CLL patients showing del-3′IGH had a significantly shorter TFT than the non-del-3′IGH subgroup (median 45 vs. 75 months, $p = .004$), especially when compared with low-risk cytogenetics del(13q) (45 vs. 184 months, $p < .001$) or normal FISH (45 vs. 116 months, $p < .001$) (Figure 1C,D). By contrast, del-3′IGH CLL patients exhibited a similar prognosis to those with trisomy 12 (45 vs. 26 months, $p = .893$). It should be noted that del-3′IGH was detected in a subset of CLL patients categorized as normal FISH with the standard four-probe panel, showing these patients a significantly shorter TFT (5 vs. 116 months, $p < .001$) (Figure 1E). The addition of del-3′IGH to trisomy 12 also contributed to a decreased TFT close to the statistical significance (1 vs. 26 months, $p = .062$) (Figure 1F). In terms of OS, we did not observe statistically significant differences in comparison to the control group (128 months vs. median not reached, $p = .578$) (Figure S2).

3.2 | Mutations in *NOTCH1*, *ATM*, *TRAF3*, and *RAS* signaling pathway genes are enriched in del-3′IGH CLL patients

NGS analyses revealed that 81% of patients harbored at least one mutation in any of the 54 genes included in the NGS panel, and the median of mutations per patient was 2 (range: 0–7). The most recurrently mutated genes were *NOTCH1* (20%), *IGLL5* (12%), *SF3B1* (11%), *ATM* (11%), *TP53* (11%), *POT1* (8%), *RPS15* (7%), *XPO1* (5%), and *NFKBIE* (5%).

In the del-3′IGH subgroup, we identified 150 mutations located in 40 genes. Exactly 92.6% of patients (50 of 54) showed at least one mutation, with a median of 2 mutated genes per patient (range: 0–5). Most mutations (98 of 150) were clustered in 12 genes being the most frequently mutated: *NOTCH1* (29.6%), *ATM* (20.4%), RAS signaling pathway genes (*BRAF*, *KRAS*, *NRAS*, *MAP2K1*) (14.8%), and *TRAF3* (13%) (Figure 2A).

A total of 18 *NOTCH1* mutations were detected in 16 del-3′IGH CLL patients (29.6%), being the hotspot variant p.P2514fs in exon 34 the most recurrent mutation (16/18) (Table S5). Of note, there was a trend toward the enrichment of *NOTCH1* mutations in del-3′IGH CLL patients (29.6% vs. 19.6%, $p = .09$) (Figure 2A). The presence of *NOTCH1* mutations was significantly associated with del-3′IGH in comparison to the control group not involving trisomy 12 (29.6% vs. 14.8%, $p = .016$). Despite the association of *NOTCH1* mutations with trisomy 12 previously described in other cohorts, 75% (12/16) of del-3′IGH patients harboring these mutations did not show this additional cytogenetic alteration, suggesting that the enrichment of *NOTCH1* mutations in del-3′IGH CLLs is independent of the presence of additional trisomy 12 (Figure 2B).

The second most commonly mutated gene was *ATM* (20.4%). Variants identified in this case included missense substitutions (4/15), frameshift deletions/insertions (5/15), stop-gain (4/15), and splice-site mutations (2/15) (Table S5). Interestingly, mutations in this gene were significantly associated with the del-3′IGH (20.4% vs. 9.8%, $p = .037$) (Figure 2A). In terms of cytogenetic alterations, del-3′IGH CLLs with *ATM* mutations carried 13q deletion (6/11) and/or 11q deletion (4/11), but none of them showed trisomy 12 (Figure 2B).

In addition, we observed a high mutational rate in RAS signaling pathway genes in this subgroup (*BRAF*, *KRAS*, *NRAS*, *MAP2K1*) (8/54, 14.8%). This mutational frequency was higher than that observed in the control group (14.8% vs. 8.1%, $p = .13$) (Figure 2A), and similar to the one of CLLs with trisomy 12 (14.8% vs. 18.7%, $p = .61$). A total of 10 mutations were located in BRAF (5/10), *KRAS* (2/10), *NRAS* (2/10), and *MAP2K1* (1/10), mainly at hotspots (*BRAF*: G469A/R, V600E, K601E, *KRAS*: G13D, *NRAS*: Q61R/H). Moreover, these variants were present at low variant allele frequencies (VAFs) (1.85%–28%) (Table S5), and samples with these mutations displayed a higher number of mutated genes than the rest of del-3′IGH CLLs (median 4 vs. 2, $p = .001$). However, only 38% of CLL patients with RAS-related mutations had additional FISH cytogenetic alterations, and indeed, only one of them carried additional trisomy 12 (Figure 2B).

3.3 | Del-3′IGH CLLs frequently exhibit a biallelic *TRAF3* inactivation by deletion and mutation

TRAF3 showed a higher mutational frequency (7/54, 13%) within del-3′IGH CLL patients compared with the control group (1/263, 0.4%) ($p < .001$) (Figure 2A), and to previously reported large cohorts of CLL patients[21,25] (0.5%–1%). A total of 20 *TRAF3* mutations were detected in seven samples (Figure 2C). Interestingly, most *TRAF3*-mutated patients carried more than one mutation in this gene (5/7), with a median of 2 (range: 1–6) and at low VAFs (15/20 with VAF <15%) (Table S6). Regarding the type of mutations, whereas 11 out of 20 were frameshift insertion/deletion or encoded for a stop codon that led to truncated proteins, the remaining (9/20) were missense substitutions annotated as pathogenic by the bioinformatic predictors

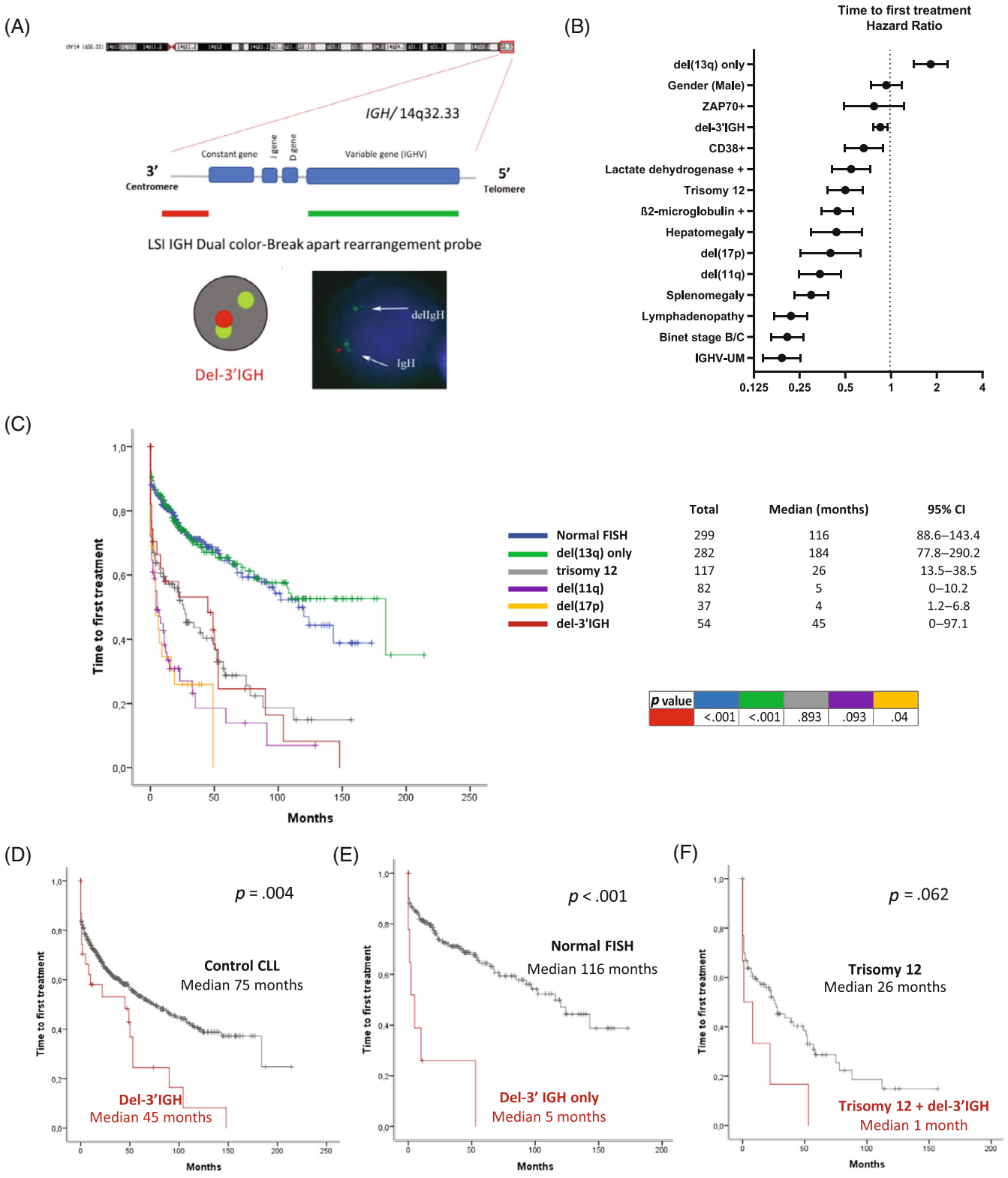

FIGURE 1 Deletion of the centromeric side of *IGH* constant region (del-3′IGH) and its clinical impact on chronic lymphocytic leukemia (CLL). (A) Schematic interphase FISH signal pattern of 3′IGH deletion (del-3′IGH) obtained with the IGH break-apart probe in CLL. (B) Hazard ratios of clinical–biological variables for time to first treatment (TFT) of CLL patients in univariate Cox regression analyses ($n = 871$). (C) TFT according to the cytogenetic alterations (del(13q), del(11q), del(17p), trisomy 12, del-3′IGH). Cytogenetic-risk classification is described in the Supplementary material. (D) TFT of del-3′IGH group versus control group in CLL. Control group represents those patients without del-3′IGH. (E) Clinical impact of del-3′IGH as the only abnormality in the context of normal FISH. (F) Clinical impact of the co-occurrence of del-3′IGH and trisomy 12 when compared with trisomy 12 as the sole abnormality. FISH, fluorescence in situ hybridization. [Color figure can be viewed at wileyonlinelibrary.com]

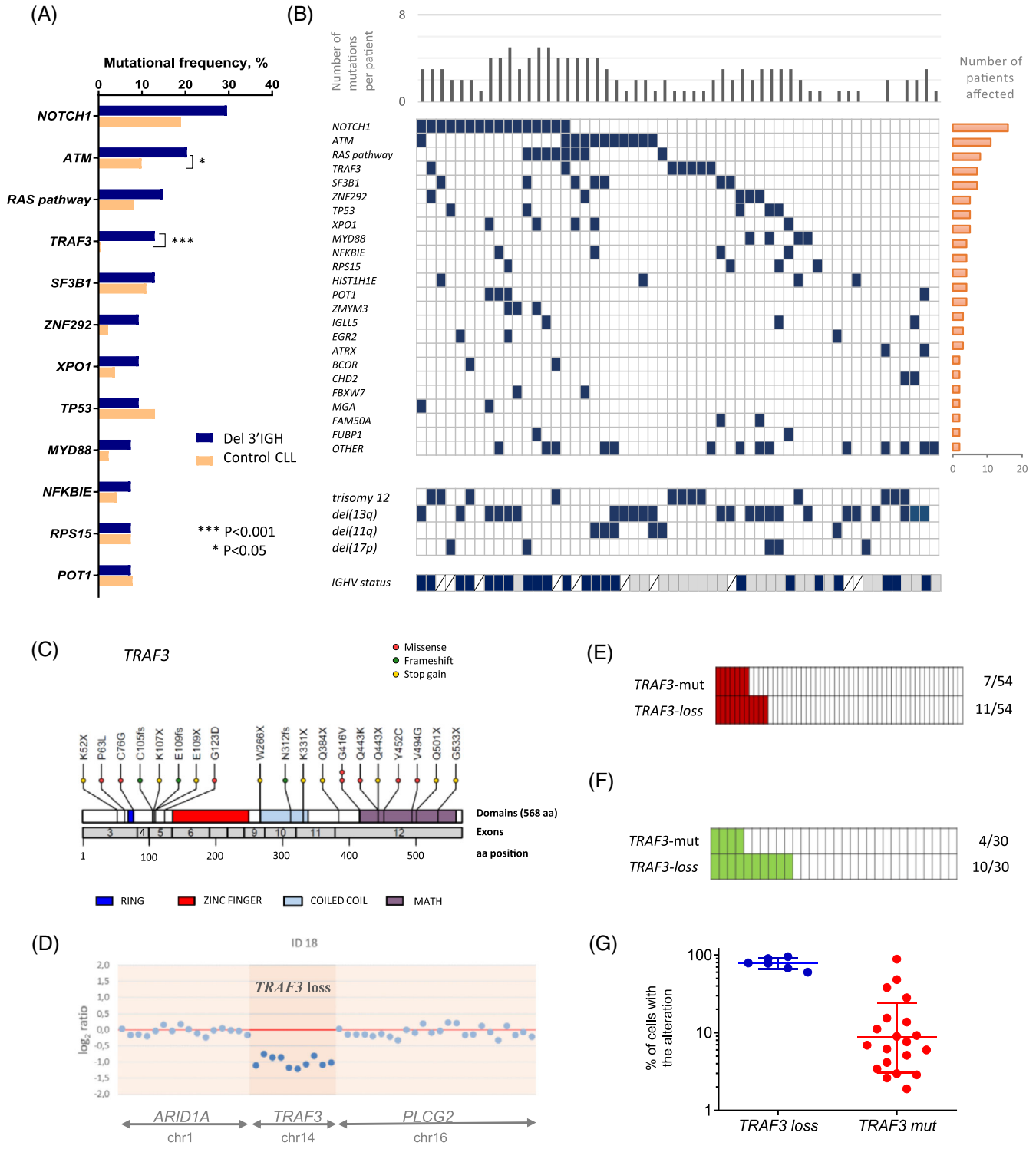

FIGURE 2 Mutational landscape of CLLs with del-3′IGH. (A) Mutational frequencies of CLL genes in del-3′IGH (blue bars) versus control group (orange bars) ($n = 317$). (B) Waterfall plot of genetic and cytogenetic landscape of 54 patients carrying del-3′IGH. Mutations and cytogenetic alterations in the heat map are shown in blue. For the IGHV status, light blue refers to mutated IGHV and dark blue to unmutated IGHV. Crossed lines represent missing data. Number of patients with each mutation are shown at the right orange chart bar and number of mutations per patient are shown in the gray chart bar above the heat map. (C) *TRAF3* mutations identified in 54 del-3′IGH CLLs. Positions of coding mutations are indicated according to the amino acid (aa) change at the protein level (Transcript: ENST00000392745/NM_145725). Number of cases are denoted by circles in each mutation line and the color of the circles indicates the mutation subtype (missense, frameshift or nonsense/stop gain). (D) Dot plot representation of *TRAF3* copy number (CN) loss in one CLL patient (all del-3′IGH cases with a *TRAF3* loss are shown in Figure S3). The *x*-axis represents the targets of chromosomes 1, 14, and 16 included in the custom NGS panel (*ARID1A*, *TRAF3*, and *PLCG2*, respectively). The y-axis shows the values of the $\log_2$ ratio. The red line indicates the reference. (E) *TRAF3* alterations by mutation and/or deletion (red bars) in our cohort of del-3′IGH CLL patients. (F) *TRAF3* alterations by deletion and/or mutation in the validation cohort[25] (green bars) (Table S8). (G) Percentage of altered cells with *TRAF3* loss (blue, % del-3′IGH cells by FISH) or *TRAF3* mutation (red, % variant allele frequency by NGS). Mean value is represented by a horizontal bar and standard deviation is represented by vertical bars. FISH, fluorescence in situ hybridization [Color figure can be viewed at wileyonlinelibrary.com]

of pathogenicity (Figure 2C, Table S5). Moreover, *TRAF3* was the only mutated gene in four cases, while three *TRAF3*-mutated patients also exhibited other genetic mutations (Figure 2B, Table S6).

TRAF3 is located in chromosome 14q32.32 region, proximal to the centromeric side of IGH locus (14q32.33). Due to this proximity, we hypothesized that del-3′IGH could comprise *TRAF3*. Indeed, the CNV analyses with NGS data in the whole cohort of 317 CLLs identified a *TRAF3* loss in 11 patients within the del-3′IGH subgroup (11/54, 20%) (Figures 2D and S3), but only 4 in the control group (4/263, 1.5%) (Table S7). Therefore, *TRAF3* loss was significantly enriched in del-3′IGH CLL patients in comparison to the control group (20 vs. 1.5%, $p < .001$). By SNP arrays, we confirmed the presence or absence of *TRAF3* deletion observed by NGS in two del-3′IGH CLL patients with available material (Figure S1, Table S4). The size of the deletion in patient A (ID 23) comprised only 14q32.33 region (*IGH* locus), with an absence of *TRAF3* loss, while deletion in patient B (ID 28) mapped from 14q24.1 to 14q32.32, including *3′IGH* locus and *TRAF3* (as we observed in CNV analysis) (Tables S4 and S7).

Integrating both mutational and CNV information, we observed that 7 of 11 patients with a *TRAF3* loss also displayed mutations in this gene (Figure 2E, Table S6). To further validate these findings, we reviewed the mutational status of 30 cases with 14q loss from an external published cohort of CLL patients with mutational and CNV information.[25] Within those patients, 14q loss comprised *TRAF3* gene in a total of 10 cases, and 4 of them harbored mutations in this gene, corroborating the presence of biallelic *TRAF3* alterations in this subset of CLL patients (Figure 2F, Table S8).

As we confirmed that *TRAF3* could be included in the 14q32.3 deletion, we next analyzed which event was earlier in time: mutation or deletion. According to our results, the deletion was present in a higher percentage of cells (mean: 70.5 ± 6.5), while mutations occurred at low VAFs (15.6 ± 4.7), indicating that *TRAF3* mutations may appear as a second or subclonal event, leading to a biallelic inactivation of this gene (Figure 2F).

3.4 | Co-occurrence of del-3′IGH and gene mutations contributes to an adverse clinical outcome in CLL patients

Given the high incidence of mutations in poor-prognosis genes, we next wondered if the combination of del-3′IGH and these alterations could have an impact in the clinic. Interestingly, only *TRAF3* mutations exhibited a significantly shorter TFT in this subgroup of patients (median TFT: 6 vs. 51 months, $p < .001$) (Figure 3A). In addition, mutations in *NOTCH1*, *ATM*, and RAS signaling pathway refined patients' outcome in terms of OS (Figure 3B–D).

Patients harboring *NOTCH1* mutations within del-3′IGH subgroup showed a shorter median OS than *NOTCH1-wt* del-3′IGH CLLs (16 months vs. median not reached, $p = .009$) (Figure 3B). Moreover, considering the whole CLL cohort ($n = 317$), we observed that median OS of cases with *NOTCH1-mut* and del-3′IGH was shorter than NOTCH1-*wt* (16 vs. 144 months, $p = .001$), with a stronger statistical significance than the comparison between *NOTCH1-mut* without the deletion and NOTCH1-*wt* cases (120 vs. 144 months, $p = .155$) (Figure S4A).

Interestingly, *ATM* mutations had a significant negative impact on del-3′IGH CLL OS (14 vs. median not reached, $p = .04$) (Figure 3C), and the presence of mutations in the RAS signaling pathway also contributed to a shorter OS in comparison to RAS-*wt* patients (12 vs. median not reached, $p = .004$) (Figure 3D). As we observed in *NOTCH1*-mutated cases with concurrent del-3′IGH, those patients with mutations in *ATM* or in RAS signaling pathway genes that also carried del-3′IGH had a significantly reduced OS than non-del-3′IGH CLLs harboring these mutations, when compared with cases without these alterations (*ATM*: 14 months vs. median not reached, $p = .006$; 92 months vs. median not reached, $p = .99$) (RAS-related genes: 12 months vs. median not reached, $p = .001$; 41 months vs. median not reached, $p = .001$) (Figure S4B,C). Hence, these results demonstrate that concurrent del-3′IGH and mutations in the aforementioned genes could contribute to a worse outcome in CLL patients.

3.5 | *TRAF3* biallelic inactivation drives progression in del-3′IGH patients and constitutes an independent prognostic marker of TFT

Considering that *TRAF3* has an exceptional high frequency of mutation and that it is the only gene with an impact on TFT within this subgroup, we were prompted to delve into the clinical implications of *TRAF3* alterations (deletion and/or mutation). For that purpose, we determined their statistical association with clinical parameters as well as their impact in prognosis and survival when compared with the rest of del-3′IGH and the CLL control group.

In terms of clinical features, patients carrying *TRAF3* alterations had a significant decrease of white blood cells, lymphocytes, and platelets counts and lower levels of hemoglobin. A total of 45.5% of patients exhibited Binet B/C stage and 54.5% were IGHV-UM (Table S9). Notably, all *TRAF3*-altered patients (with mutations and/or deletion) received treatment during their follow-up and showed a significantly shorter median TFT than the rest of del-3′IGH patients (6 vs. 54 months, $p < .001$) (Figure S5A). By contrast, we found no significant statistical differences between median OS of *TRAF3*-altered patients and that of the rest of del-3′IGH (68 vs. 144 months, $p = .14$) (Figure S5B).

As expected, CLL-IPI and IPS-E score systems were able to stratify patients in terms of TFT in the whole cohort ($n = 317$). However, focusing on the del-3′IGH subgroup, CLL-IPI nor IPS-E discriminate risk subgroups with different TFT. Strikingly, the presence of *TRAF3* alterations was an independent risk factor in the multivariate analyses, not only in the whole cohort but also in del-3′IGH patients ($n = 54$) (Figure S6).

In this work, we described a pathological mechanism well known in CLL, consistent with a gene dysfunction caused by a biallelic alteration (deletion and mutation). For this reason, we next carried out an integrated clinical analysis to determine the prognosis impact of biallelic *TRAF3* alterations with respect to other biallelic inactivation phenomena previously associated with an adverse prognosis (biallelic

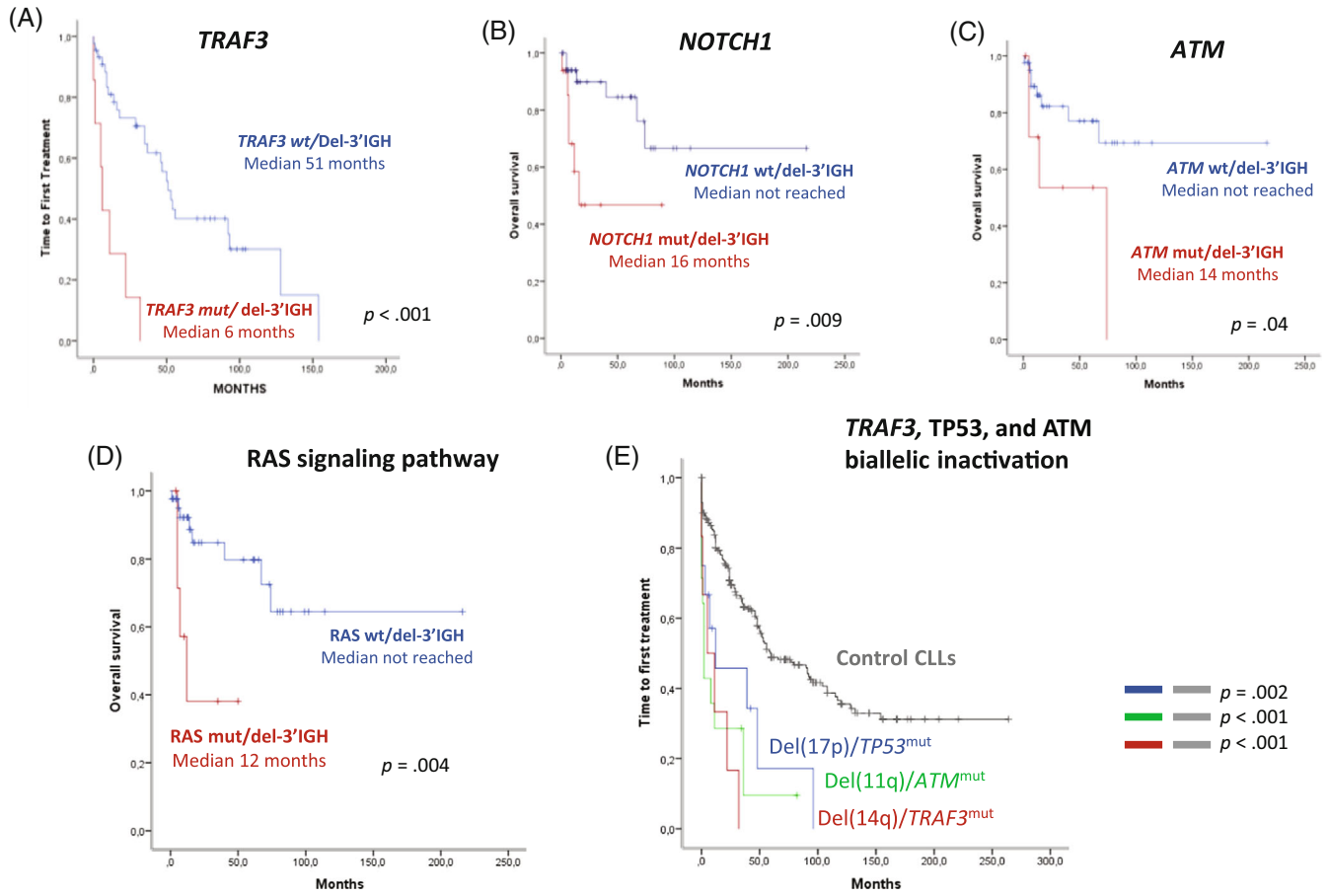

FIGURE 3 Clinical outcome of CLL patients according to the co-occurrence of del-3′IGH and different genetic alterations. (A) TFT of del-3′IGH CLL patients according to the presence of *TRAF3* mutations ($n = 54$). OS of del-3′IGH CLL patients with (B) *NOTCH1* mutations, (C) *ATM* mutations, and (D) mutations in the RAS signaling pathway ($n = 54$). (E) Clinical impact of biallelic inactivation by deletion and mutation of *TP53*, *ATM*, and *TRAF3* in the TFT of CLL patients ($n = 317$) [Color figure can be viewed at wileyonlinelibrary.com]

TP53 (del(17p) and *TP53* mutations) and *ATM* (del(11q) and *ATM* mutations) in the entire CLL cohort ($n = 317$). Our results showed that a total of 16 out of 317 CLLs had a biallelic loss of *TP53* by deletion and mutation, while 14 patients had *ATM* biallelic inactivation (Table S2). All biallelic alterations analyzed had a significant negative impact on TFT (median *TRAF3*: 5 vs. 59 months, $p < .001$, *ATM*: 2 vs. 59, $p < 0.001$ and *TP53*: 12 vs. 59, $p = .002$) (Figure 3E). Interestingly, median TFT of biallelic *TRAF3* inactivation was similar to that of biallelic *ATM* and *TP53* loss, identifying a novel subgroup with inferior outcome. Moreover, the presence of biallelic *TRAF3* alteration was an independent risk factor in the multivariate analyses, suggesting this gene as a new potential biomarker of prognosis (HR = 0.21, 95% CI = 0.05–0.85, $p = .029$) (Table 1).

TABLE 1 Multivariate Cox model analysis of TFT in CLL patients ($n = 317$)

		95% CI		
	HR	Lower	Upper	*p*
Binet stage B/C	0.16	0.08	0.32	**<.001**
IGHV-unmutated	0.36	0.20	0.62	**<.001**
CD38+	0.72	0.41	1.28	.267
High LDH	0.69	0.35	1.37	.289
High β2-M	1.00	0.56	1.80	.988
Hepatomegaly	0.32	0.10	1.04	.058
Splenomegaly	0.95	0.47	1.92	.896
Biallelic *TP53* alteration	0.19	0.04	0.88	**.034**
Biallelic *ATM* alteration	0.75	0.9	1.93	.549
Biallelic *TRAF3* alteration	0.21	0.05	0.85	**.029**

Note: Significant values are shown in bold.
Abbreviations: CI, confidence interval; CLL, chronic lymphocytic leukemia; HR, hazard ratio; LDH, lactate dehydrogenase level; TFT, time to first treatment; β2M, β2-microglobulin level.

4 | DISCUSSION

Chromosomal abnormalities involving 14q are recurrently observed in B-cell neoplasms.[6] In CLL, interstitial 14q deletions are found in ≈5% of

cases and have a variable size ranging from 14q24 to 14q32.33 regions, being the *IGH* locus the most frequent breakpoint at the telomeric side.[4,5,17] Here, we report the largest CLL cohort with a 3′IGH deletion analyzed by FISH and NGS. Specifically, 54 out of 871 CLL patients (6.2%) had a del-3′IGH, representing a group characterized by an enrichment of *TRAF3* alterations, which had a negative impact in prognosis.

According to our results, the deletion of 3′IGH was monoallelic and was present in a high percentage of cells in most cases (median: 65%). This alteration occurred simultaneously with other cytogenetic alterations in 76% of cases, being the most frequent in the del(13q) and trisomy 12. This enrichment of trisomy12 has been previously reported, not only in CLL with del(14q),[4,5,17,35] but also in other neoplasms with this aberration,[6] and furthermore, with other *IGH* abnormalities such as *IGH* translocations,[13,16,36–39] suggesting a strong cooperation between both genetic events.

With respect to the clinical characteristics of del-3′IGH CLL patients, the presence of the deletion had a negative impact on TFT, especially when compared with low-risk cytogenetics, and quite similar to that of the intermediate-risk subgroups (Figure 1C). Previous data also showed an adverse clinical impact of del(14q), although the prognosis was closer to high-risk (del(11q) and del(17p)) than to intermediate-risk cytogenetics.[5] Unlike previous studies,[5,17] we did not observe a significant association between del-3′IGH and IGHV-UM (Table S1). Nevertheless, we did find that del-3′IGH CLLs without additional cytogenetic abnormalities showed a marked poorer outcome in terms of TFT than patients with normal FISH, corroborating the clinical impact of the del-3′IGH in CLL (Figure 1E). With respect to trisomy 12, it is important to highlight that those patients with both alterations (trisomy 12 and del-3′IGH) exhibited shorter TFT than CLLs with trisomy 12 as the sole abnormality (Figure 1F), suggesting that the prognosis observed in the entire del-3′IGH cohort is independent of the trisomy 12 co-occurrence.[5,17] Therefore, this study highlights the value of including the *IGH* probe in the CLL FISH panel to improve CLL prognostic stratification, since it allows us to detect two types of *IGH* abnormalities (deletion and rearrangement), both with a clinical impact.[5,11,13,14,17,40] Thus, this study contributes a significant step forward in the improvement of CLL prognostication.[41]

For a deeper understanding of the molecular pathogenesis of del-3′IGH CLLs, we assessed the mutational profile of this subgroup by NGS. Interestingly, del-3′IGH CLLs harbored a median of two mutations per patient and a high incidence of mutations in *NOTCH1* (30%), *ATM* (20%), *TRAF3* (13%), and RAS signaling pathway genes (15%) (*BRAF*, *KRAS*, *NRAS*, *MAP2K1*) (Figure 2). These results are consistent with the enrichment of *NOTCH1* mutations previously identified in 14q-deleted CLLs.[17] The presence of *TRAF3* mutations in patients with del(14q) has also been described in some B-cell neoplasms,[20] but to our knowledge, this is the first time that it has been described a remarkably high mutational rate within del-3′IGH CLLs as well as a significant association between *TRAF3* mutations and del(14q).

As *NOTCH1* and *RAS* signaling pathway mutations have been extensively related to trisomy 12,[42–48] the mutational frequencies observed in our del-3′IGH cohort could suggest that this entity exhibits a similar mutational pattern. However, the presence of high mutational rates in *ATM* and *TRAF3* identifies a distinct genetic profile with multiple biological pathways affected, involved in proliferative and pro-survival functions as well as in DNA damage repair. Notably, the alteration of multiple pathways and the mutational burden have been previously related to shorter TFT and OS.[25] In our series, the presence of mutations in *NOTCH1*, *ATM*, and RAS pathway within del-3′IGH subgroup had a negative impact on the OS, whereas only *TRAF3* mutations were able to stratify del-3′IGH prognosis in terms of TFT (Figures 3 and S4).

TRAF3 is a negative regulator of the non-canonical NF-κB pathway, and their mutations may lead to a constitutive NF-κB activation.[49] Dysregulation of the non-canonical NF-κB signaling has been shown to play an important role in B-cell transformation and CLL pathogenesis, especially due to *BIRC3* alterations.[33,50–52] Besides *TRAF3* mutations, we also detected mutations in other genes involved in this pathway, more related to the canonical NF-κB signaling (*MYD88* and *NFKBIE*), which may indicate a deregulation of both canonical and non-canonical pathways in del-3′IGH CLL patients. Remarkably, we did not observe co-occurrences of mutations in NF-κB pathway-related genes, and most *TRAF3* mutations appeared as the only mutated gene within this pathway, suggesting mutual exclusive phenomena.

TRAF3 is located at chromosome 14q32.32, proximal to the IGH locus, and it is frequently included in the 14q deletion observed in CLL and other B-cell neoplasms.[4,20] Indeed, our results show that both loss and mutations of *TRAF3* are significantly enriched in del-3′IGH CLL patients. Additionally, we reported that *TRAF3* mutations appeared at lower VAFs than FISH IGH-deleted cells (Figure 2F), suggesting that the mutation could be an acquired secondary event in CLL evolution. The combination of deletion and mutation results in biallelic inactivation of *TRAF3*, which resembles the mechanism of other CLL driver genes dysfunction with a relevant clinical significance in the disease (*TP53* and *ATM*). Besides the clinical impact of *TP53* monoallelic alterations (deletion or mutation) in CLL patients, it has been demonstrated that double null of this gene contributes to a marked poor outcome.[53–56] Regarding *ATM* alterations, latest studies may indicate a stronger negative impact when combined *ATM* loss by 11q deletion and mutations in the remaining allele,[48,57,58] although there is still some controversy. In our cohort, our results showed that biallelic inactivation of *TP53* and *ATM* had a strong negative impact in the TFT of CLL patients and, interestingly, deletion and mutation of *TRAF3* also contributed to a poor outcome, in line with those double-hit alterations (Figure 3E). Moreover, multivariate analysis confirmed that *TRAF3* alterations constitute an independent risk factor of TFT, also in models with prognostic indexes (CLL-IPI and IPS-E) used in clinical practice (Tables 1 and S6), which could help physicians predict the prognosis of CLL patients and guide follow-up approaches. Taken together, our results also suggest that the biallelic inactivation of *TRAF3* could be driving CLL progression, especially in this subgroup of patients with del 3′-IGH.

Taking into account that this study was retrospective and more than 90% of patients received chemotherapeutic regimens, several limitations were found to answer further questions. It would be of great interest to assess the response to novel drugs according to *TRAF3* status in CLL, as well as to evaluate its potential value as a

prognostic and predictive biomarker in the era of novel agents.[41,59] In addition, performing a sequential genetic analysis during disease evolution could help to understand when the del-3′IGH usually appears and if this cytogenetic alteration plays a role as an early or late alteration in CLL. Moreover, 14q deletion including del-3′IGH may encompass other genes (apart from those analyzed in this study) that could also influence CLL pathogenesis and prognosis, and further investigations on the molecular landscape of this entity would be relevant.

In conclusion, a specific subgroup of CLLs carrying del-3′IGH was characterized by a distinct genetic profile harboring mutations affecting multiple biological pathways (NOTCH, RAS, DNA damage response, and NF-κB signaling), which also had a negative impact in CLL prognosis. Moreover, *TRAF3* biallelic inactivation by deletion and mutation, highly enriched in this del-3′IGH of CLL patients, contributes to a marked poor outcome in this subgroup of patients. Taken together, our study provides new insights into the mechanisms of pathogenesis of this CLL cytogenetic subgroup, as well as additional clinical information regarding the combination of different genetic alterations that suggests the emergence of novel molecular biomarkers. In addition, these results also manifest the importance of the IGH assessment, as it allows to classify CLL patients with poor outcomes that would be considered as low-risk cytogenetics otherwise.

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CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

Claudia Pérez-Carretero and María Hernández-Sánchez designed research, performed sample selection, carried out NGS and statistical analyses, and drafted the manuscript. Teresa González performed sample selection, FISH analyses, and provided clinical and immunophenotypic data. Miguel Quijada-Álamo contributed to data analysis and interpretation of the results and critically reviewed the manuscript. Marta Martín-Izquierdo, Sandra Santos-Mínguez, Cristina Miguel-García performed NGS studies and data analysis. María-Jesús Vidal, Alfonso García-De-Coca, Josefina Galende, Emilia Pardal, Carlos Aguilar, Manuel Vargas-Pabón, Julio Dávila, Isabel Gascón-Y-Marín provided patients' clinical data. José-Ángel Hernández-Rivas provided clinical data and critically reviewed the manuscript. Rocío Benito designed sequencing studies and contributed to data analysis. Ana-Eugenia Rodríguez-Vicente performed SNP arrays studies, data analysis, and together with Jesús-María Hernández-Rivas conceived the study, designed and supervised the research, and critically reviewed and approved the final version of the manuscript. All authors discussed the results and revised the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Nowakowski GS, Dewald GW, Hoyer JD, et al. Interphase fluorescence in situ hybridization with an IGH probe is important in the evaluation of patients with a clinical diagnosis of chronic lymphocytic leukaemia. *Br J Haematol*. 2005;130(1):36-42.
2. Berkova A, Pavlistova L, Babicka L, et al. Combined molecular biological and molecular cytogenetic analysis of genomic changes in 146 patients with B-cell chronic lymphocytic leukemia. *Neoplasma*. 2008;55(5):400-408.
3. Lu G, Kong Y, Yue C. Genetic and immunophenotypic profile of IGH@ rearrangement detected by fluorescence in situ hybridization in 149 cases of B-cell chronic lymphocytic leukemia. *Cancer Genet Cytogenet*. 2010;196(1):56-63.
4. Pospisilova H, Baens M, Michaux L, et al. Interstitial del(14)(q) involving IGH: a novel recurrent aberration in B-NHL. *Leukemia*. 2007;21(9):2079-2083.
5. Reindl L, Bacher U, Dicker F, et al. Biological and clinical characterization of recurrent 14q deletions in CLL and other mature B-cell neoplasms. *Br J Haematol*. 2010;151(1):25-36.
6. Mitelman F, Johanson B, Mertens F. Mitelman Database of Chromosome Aberrations in Cancer. 2014. http://cgap.nci.nih.gov/Chromosomes/Mitelman.
7. Damle RN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood*. 1999;94(6):1840-1847.

8. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood*. 1999;94(6):1848-1854.
9. Mayr C, Speicher MR, Kofler DM, et al. Chromosomal translocations are associated with poor prognosis in chronic lymphocytic leukemia. *Blood*. 2006;107(2):742-751.
10. Van Den Neste E, Robin V, Francart J, et al. Chromosomal translocations independently predict treatment failure, treatment-free survival and overall survival in B-cell chronic lymphocytic leukemia patients treated with cladribine. *Leukemia*. 2007;21(8):1715-1722.
11. Cavazzini F, Hernandez JA, Gozzetti A, et al. Chromosome 14q32 translocations involving the immunoglobulin heavy chain locus in chronic lymphocytic leukaemia identify a disease subset with poor prognosis. *Br J Haematol*. 2008;142(4):529-537.
12. Jimenez-Zepeda VH, Chng WJ, Schop RF, et al. Recurrent chromosome abnormalities define nonoverlapping unique subgroups of tumors in patients with chronic lymphocytic leukemia and known karyotypic abnormalities. *Clin Lymphoma Myeloma Leuk*. 2013;13(4):467-476.
13. Pérez-Carretero C, Hernández-Sánchez M, González T, et al. Chronic lymphocytic leukemia patients with IGH translocations are characterized by a distinct genetic landscape with prognostic implications. *Int J Cancer*. 2020;147(10):2780-2792.
14. Davids MS, Vartanov A, Werner L, Neuberg D, Dal Cin P, Brown JR. Controversial fluorescence in situ hybridization cytogenetic abnormalities in chronic lymphocytic leukaemia: new insights from a large cohort. *Br J Haematol*. 2015;170(5):694-703.
15. Hwang Y, Lee JY, Mun YC, Seong CM, Chung WS, Huh J. Various patterns of IgH deletion identified by FISH using combined IgH and IgH/CCND1 probes in multiple myeloma and chronic lymphocytic leukemia. *Int J Lab Hematol*. 2011;33(3):299-304.
16. Leeksma AC, Baliakas P, Moysiadis T, et al. Genomic arrays identify high-risk chronic lymphocytic leukemia with genomic complexity: a multi-center study. *Haematologica*. 2021;106(1):87-97.
17. Cosson A, Chapiro E, Belhouachi N, et al. 14q deletions are associated with trisomy 12, NOTCH1 mutations and unmutated IGHV genes in chronic lymphocytic leukemia and small lymphocytic lymphoma. *Genes Chromosomes Cancer*. 2014;53(8):657-666.
18. Wlodarska I, Matthews C, Veyt E, et al. Telomeric IGH losses detectable by fluorescence in situ hybridization in chronic lymphocytic leukemia reflect somatic VH recombination events. *J Mol Diagn*. 2007;9(1):47-54.
19. Quintero-Rivera F, Nooraie F, Rao PN. Frequency of 5'IGH deletions in B-cell chronic lymphocytic leukemia. *Cancer Genet Cytogenet*. 2009;190(1):33-39.
20. Nagel I, Bug S, Tönnies H, et al. Biallelic inactivation of TRAF3 in a subset of B-cell lymphomas with interstitial del(14)(q24.1q32.33). *Leukemia*. 2009;23(11):2153-2155.
21. Landau DA, Tausch E, Taylor-Weiner AN, et al. Mutations driving CLL and their evolution in progression and relapse. *Nature*. 2015;526(7574):525-530.
22. Hallek M, Cheson BD, Catovsky D, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood*. 2018;131(25):2745-2760.
23. Group IC-Iw. An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data. *Lancet Oncol*. 2016;17(6):779-790.
24. Condoluci A, Terzi di Bergamo L, Langerbeins P, et al. International prognostic score for asymptomatic early-stage chronic lymphocytic leukemia. *Blood*. 2020;135(21):1859-1869.
25. Puente XS, Beà S, Valdés-Mas R, et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia. *Nature*. 2015;526(7574):519-524.
26. González MB, Hernández JM, García JL, et al. The value of fluorescence in situ hybridization for the detection of 11q in multiple myeloma. *Haematologica*. 2004;89(10):1213-1218.
27. Quijada-Álamo M, Hernández-Sánchez M, Robledo C, et al. Next-generation sequencing and FISH studies reveal the appearance of gene mutations and chromosomal abnormalities in hematopoietic progenitors in chronic lymphocytic leukemia. *J Hematol Oncol*. 2017;10(1):83.
28. Hernández-Sánchez M, Rodríguez-Vicente AE, y Marín IG, et al. DNA damage response-related alterations define the genetic background of patients with chronic lymphocytic leukemia and chromosomal gains. *Exp Hematol*. 2019;72:9-13.
29. Quijada-Álamo M, Hernández-Sánchez M, Alonso-Pérez V, et al. CRISPR/Cas9-generated models uncover therapeutic vulnerabilities of del(11q) CLL cells to dual BCR and PARP inhibition. *Leukemia*. 2020;34:1599-1612.
30. Quijada-Álamo M, Pérez-Carretero C, Hernández-Sánchez M, et al. Dissecting the role of TP53 alterations in del(11q) chronic lymphocytic leukemia. *Clin Transl Med*. 2021;11(2):e304.
31. Feng Y, Chen D, Wang GL, Zhang VW, Wong LJ. Improved molecular diagnosis by the detection of exonic deletions with target gene capture and deep sequencing. *Genet Med*. 2015;17(2):99-107.
32. Bastida JM, Lozano ML, Benito R, et al. Introducing high-throughput sequencing into mainstream genetic diagnosis practice in inherited platelet disorders. *Haematologica*. 2018;103(1):148-162.
33. Quijada-Álamo M, Hernández-Sánchez M, Rodríguez-Vicente AE, et al. Biological significance of monoallelic and biallelic BIRC3 loss in del(11q) chronic lymphocytic leukemia progression. *Blood Cancer J*. 2021;11(7):127.
34. Parker H, Rose-Zerilli MJ, Parker A, et al. 13q deletion anatomy and disease progression in patients with chronic lymphocytic leukemia. *Leukemia*. 2011;25(3):489-497.
35. Roos-Weil D, Nguyen-Khac F, Chevret S, et al. Mutational and cytogenetic analyses of 188 CLL patients with trisomy 12: a retrospective study from the French Innovative Leukemia Organization (FILO) working group. *Genes Chromosomes Cancer*. 2018;57(11):533-540.
36. Martín-Subero JI, Ibbotson R, Klapper W, et al. A comprehensive genetic and histopathologic analysis identifies two subgroups of B-cell malignancies carrying a t(14;19)(q32;q13) or variant BCL3-translocation. *Leukemia*. 2007;21(7):1532-1544.
37. Chapiro E, Radford-Weiss I, Bastard C, et al. The most frequent t(14;19)(q32;q13)-positive B-cell malignancy corresponds to an aggressive subgroup of atypical chronic lymphocytic leukemia. *Leukemia*. 2008;22(11):2123-2127.
38. Nguyen-Khac F, Chapiro E, Lesty C, et al. Specific chromosomal IG translocations have different prognoses in chronic lymphocytic leukemia. *Am J Blood Res*. 2011;1(1):13-21.
39. Fang H, Reichard KK, Rabe KG, et al. IGH translocations in chronic lymphocytic leukemia: clinicopathologic features and clinical outcomes. *Am J Hematol*. 2019;94(3):338-345.
40. Harris RA, Stevens JM, Pickering DL, et al. Frequency, variations, and prognostic implications of chromosome 14q32 deletions in chronic lymphocytic leukemia. *Leuk Res*. 2021;110:106665.
41. Pérez-Carretero C, González-Gascón-Y-Marín I, Rodríguez-Vicente AE, et al. The evolving landscape of chronic lymphocytic leukemia on diagnosis, prognosis and treatment. *Diagnostics*. 2021;11(5):853.
42. López C, Delgado J, Costa D, et al. Different distribution of NOTCH1 mutations in chronic lymphocytic leukemia with isolated trisomy 12 or associated with other chromosomal alterations. *Genes Chromosomes Cancer*. 2012;51(9):881-889.
43. Del Giudice I, Rossi D, Chiaretti S, et al. NOTCH1 mutations in +12 chronic lymphocytic leukemia (CLL) confer an unfavorable prognosis, induce a distinctive transcriptional profiling and refine the intermediate prognosis of +12 CLL. *Haematologica*. 2012;97(3):437-441.
44. Balatti V, Bottoni A, Palamarchuk A, et al. NOTCH1 mutations in CLL associated with trisomy 12. *Blood*. 2012;119(2):329-331.

45. Weissmann S, Roller A, Jeromin S, et al. Prognostic impact and landscape of NOTCH1 mutations in chronic lymphocytic leukemia (CLL): a study on 852 patients. *Leukemia*. 2013;27(12):2393-2396.
46. Giménez N, Martínez-Trillos A, Montraveta A, et al. Mutations in the RAS-BRAF-MAPK-ERK pathway define a specific subgroup of patients with adverse clinical features and provide new therapeutic options in chronic lymphocytic leukemia. *Haematologica*. 2019;104(3):576-586.
47. Vendramini E, Bomben R, Pozzo F, et al. KRAS, NRAS, and BRAF mutations are highly enriched in trisomy 12 chronic lymphocytic leukemia and are associated with shorter treatment-free survival. *Leukemia*. 2019;33(8):2111-2115.
48. Blakemore SJ, Clifford R, Parker H, et al. Clinical significance of TP53, BIRC3, ATM and MAPK-ERK genes in chronic lymphocytic leukaemia: data from the randomised UKLRF CLL4 trial. *Leukemia*. 2020;34(7):1760-1774.
49. Moore CR, Edwards SK, Xie P. Targeting TRAF3 downstream signaling pathways in B cell neoplasms. *J Cancer Sci Ther*. 2015;7(2):67-74.
50. Moore CR, Liu Y, Shao C, Covey LR, Morse HC, Xie P. Specific deletion of TRAF3 in B lymphocytes leads to B-lymphoma development in mice. *Leukemia*. 2012;26(5):1122-1127.
51. Bushell KR, Kim Y, Chan FC, et al. Genetic inactivation of TRAF3 in canine and human B-cell lymphoma. *Blood*. 2015;125(6):999-1005.
52. Diop F, Moia R, Favini C, et al. Biological and clinical implications of. *Haematologica*. 2020;105(2):448-456.
53. Malcikova J, Smardova J, Rocnova L, et al. Monoallelic and biallelic inactivation of TP53 gene in chronic lymphocytic leukemia: selection, impact on survival, and response to DNA damage. *Blood*. 2009;114(26):5307-5314.
54. Zenz T, Vollmer D, Trbusek M, et al. TP53 mutation profile in chronic lymphocytic leukemia: evidence for a disease specific profile from a comprehensive analysis of 268 mutations. *Leukemia*. 2010;24(12):2072-2079.
55. Yu L, Kim HT, Kasar S, et al. Survival of Del17p CLL depends on genomic complexity and somatic mutation. *Clin Cancer Res*. 2017;23(3):735-745.
56. Campo E, Cymbalista F, Ghia P, et al. Aberrations in chronic lymphocytic leukemia: an overview of the clinical implications of improved diagnostics. *Haematologica*. 2018;103(12):1956-1968.
57. Skowronska A, Parker A, Ahmed G, et al. Biallelic ATM inactivation significantly reduces survival in patients treated on the United Kingdom leukemia research fund chronic lymphocytic leukemia 4 trial. *J Clin Oncol*. 2012;30(36):4524-4532.
58. Lozano-Santos C, García-Vela JA, Pérez-Sanz N, et al. Biallelic ATM alterations detected at diagnosis identify a subset of treatment-naïve chronic lymphocytic leukemia patients with reduced overall survival similar to patients with p53 deletion. *Leuk Lymphoma*. 2017;58(4):859-865.
59. González-Gascón-Y-Marín I, Muñoz-Novas C, Rodríguez-Vicente AE, et al. From biomarkers to models in the changing landscape of chronic lymphocytic leukemia: evolve or become extinct. *Cancers*. 2021;13(8):1782.

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