Heterogeneity in \textit{BIRC3} and \textit{ATM} Alterations in del(11q) Chronic Lymphocytic Leukemia

Camila Galvano (camgalvano@gmail.com)
IMEX: CONICET-Academia Nacional de Medicina Instituto de Medicina Experimental
https://orcid.org/0000-0001-7702-3129
Andrea Krzywinski
IMEX: CONICET-Academia Nacional de Medicina Instituto de Medicina Experimental
Carmen Stanganelli
Academia Nacional de Medicina
Cecilia M. Rodríguez
Universidad Nacional de Córdoba Facultad de Ciencias Médicas: Universidad Nacional de Cordoba Facultad de Ciencias Medicas
Viviana Heller
Universidad Nacional de Córdoba Facultad de Ciencias Médicas: Universidad Nacional de Cordoba Facultad de Ciencias Medicas
Marcela Miodosky
Instituto de Oncologia Angel H Roffo
Silvana Cugliari
Instituto de Oncologia Angel H Roffo
Raimundo Bezares
Acute Care General Hospital Dr Teodoro Alvarez: Hospital General de Agudos Dr Teodoro Alvarez
Irma Slavutsky
IMEX: CONICET-Academia Nacional de Medicina Instituto de Medicina Experimental

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Abstract

Deletion 11q22 [del(11q)] is among the most common chromosomal alteration in chronic lymphocytic leukemia (CLL). This anomaly is highly variable in size and may be distinguished in large and small deletions. The minimal deleted region on 11q usually includes ATM gene and can also encompass the BIRC3 locus, located 6 Mb centromeric to ATM, in approximately 80% of cases. In this study, we have evaluated BIRC3 and ATM losses and their association with adverse prognostic factors in CLL patients. Thirty-three out of 200 patients (16.5%) sequentially studied in our laboratory harbored del(11q). Our study shows a predominant presence of concomitant ATM and BIRC3 deletions but with differences in the percentage of abnormal cells: 54.5% of cases had similar ATM and BIRC3 percentages, 9.1% showed higher frequency of ATM deletion and 21.2% increased percentage of BIRC3 loss, indicating clonal evolution. Interestingly, 9.1% patients presented only BIRC3 deletion and 6.1% cases showed only ATM loss, suggesting the inclusion of BIRC3 analysis in the routine FISH laboratory screening. A strong association between ATM and BIRC3 deletions with TP53 loss (48.5%), complex karyotypes (36%), unmutated IGHV (72.7%) and intratumor heterogeneity (51.5% patients) was observed. A short time to first treatment (p<0.0001) in cases with del(11q) compared to patients with 13q14 deletion single and without cytogenetic alterations was also found, highlighting the increased genomic instability and poor outcome present in this subgroup of patients.

Introduction

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world, accounting for nearly 30% of adult leukemias. It is a complex heterogeneous disease, characterized by the accumulation of malignant mature clonal B-cells in the blood, bone marrow and lymphoid tissues. The clinical course of the disease is highly variable with a wide range of survival, from a few months to more than a decade after diagnosis [1, 2]. Several biomarkers have been identified allowing the subdivision of CLL into clinical relevant subgroups. Particularly, the analysis of recurrent cytogenetic and FISH (fluorescence in situ hybridization) alterations have proved to be important tools in the biologic characterization of the disease, being the most common: deletions 13q14 [del(13q)], 11q22 [del(11q)] and 17p13 [del(17p)] as well as trisomy 12. Among them, patients with del(13q) as a single alteration have a better outcome, while those with del(11q) or del(17p) show the shortest median survival and, cases with trisomy 12 have an intermediate prognosis [3, 4]. Furthermore, some specific cytogenetic patterns provide additional information on clinical evolution and outcome, such as complex karyotypes (CK), associated to an unfavorable prognosis [5, 6]. In addition, the IGHV (immunoglobulin heavy variable) mutational status allowed to divide the CLL into two major subtypes, mutated (M), associated to favorable outcome and unmutated (U), related to adverse prognosis [7].

By FISH analysis, del(11q) is present in about 11-17% of CLL patients [3,4] associated to bulky lymphadenopathy, progressive disease and poor outcome [8, 9]. As known, this alteration is highly variable in size and may be distinguished in classical or large deletions (more frequent) and atypical or small deletions [10, 11]. In almost all cases, the deletion involves the ATM (Ataxia telangiectasia mutated) gene, located at 11q22.3. The introduction of next generation sequencing revealed the concomitant involvement of other genes, like BIRC3 (baculoviral IAP repeat-containing protein 3) mapped at 11q22.2, 6Mb centromeric to the ATM locus [12-14]. Rose-Zerilli et al [13] found that, in most cases, ATM and BIRC3 genes are lost as a part of a single event, meanwhile Alhourani et al [14] observed wide variations in the clone size with deletions of these genes, suggesting clonal evolution. In addition, there are controversial results with respect to the relationship between del(11q) and del(17)(p13), where the TP53 (Tumor Protein P53) gene is located. Some authors found a low frequency of TP53 alterations in cases with del(11q) [12, 14] while others have reported the co-occurrence of these anomalies in the same clone [15-17]. In this study, we have evaluated BIRC3 and ATM losses in CLL patients and their relation with adverse prognostic factors of the disease. A strong association with TP53 deletion, complex karyotypes, U-IGHV and intratumor heterogeneity was observed.
Material And Methods

Patients

From a total of 200 CLL patients sequentially studied in our laboratory between 2015 to 2020, 33 cases (16.5%) harbored del(11q). Patients were diagnosed according to the International Workshop on CLL (iwCLL) guidelines [18]. To compare clinical parameters, we included two control groups: 30 cases with normal karyotype and FISH analysis and 45 patients with del(13q) as the only abnormality. Clinical-biological characteristics of patients are summarized in Table 1. In our cohort, 58.6% of patients were at diagnosis or untreated before the genetic assessment. The study was approved by the local Ethics Committee. All patients provided their written informed consent.

| Clinical characteristics | del(11q) | No alterations | P value | del(13q) | P value |
|--------------------------|---------|---------------|---------|----------|---------|
| N° of patients (n)       | 33      | 30            | 0.84    | 45       | 0.019   |
| Sex F/M                  | 11/22   | 18/12         |         | 16/19    |         |
| Mean age (years; range)  | 69.5 (36-86) | 64.5 (45-87) |         | 63.2 (36-82) |         |
| Rai stages (%)           |         |               | 0.005   |         | <0.0001 |
| 0                        | 18.3    | 38.5          |         | 41.5     |         |
| I-II                     | 41.7    | 50            |         | 51.2     |         |
| III-IV                   | 50      | 11.5          |         | 7.3      |         |
| WBC count (x10⁹/L) mean  | 72      | 37.1          | 0.260   | 44       | 0.04    |
| Hemoglobin (g/L) mean    | 12.6    | 13.1          | 0.450   | 13.2     | 0.348   |
| Lymphocytes (x10⁹/L) mean| 43.5    | 31.1          | 0.656   | 36.7     | 0.572   |
| Platelets (x10⁹/L) mean  | 170.6   | 186.2         | 0.703   | 172.4    | 0.594   |
| LDH (U/L) mean           | 451.1   | 107.1         | 0.501   | 334      | 0.168   |
| B2M (mq/L) mean          | 4.1     | 2.7           | 0.034   | 2.8      | 0.014   |

del: deletion; F: female; M: male; WBC: white blood cells; LDH: lactate dehydrogenase; B2M: b2microglobulin.

Cytogenetics and FISH analysis

Cytogenetic and FISH analyses were performed on stimulated peripheral blood lymphocyte cultures as previously described [19]. G-banding technique was used. Chromosome abnormalities were reported in accordance with the International System for Human Cytogenomic Nomenclature (ISCN) [20]. For FISH analysis, the following DNA probes were used: EN12, OLE13q14 D13S319, OLE11q22.3 ATM, OLE17p13.1 TP53 and ODF11q18q IAP (BIRC3)/MAL1T1 (LIVE-Lexel, Buenos Aires, Argentina), according to the manufacturer’s protocols. For each probe, at least two hundred interphase nuclei were evaluated. The cut-offs for positive values (mean of normal controls plus 3 standard deviations) determined from ten cytogenetically normal donors were as follows: 3%, 10%, 7.5%, 5.5% and 5.5% for trisomy 12, and monosomies of D13S319, ATM, TP53 and BIRC3, respectively. Image acquisition was performed using the CytoVision applied Imaging System (Leica Biosystems, Germany).
IGHV Mutational Status

For IGHV analysis, polymerase chain reaction (PCR) and bidirectional sequencing were performed as previously described [21]. Sequences were analyzed using the IMGT database, the IMGT/V-Quest and the IMGT/JunctionAnalysis (http://www.imgt.org/IMGT_vquest/analysis). IGHV sequences with <98% homology with respect to the germ line counterpart were considered as M, whereas those with homology of 98% or higher were classified as U.

Statistical analysis

Statistical analysis was performed using the SPSS statistical package (Version 24.0). The comparison of data between subgroups was performed using the Student t test (for quantitative variables) and the \( \chi^2 \) or Fisher’s exact test (for categorical variables). Time to first treatment (TTFT), calculated from the date of diagnosis to the date of starting therapy or the date of the last follow-up for patients not receiving treatment, was estimated by the Kaplan-Meier method and compared with the Log-rank test. For all tests, \( p<0.05 \) was regarded as statistically significant.

Results

Cytogenetics and FISH analysis

Cytogenetics and FISH analysis of 33 CLL patients with del(11q) are presented in Table 2. From them, 8 (24.2%) showed normal karyotypes (NK) while 25 (75.8%) exhibited chromosome alterations: 64% (16/25) had simple karyotypes (SK; 1 or 2 abnormalities) and 36% (9/25) complex karyotypes (CK; 3 or more alterations). By cytogenetics analysis, del(11q) was observed in 17 cases (51.5%), in 8 of them as a single abnormality.
Table 2
Cytogenetics and FISH analysis in CLL patients with del(11q)

| Case | Age/Sex | Karyotype | FISH (%) | IGHV (%) |
|------|---------|-----------|----------|----------|
|      |         |           | T12      | del(13q) | delATM  | delBIRC3 | delTP53 |
| 1    | 56/F    | 46,XX     | 23.0     | N        | 13.3    | 10.7     | 6.4     | U (100) |
| 2    | 54/M    | 46,XY     | N        | 75.6     | N       | 18.7     | 7.6     | -       |
| 3    | 74/F    | 46,XX     | N        | N        | 9.8     | 28.8     | 6.1     | -       |
| 4    | 80/M    | 46,XY     | N        | 82.0     | 20.0    | 11.0     | 70.7    | U (100) |
| 5    | 69/M    | 46,XY     | N        | 34.5     | 68.7    | 73.6     | N       | U (100) |
| 6    | 69/M    | 46,XY     | N        | 37.5     | 43.0    | 64.5     | N       | U (99.0) |
| 7    | 72/M    | 46,XY     | N        | N        | 47.4    | 88.5     | 9.3     | M (93.8) |
| 8    | 67/M    | 46,XY     | N        | 18.2     | 8.6     | N        | 8.4     | M (91.9) |
| 9    | 80/F    | 46,XX,del(13)(q12q22) [6]/46,XX[14] | N | x2:78.8 | 8.1     | 10.4     | 7.0     | M (96.0) |
| 10   | 85/F    | 47,XX,+20[3]/46,XX[13] | N | 93.3   | N       | 25.5     | 10.5    | -       |
| 11   | 84/M    | 46,XY,del(6)(q23) [5]/46,XYt(6;17)(q27;q11) [1]/46,XY[9] | 11.3 | 73.3   | 69.1    | 91.0     | N       | -       |
| 12   | 64/M    | 46,XY,del(13)(q14q22), der(18)t(3;18)(p21;q23) [cp3]/46,XY[7] | N | 52.2   | 46.9    | 17.0     | N       | -       |
| 13   | 80/F    | 46,XX,del(11)(q22q23) [2]/46,XX[13] | N | N       | N       | 18.2     | N       | U (100) |
| 14   | 76/F    | 46,XX,del(11)(q21) [3]/46,XX[7] | N | 19.0   | 12.1    | 8.5      | 9.8     | -       |
| 15   | 61/F    | 46,XX,del(11)(q21) [3]/46,XX[14] | N | 98.4   | 93.9    | 83.0     | N       | -       |
| 16   | 86/F    | 46,XX,del(11)(q21) [11]/46,XX[4] | N | x1: 9.5/x2: 52.9 | 48.0 | 59.9 | 5.7 | - |
| 17   | 62/M    | 46,XY,del(11)(q21q23) [6]/46,XY[12] | N | N       | 79.7    | 75.7     | N       | U (100) |
| 18   | 36/M    | 46,XY,del(11)(q22) [3]/46,XY[21] | N | 83.9   | 26.6    | 24.0     | N       | M (97.5) |

FISH: fluorescence in situ hybridization; IGHV: immunoglobulin heavy variable; T: trisomy; del: deletion; F: female; M: male; NA: not available; N: no alteration; x1: monoallelic deletion. x2: biallelic deletion. M: Mutated; U: unmutated: *: at diagnosis; FCR: fludarabine, cyclophosphamide, rituximab; BR: bendamustine, rituximab.

** IGHV3-21 Subset #2
| Case | Age/Sex | Karyotype | FISH (%) | IGHV (%) |
|------|---------|-----------|----------|----------|
|      |         |           | T12      | del(13q) | delATM  | delBIRC3 | delTP53 |
| 19   | 49/M    | 46,XY,del(11)(q21) [12]/46,XY[3] | N         | N         | 67.9    | 77.0     | N        | U (100) |
| 20   | 64/M    | 46,XY,del(11)(q21) [12]/46,XY[8] | 7.2       | 46.0      | 57.4    | 62.0     | N        | U (99.7) |
| 21   | 64/M    | 45,XY,der(9)t(9;15) (p22;q11),del(11)(q21) [15]/46,XY[5] | N         | 67.8      | 84.4    | 85.4     | N        | U (99.5) |
| 22   | 79/M    | 46,XY,del(11)(q21),del(13) (q12q14)[10]/46,XY[10] | N         | 94.0      | 93.7    | 93.2     | N        | -        |
| 23   | 79/M    | 46,XY,del(11)(q21) [15]/47,XY,+i(2)(p10),del(11) (q21)[4]/46,XY[3] | 7.4       | N         | 37.1    | 58.9     | 12.0     | -        |
| 24   | 57/M    | 45,XY,del(17;18)(q10;q10) [4]/46,XY,del(11)(q21) [2]/46,XY[20] | N         | 10.7      | 56.9    | 45.8     | 8.4      | U (100) |
|      |         |           |          |          |         |          |          |          |
|      |         |           |          |          |         |          |          |          |
|      |         |           |          |          |         |          |          |          |
|      |         |           |          |          |         |          |          |          |
| 25   | 60/M    | 46,XY,add(12)(q24) [3]/46,XY,der(1)t(1;11) (q12;q23)[3]/45-47,XY+der(4)t(1;4) (q21;q12),-5,+der(13)t(10;13) (q11;q14),der(14) t(3;14) (p13;q24),der(19)t(4;19) (q21;q13),del(22) (q11) [cp12]/46,XY[9] | N         | 96.4      | 9.3     | 5.6      | 66.1     | U (99.6) |
| 26   | 70/M    | 46,47,XY,+der(3)t(3;9) (p12;q13),del(6)(q25),del(7) (q32),-8, +13,+15,-20[cp10]/46,XY[6] | N         | 91.4      | 17.9    | 14.5     | 30.9     | M (95.4) |
| 27   | 64/F    | 46,XX,del(2;13)(q25;q13) [2]/44,XX,-3,+der(4)t(4;12) (q31;q11), -13,der(17) t(17;21) (p11;q11),-18,der(22)t(18;22) (q11;p11 )[5]/46,XX[23] | 76.6      | x1:80.3/x2: 15.0 | 12.1 | N | 88.4 | U (100) |
| 28   | 78/M    | 46,XY,1,fis(1)(p10),+fis(1) (q10),-6,der(9)t(9;14) (q32;q11), +del(16) (q13),add(17)(q21) [13]/46,XY[7] | N         | 14.3      | 28.4    | 34.3     | N        | U (100) |

FISH: fluorescence in situ hybridization; IGHV: immunoglobulin heavy variable; T: trisomy; del: deletion; F: female; M: male; NA: not available; N: no alteration; x1: monoallelic deletion. x2: biallelic deletion; M: Mutated; U: unmutated: *: at diagnosis; FCR: fludarabine, cyclophosphamide, rituximab; BR: bendamustine, rituximab.

**IGHV3-21 Subset #2**
| Case | Age/Sex | Karyotype | FISH (%) | IGHV (%) |
|------|---------|-----------|----------|----------|
|      |         |           | T12     | del(13q) | delATM | delBIRC3 | delTP53 |      |
| 29   | 79/F    | 46,XX,del(7)<br>(q32q36),del(11)<br>(q21),del(13)(q12q14),i(21)(q10)<br>[cp15]/46,XX,t(2;8)<br>(p11.2;q24),del(6)<br>(q23q25),t(7;12)(q36;p13),<br>del(11)(q21),del(13)<br>(q12q14),i(21)(q10)[cp2]/46,XX,del(11)(q21),del(13)<br>(q12q14),der(21)t(X;21)<br>(p11,p11)[cp2] | N  | x1: 75.3/x2: 6.8 | 64.1 | 70.8 | N | M (97.7)** |
| 30   | 72/M    | 46,XY,del(11)(q21)<br>[10]/47,XY,del(11)<br>(q21),+22[4]/46,XY,t(1;4)<br>(q42;q21),del(11)(q21)<br>[3]/46,XY[5] | N  | N | 48.8 | 67.9 | N | U (100) |
| 31   | 67/M    | 45,XY,del(6)(q25),del(11)<br>(q21),-20[7]/46,XY[11] | N  | 11.0 | 92.2 | 90.2 | N | U (100) |
| 32   | 78/M    | 44-46,XY,del(6)(q25),t(8;14)<br>(q24;q32),del(11)<br>(q21),del(13)<br>(q12q14),der(22)t(18;22)<br>(q11,p11)[cp18] | N  | 96.1 | 73.3 | 64.7 | N | - |
| 33   | 80/F    | 46-47,X,-X,del(6)<br>(q23q25),del(11)<br>(q21),+12[cp14]/46,XX[5] | 63.8 | N | 71.8 | 65.7 | 6.0 | U (100) |

FISH: fluorescence in situ hybridization; IGHV: immunoglobulin heavy variable; T: trisomy; del: deletion; F: female; M: male; NA: not available; N: no alteration; x1: monoallelic deletion. x2: biallelic deletion; M: Mutated; U: unmutated; *: at diagnosis; FCR: fludarabine, cyclophosphamide, rituximab; BR: bendamustine, rituximab.

** IGHV3-21 Subset #2

On the contrary, by FISH analysis using both ATM and BIRC3 DNA probes, all patients showed del(11q). From them, BIRC3 deletion was observed in 93.9% of cases while ATM deletion was found in 90.9% of patients. The mean percentage of clonal cells was 49.8% (range: 5.6-93.2%) and 47% (range: 8.1-93.9%) for BIRC3 and ATM deletions, respectively. The distribution of ATM and BIRC3 deletions is shown in Figure 1. Similar ATM and BIRC3 percentages (considered as a 10% difference) were found in 54.5% of cases, 9.1% had higher frequency of ATM deletion and 21.2% showed increased frequency of BIRC3 loss, indicating clonal evolution. Interestingly, 3 (9.1%) patients presented only BIRC3 deletion and 2 (6.1%) cases showed only ATM loss.

The analysis of the other FISH alterations showed that 72.7% of cases had del(13q) (16.7% biallelic), 48.5% exhibited del(17p) and 18.2% showed trisomy 12 (Table 2). When the number of alterations in addition to ATM and BIRC3 lesions was analyzed, we found that only 4 patients (12.1%) had del(11q) alone, 13 (39.4%) showed one additional FISH abnormality, 15 (45.5%) had 2 more FISH alterations and only 1 (3%) showed 3 FISH lesions; as a total, 48.5% (16/33) of cases had ≥2 FISH abnormalities (Figure 2). Furthermore, when the percentage of tumor cells involved in each aberration among the patients with more than one alteration was evaluated, there was a wide variation in the clone size indicating the presence of at least two different cytogenetic clones, reflecting intratumor heterogeneity, defined as the variable expression of different markers within the same tumor [22]. As reference, we considered the presence of two clones when
the difference between probes was more than 30% [23]. This situation was observed in 54.5% (18/33) of cases with del(11q) (Table 2), value significantly higher than those found in the remaining patients of our series (32.4%) (p=0.03) (data not shown), supporting the association of this subgroup with genomic instability.

**Analysis of prognostic factors**

IGHV rearrangements were evaluated in 22 patients, 72.7% of them showed U-IGHV, percentage significantly different with respect to our complete published series of CLL patients that showed 42% U-IGHV cases (p=0.009) [24]. No particular involvement of VH family or IGHV gene rearrangement was observed. Case 29 of Table 2 with M-IGHV expressing the IGHV3-21*01 gene rearrangement showed stereotyped subset #2, which is associated to short TTFT and the presence of 11q and 13q deletions, like our patient [25, 26].

As previously mentioned, for the analysis of clinical characteristics, our cohort of CLL patients with del(11q) was compared to cases without cytogenetics and FISH alterations and patients with del(13q) as the only abnormality (Table 1). Patients with del(11q) showed significant increase of advanced Rai stages (p=0.005), higher white blood cell count (p=0.04) and Beta2 microglobulin levels (p=0.034) compared to the other groups. Furthermore, a short TTFT in cases with del(11q) with respect to cases with del(13q) single and those without alterations was found (p<0.0001) (Figure 3).

**Discussion**

In this study, we have evaluated *BIRC3* and *ATM* deletions in CLL patients in order to analyze their frequency and association with prognostic factors of the disease. *BIRC3* gene belongs to the IAP (*Inhibitor of apoptosis*) family; it is mainly expressed in lymphoid tissue, particularly in spleen and peripheral blood lymphocytes. It is a negative regulator of the non-canonical NF-kB (*Nuclear factor kappa B*) signaling pathway [27, 28]. BIRC3 protein cooperates in the same complex with TRAF2 and TRAF3 (*tumor necrosis factor receptor-associated factors 2 and 3*) that negatively regulates MAP3K14 (*mitogenactivated protein kinase 14*), a central activator of non-canonical NF-kB pathway. Furthermore, it is involved in the maintenance of TP53 levels, through MDM2 (*Mouse Double Minute 2, Human Homolog*) modulation and, its suppression contributes to neoplastic progression [29]. *BIRC3* mutations/deletions are infrequently detected in CLL at diagnosis (4%) but they reached 24% in fludarabine-refractory patients [12, 30]. On the other hand, *ATM* gene, a member of the PI3K (*phosphatidylinositol-3 kinase*) family, has a key role in protect the genome integrity by regulating the cell-cycle, preventing the DNA damage, activating DNA-repair pathways and inducing apoptosis if the DNA damage cannot be repaired [31]. *ATM* deficiency allows the accumulation of genetic alterations related to genomic instability and is associated with a predisposition to lymphoid malignancies [32, 33]. In addition, *ATM* is a p53 regulator and its inactivation is associated to p53 dysfunction [34].

The analysis of our data showed, in agreement with previous results [11, 14, 33, 35], an increased presence of large deletions in 11q22, given that 84.8% of cases had *BIRC3* and *ATM* losses. Among them, 3 patients showed higher frequency of *ATM* deletion than *BIRC3* loss and 7 cases had higher frequency of *BIRC3* deletion than *ATM* loss, indicating the presence of two separated deletion events, resulting in clonal evolution. Alhourani et al [14] also observed a similar finding in a small number of cases. It is also interesting to note the presence of 3 patients with only *BIRC3* deletion and 2 cases with only *ATM* loss. In concordance with our data, Rose-Zerilli et al [13] found one case with *BIRC3* deletion without concomitant loss of *ATM*. These findings show the heterogeneity of del(11q) and suggest the importance to consider the inclusion of the analysis of *BIRC3* deletions in the routine laboratory screening of CLL patients. Besides, our cohort showed a high frequency of CK, confirming previous reports [36, 37] and reflecting the high complexity observed in CLL cases with del(11q) as well as their influence in progression of the disease and poor outcome [19, 35, 38].

Concerning FISH analysis, 48.5% of cases showed two or more abnormalities, being del(13q) the most frequent, followed by del(17p) and trisomy 12. Data of the literature support our findings for del(13q) and trisomy 12 [12, 13, 17, 32], but there are controversial results about del(17p). Some reports showed low frequency of del(17p) in cases with del(11q) [13,
suggesting that both alterations cannot coexist [12, 34]. On the contrary, other authors found association of monoallelic deletions in both genes in 25.5%-43% of cases [15, 17], supporting the co-occurrence of these alterations in CLL patients, which was associated with more aggressive disease and poor outcome [16, 17]. Simultaneously, 54.5% of cases showed intratumor heterogeneity, phenomenon related to genomic instability and disease progression [23, 39]. Although it is not clear the exact mechanism involved in the generation of intratumor heterogeneity, different reports suggest that it could be originated by genetic disorders within the tumor cells and/or under the influence of changes in the tumor microenvironmental conditions [22, 40]. Interestingly, Yi et al [23] found that the integrated analysis of the number of cytogenetic alterations and the intratumor cytogenetic heterogeneity provide a better information for prognostic stratification of CLL patients than the conventional model [3, 4]. More studies will be necessary to confirm these results.

Regarding the association with prognostic factors, our series showed 72.7% of patients with U-IGHV [7, 20, 36], intermediate value between those found in previous studies (68-89%) [17, 33, 41, 42]. Simultaneously, a significantly shorter TTFT in patients with del(11q) compared to cases with del(13q) single and patients without cytogenetic and FISH alterations was observed [3, 4, 42, 43]. Even though the introduction of ibrutinib therapy has improved the clinical outcome of patients with del(11q) [44, 45], recent studies [30, 46] showed the lack of response to ibrutinib but not to venetoclax in CLL cells with BIRC3 disruption, making its detection relevant for treatment decisions. In addition, Asslaber et al [47] found that patients with low BIRC3 expression had rapid disease progression and short TTFT, associated to an altered NK-kB pathway, upregulation of antiapoptotic BCL-2 family members, and high sensitivity to venetoclax treatment in vitro. More recently, different authors reported the presence of BIRC3 gene inactivation (~5% of cases) [48, 49], suggesting that these patients would represent a subgroup with the worst outcome following initial chemotherapy treatment, reinforcing the importance to identify this abnormality in order to refine the risk stratification of CLL patients and highlighting the benefit of novel agents-based therapy.

Our study has some limitations, as the low number of our cohort and the retrospective nature of the analysis. Despite these limitations, our analysis shows concomitant ATM and BIRC3 deletions, with a number of patients with different clonal frequency indicating the presence of clonal evolution, as well as cases with only ATM or BIRC3 deletions, suggesting the importance to consider the inclusion of BIRC3 analysis in the routine FISH laboratory screening of CLL patients. In addition, a strong association between ATM and BIRC3 deletions with TP53 loss, U-IGHV, complex karyotypes, and intratumor heterogeneity was observed, highlighting the increased genomic instability present in this group of patients.

**Declarations**

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**Competing interests**

The authors have non-financial interests to disclosure.

**Author contributions**

All the authors contributed to the study conception and design. Material preparation, laboratory data collection, analysis and interpretation of data were performed by Camila Galvano, Andrea Krzywinski, Carmen Stanganelli and Cecilia Rodríguez. Clinical data collection and follow up of patients were performed by Viviana Heller, Marcela Miodosky, Silvana Cugliari and Raimundo Bezares. The first draft of the manuscript was written by Camila Galvano and Irma Slavutsky and
all authors commented on previous versions of the manuscript. All authors read and approved the final version of the manuscript.

**Data Availability**

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics approval**

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the National Academy of Medicine (May 8, 2019/ № 04202018 IR43). All patients provided their written informed consent.

**Consent to participate**

Informed consent was obtained from all individual participants included in the study.

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Figures

**Figure 1**

Distribution of *BIRC3* and *ATM* deletions in CLL patients.
Figure 2

Distribution of FISH alterations in CLL patients with BIRC3 and ATM deletions.
Figure 3

Time to first treatment (TTFT) in CLL patients with del(11q), del(13q) alone and cases without alterations (w/alt) showing the poor outcome of cases with del(11q) (p<0.0001).