Late appearance of the 11q22.3-23.1 deletion involving the ATM locus in B-cell chronic lymphocytic leukemia and related disorders.

Clinico-biological significance

Background and Objectives. Chromosome 11q22.3-23.1 deletions involving the ataxia-teleangiectasia mutated (ATM) locus (11q-/-ATM +/-) are detected at diagnosis in 10-20% of cases of B-cell chronic lymphocytic leukemia (CLL) and are associated with a relatively aggressive disease. The aim of this study was to ascertain whether 11q-/-ATM +/- may appear late during the course of the disease and to analyze its possible correlation with disease evolution.

Design and Methods. Eighty-two patients with CLL and related disorders, i.e. CLL/PL and prolymphocytic leukemia (PLL), without 11q- at diagnosis were sequentially ascertained at 1-2 year intervals by conventional cytogenetic analysis (CCA) and fluorescence in situ hybridization (FISH), using an ATM-specific probe.

Results. Eight patients acquired a submicroscopic 11q deletion 13-43 months after diagnosis: the diagnosis at presentation was CLL in 3 cases, CLL/PL in 3 cases and PLL in 2 cases. A 13q14 deletion preceeded the development of 11q- in four patients; additional aberrations included +12 (three cases), 17p13 deletion and 6q21 deletion (one case each). The acquisition of the 11q deletion was more frequently found in those patients presenting with CLL/PL and PLL than typical CLL (p=0.0016) and with splenomegaly (p=0.003). Follow-up data showed that karyotype evolution (p=0.009) and cytological transformation (p<0.001) were associated with the acquisition of this cytogenetic lesion. The variables predicting for a shorter survival in this series included the 11q deletion (p=0.03), along with other classical clinicobiological parameters (performance status, advanced stage, splenomegaly, elevated serum β2 microglobulin and lactate dehydrogenase levels.

Interpretation and Conclusions. a) Submicroscopic 11q deletion involving the ATM locus may, in some instances, represent a secondary change in CLL, CLL/P and PLL, suggesting that sequential FISH analyses are necessary to detect this chromosome anomaly in some patients; b) the acquisition of 11q- /ATM deletion may play a role in determining cytological transformation and disease progression of CLL and related disorders.

Key words: 11q deletion, ATM locus, chronic lymphocytic leukemia.

Acquired chromosome deletions involving the ataxia-teleangiectasia mutated (ATM) locus at bands 11q22.3-23.1, have been frequently observed in T-cell prolymphocytic leukemia (PLL); in B-cell chronic lymphocytic leukemia (CLL), in which they identify an aggressive disease having karyotype instability and in mantle cell lymphoma (MCL). Albeit at a lower frequency, other forms of low-grade and high grade B-cell non-Hodgkin's lymphoma (NHL) have also been found to carry a deletion at this locus, the presence of which was associated with an inferior prognosis.

Though chromosome 11q22.3-23.1 deletion is an early cytogenetic event predisposing to or initiating the neoplastic transformation in B-CLL, the following lines of evidence point to a possible role for acquired 11q deletion involving the ATM locus in the progression of some B-lymphoid neoplasias: i) 11q22-23 deletion was reported as a secondary change in NHL; ii) some CLL carried the 11q22-23 deletion in a minority of cells; iii) in BCL1-rearranged MCL, the 11q deletion was
found to be confined to a sideline.9  
To investigate the possible role of 11q deletion involving the ATM locus (11q-/ATM +/−) as a secondary chromosome lesion in B-CLL and related disorders, we studied 82 patients at diagnosis and during the course of their disease by conventional cytogenetic analysis (CCA) and fluorescence in situ hybridization (FISH).

**Design and Methods**

**Patient selection**  
Approximately 200 patients with B-cell CLL, atypical CLL (including CLL/PL and CLL mixed-cell type) and PLL were studied by cytogenetic analysis at the Institute of Hematology, University of Ferrara between 1993 and 2000.

Sequential chromosome analyses and interphase FISH studies using an 11q22-23/ATM probe were performed before therapy and at 1-2 year intervals in those patients fulfilling any of the following requirements: a) they presented with a high white blood cell (WBC) count (WBC > 50,000 × 10⁹/L) or, b) they were diagnosed in advanced stage, i.e. stage II-IV according to Rai13 or stage B/C according to Binet14 or, c) they had a therapy-demanding disease. A total of 98 patients were studied: 40 patients were analyzed on two occasions, 36 on three occasions and 22 on four occasions. Sixteen patients were excluded because they had an 11q- chromosome by cytogenetic/FISH analysis at the first examination; the remaining 82 patients without 11q deletion at diagnosis form the basis of the present report.

**Diagnosis and clinical follow-up**  
Diagnosis was based on the examination of peripheral blood (PB) smears and on the results of immunophenotyping. All cases had either the classical morphology (typical CLL) or they presented 10-55% large lymphocytes or prolymphocytes (atypical CLL, including CLL mixed-cell type and CLL/PL), or they had more than 55% prolymphocytes (B-PLL).15 The presence of more than 40% CD5+/CD19+ lymphocytes was a minimum diagnostic requirement. Other markers, tested in all cases by cytofluorimetric analysis as previously described,16 included the CD23, CD22, CD25, CD11c, CD10 and CD103 antigens, the FM7 monoclonal antibody and the expression of surface immunoglobulins.

All patients underwent a physical examination, chest X-ray film and abdomen ultrasonography as part of the diagnostic work-up. They were followed on an outpatient basis at regular 2-6 month intervals by the following investigations: complete blood count, routine biochemical profile, medical examination and, when indicated, radiological investigations. Histologic studies were performed for diagnostic purposes when the development of Richter’s syndrome was suspected.

Indications for treatment included a rise of the WBC count with a <12 month lymphocyte doubling time (LDT), the development of anemia, neutropenia or thrombocytopenia due to BM infiltration or autoimmune phenomena and disease progression in the Rai/Binet staging system.13,14 The patients were treated according to guidelines in use at our Institution during the study period. Intermittent chlorambucil administration was used as first line therapy in the majority of cases. In young patients with CLL and in those patients presenting with PLL treatment included cyclophosphamide, vincristine, prednisone, with or without an anthracycline drug [C(H)OP regimens]. Fludarabine has been used since 1995 in refractory or relapsing patients and, since 1998, as front-line therapy in selected cases.

**Cytogenetic studies**

Conventional chromosome analysis was performed on representative PB samples at diagnosis and during follow-up in all cases, according to methods previously described.17 The presence of the 11q22-23/ATM deletion was studied in all cases using the λEMBL3 clones 19 and 65, spanning an area of approximately 40 kb within the middle portion of the ATM gene. Because FISH is more sensitive than cytogenetic analysis, 8 patients having the 11q/ATM deletion were studied using the following panel of locus-specific probes: the C21 cosmid, recognizing DNA sequences between the Rb gene and the D13S25 marker at 13q14, isolated as previously described;18 a 6q21 probe prepared by B. Schlegelberger (Institute for Human Genetics, Kiel, Germany);19 a 17p13.3 cosmid recognizing DNA sequences between the Rb gene and the D13S25 marker at 13q14, isolated as previously described;18 a 6q21 probe prepared by B. Schlegelberger (Institute for Human Genetics, Kiel, Germany);19 a 17p13.3 cosmid recognizing p53 gene sequences, made available by F. Birg (Institut de Cancérologie e d’Immunologie de Marseille, INSERM 119, Marseille, France) in the...
context of the Biomed I program, E.U. concerted action for cytogenetic diagnosis of hematologic malignancies (project leader: A. Hagemeijer, Centre for Human Genetic, K.U.L., Leuven, Belgium). A chromosome-12-specific centromeric probe was purchased (Oncor, Gaithersburg, MD, USA) to study trisomy 12 in interphase cells.

**Hybridization and signal screening.** The hybridization protocol was described in detail in previous studies. Co-hybridization of the test probe with a control probe was performed by dual color FISH. To prevent false-positive results due to inefficient hybridization, signal screening was performed on slides with a high hybridization efficiency, having >80% interphase cells showing two signals with the control probe. The evaluation was performed using a fluorescence microscope (Nikon Italia, Florence, Italy); 200 cells with well-delineated signals were observed and images were captured with a couplecharged camera device (Cytovision, Nikon Italy).

Using 5 normal control samples, less than 1% of cells showed 3 signals (false trisomy or false rearrangement) and less than 5% of cells showed 1 signal (false deletion) with each probe. Because CLL cells may undergo apoptosis in vitro and consequently false positive results may be obtained by interphase FISH due to DNA fragmentation, probes detecting chromosome regions usually not involved in CLL (i.e. 5q31 and 7q31) were tested in 5 CLL samples. Less than 6% of cells showed 1 signal (false deletion) and less that 2% of cells showed 3 signals (false trisomy): the cut-off point for the recognition of trisomy and deletion was therefore set at 3% and 12%, respectively.

**Statistical analysis**

The chi-squared test was applied for categorical variables. Patient survival was estimated by the Kaplan-Meier method from the date of diagnosis until death due to any cause or until the patient’s last follow-up. The survival curves were statistically compared by the log-rank test. A p value of 0.05 was used as the criterion for statistical significance. Proportional hazards regression analysis was used to identify the most significant independent prognostic variables on survival and p values of less than 0.05 were considered statistically significant.

**Results**

**Cytogenetic and FISH studies**

The outcome of cytogenetic investigations in 82 patients at diagnosis is shown in Table 1. Five patients with the t(11; 14)(q13; q32) were included because they fulfilled the FAB criteria for the diagnosis of CLL/PL (4 cases with 10-55% prolymphocytes in the peripheral blood smear) and of PLL (1 case with >55% prolymphocytes in the PB). In all cases the phenotypic score according to Matutes et al. was 3 or more points (1 point for each of the following: CD5+, CD23+, CD22+weak, sIg+weak, FMC7-).

Eight patients were found to have acquired an 11q22-23 deletion 20-43 months after diagnosis. The karyotype and the results of FISH studies in these patients are presented in Table 2. A change of the karyotype concomitant with the appearance of the 11q- was observed in four patients (cytogenetic evolution with additional aberrations in case #2 and appearance of an abnormal clone in cases #1, 7 and 8, which had previously had a normal karyotype).

**Hematologic features**

Fifty-eight patients presented with CLL, 21 were classified as having atypical CLL according to the FAB criteria (i.e. they showed 10% to 55% large lymphocytes or prolymphocytes in the PB smear), 3 had prolymphocytic leukemia (PLL).

Seventy-six patients presented in Rai stages 0-II, 6 presented in stages III-IV. The 3 patients with PLL presented with marked lymphocytosis (26-120×10⁹/L) and splenomegaly. The salient hematologic features at diagnosis in the two groups, i.e. those developing the 11q22-23/ATM deletion and those without 11q deletion are summarized in Table 3. There was not a correlation between the administration of chemotherapy and the development of the 11q22-23/ATM deletion and the presence of splenomegaly.

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**Table 1. Outcome of cytogenetic investigations at diagnosis in 82 patients with B-CLL and related disorders.**

<table>
<thead>
<tr>
<th>Anomaly</th>
<th>No. of cases</th>
<th>Cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. typical / atypical or PLL</td>
</tr>
<tr>
<td>+12</td>
<td>9</td>
<td>6/3</td>
</tr>
<tr>
<td>13q-</td>
<td>7</td>
<td>5/2</td>
</tr>
<tr>
<td>t(11; 14)(q13; q32)</td>
<td>5</td>
<td>0/5</td>
</tr>
<tr>
<td>6q-</td>
<td>4</td>
<td>2/2</td>
</tr>
<tr>
<td>17p-</td>
<td>4</td>
<td>1/3</td>
</tr>
<tr>
<td>Other abnormal</td>
<td>14</td>
<td>11/3</td>
</tr>
<tr>
<td>Normal</td>
<td>25</td>
<td>22/3</td>
</tr>
<tr>
<td>No mitosis</td>
<td>14</td>
<td>11/3</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>58/24</td>
</tr>
</tbody>
</table>

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and the CLL/PL or PLL variant were the only hematologic parameters at diagnosis that were associated with the probability of developing the 11q deletion. Analysis of follow-up data showed that the occurrence of cytological transformation, karyotype evolution and an unfavorable outcome were observed more frequently in the 11q- group.

The variables having an impact on survival in this series included performance status, stage at presentation, the presence of splenomegaly, elevated serum β2 microglobulin and lactate dehydrogenase (LDH) levels, and the development of the 11q deletion (Table 4).

At multivariate analysis the following parameters maintained their prognostic significance: LDH (p=0.007), ECOG (p=0.03) and serum β2 microglobulin (p=0.035).

### Discussion

Preliminary methodologic problems in this study were patient selection and identification of criteria defining disease evolution and transformation. Because the median follow-up in our series was relatively short compared to the natural history of stable/early stage CLL, only those patients with a relatively aggressive disease were included in this analysis. In addition, we elected to include CLL-related disorders, such as atypical CLL and PLL, because their distinction from CLL is not clear-cut in some instances, the more so that transition of CLL into cytologically more aggressive forms can be observed in up to 10-15% of the cases. Although the WHO proposal for classification of lymphoid neoplasias recognizes PLL as a distinct entity, it is likely that this condition may be heterogeneous,

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**Table 2. Outcome of cytogenetic and FISH studies in 8 patients developing 11q22-23/ATM deletion.**

<table>
<thead>
<tr>
<th>Patient/ diagnosis</th>
<th>Date</th>
<th>Therapy (*)</th>
<th>Immunophenotype</th>
<th>Karyotype</th>
<th>FISH studies (*)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient/ diagnosis</strong></td>
<td><strong>Date</strong></td>
<td><strong>Therapy (*)</strong></td>
<td><strong>Immunophenotype</strong></td>
<td><strong>Karyotype</strong></td>
<td><strong>FISH studies (*)</strong></td>
</tr>
<tr>
<td>1.AGC</td>
<td>12/94</td>
<td>CLB (5)</td>
<td>CD5/CD19+, CD23+, sIg+, CD10-</td>
<td>46,XY</td>
<td>75%</td>
</tr>
<tr>
<td>CLL/PL</td>
<td>7/98</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.BR</td>
<td>11/94</td>
<td>CLB (8)</td>
<td>CD5/CD19+, CD23+, sIg+, CD10-</td>
<td>47, idem, del(6)(q22), +7, del(18)(p11)</td>
<td>36%</td>
</tr>
<tr>
<td>3.PM</td>
<td>10/94</td>
<td>CLB (6); COP (6)</td>
<td>CD5/CD19+, CD23+, sIg+, CD10-</td>
<td>46,XX, t(11;14)(q13; q22), del(7)(q11)</td>
<td>75%</td>
</tr>
<tr>
<td>PLL</td>
<td>6/97</td>
<td>FMC7-, CD10-</td>
<td>47, idem, del(6)(q23), del(7)(q11)</td>
<td>70%</td>
<td></td>
</tr>
<tr>
<td>4.RO</td>
<td>7/93</td>
<td>CLB (4)</td>
<td>CD19/CD5+, CD23+, CD22+, sIg+, CD10-</td>
<td>46,XX, t(11;14)(q13; q22), del(7)(q11)</td>
<td>84%</td>
</tr>
<tr>
<td>PLL</td>
<td>10/95</td>
<td>FMC7+, CD10-</td>
<td>47, idem, del(6)(q23), del(7)(q11)</td>
<td>81%</td>
<td></td>
</tr>
<tr>
<td><strong>Patient/ diagnosis</strong></td>
<td><strong>Date</strong></td>
<td><strong>Therapy (*)</strong></td>
<td><strong>Immunophenotype</strong></td>
<td><strong>Karyotype</strong></td>
<td><strong>FISH studies (*)</strong></td>
</tr>
<tr>
<td>5.AZ</td>
<td>10-94</td>
<td>None</td>
<td>CD5/CD19+, CD23+, sIg+, CD10-</td>
<td>46,XX</td>
<td>-</td>
</tr>
<tr>
<td>CLL</td>
<td>11-96</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.BA</td>
<td>2-94</td>
<td>CHOP (6)</td>
<td>CD5/CD19+, CD23+, CD22+, sIg+, CD10-</td>
<td>47,XY, +3, +6 add(11)(q22),</td>
<td>85%</td>
</tr>
<tr>
<td>PLL</td>
<td>11-96</td>
<td>FMC7</td>
<td>CD5/CD19+, CD23+, CD22+, sIg+, CD10-</td>
<td>47,XY, +3, add(11)(q22), +6 add(11)(q22)</td>
<td>80%</td>
</tr>
<tr>
<td>7.FO</td>
<td>1-98</td>
<td>COP (6)</td>
<td>CD5/CD19+, CD23+, CD22+, sIg+, CD10-</td>
<td>46,XX, t(1; 20)</td>
<td>84%</td>
</tr>
<tr>
<td>PLL</td>
<td>8-99</td>
<td>fludarabine (6)</td>
<td>CD5/CD19+, CD23+, CD22+, sIg+, CD10-</td>
<td>46,XX, t(1; 20)</td>
<td>85%</td>
</tr>
<tr>
<td>8.RC</td>
<td>1-93</td>
<td>CLB (4); CHOP (6)</td>
<td>CD5/CD19+, CD23+, CD22+, sIg+, CD10-</td>
<td>46,XX, +6 add(20)(q12)</td>
<td>85%</td>
</tr>
<tr>
<td>PLL</td>
<td>9-94</td>
<td>FMC7</td>
<td>CD5/CD19+, CD23+, CD22+, sIg+, CD10-</td>
<td>47,XY, +6 add(20)(q12)</td>
<td>85%</td>
</tr>
</tbody>
</table>

*Therapy administered before the acquisition of the 11q-. (No. of courses in parentheses); CLB: chlorambucil, CHOP: cyclophosphamide, (anthracycline) vincristine and prednisone. *Percentage of interphase cells with deletion/trisomy (200 cells counted). - indicates the absence of the anomaly. *No evidence of BCL1 rearrangement by interphase FISH (data not shown).
some cases representing progression of pre-existing CLL/PL or other types of low grade non-Hodgkin’s lymphoma (NHL). Unlike NHL, sequential lymph node biopsies are not performed in CLL at relapse or evolution, the differential count of PB cells along with clinical follow-up representing useful parameters to detect disease transformation.

The late appearance of the 11q-/ATM+/- was detected by interphase FISH analysis in 8 out of 82 patients with CLL and related disorders followed for a 3-7 year period. In 6 of these patients, clonal aberrations involving other chromosomes were detected by conventional karyotyping, suggesting that the size of the 11q deletion was beyond the resolution power of banding analysis. A similar explanation or, alternatively, a low mitotic index may account for the failure of conventional cytogenetics to detect the 11q deletion in two cases with normal karyotype and with no analyzable metaphases (#4 and 5 in Table 2).

The acquisition of 11q-/ATM+/- was more frequently found in those patients diagnosed with CLL/PL and PLL (5 cases) than in typical CLL (3 cases); indeed the 11q- anomaly was detected in 3 out of 21 cases of CLL/PL 13-32 months after diagnosis, in 2/3 PLL after 20 and 31 months and 3/58 typical CLL after 19-43 months.

The first consideration stemming from these data is that submicroscopic 11q deletions involving the ATM locus may develop in CLL and related disorders not only early in the natural history of the abnormal clone, but during the course of the disease as well. This may have practical implications in two settings: a) typical CLL, especially in young patients who may require sequential molecular cytogenetic investigations for the detection of the late-appearing 11q- chromosome, this anomaly having been convincingly associated with poor prognosis, b) the CLL/PL variant, which shows an heterogeneous clinical course sometimes marked by abrupt progression, possibly associated with the development of specific chromosome lesions.

There are several observations scattered in the literature that concur with our findings in suggesting that the 11q- chromosome may represent a secondary event in some patients with B-cell chronic lymphoproliferative disorders: i) one patient with +12 at presentation was found to have acquired an 11q deletion 3 years after diagnosis in a study by Oscier et al. who analyzed cytogenetic data in 18 cases of CLL with karyotype evolution; ii) some patients with 11q- and cytogenetically unrelated clones have been observed in CLL, iii) only a minority of clonal B-cells were found to carry 11q-/ATM+/- in some cases of CLL, 12, 27 iv) 11q22-23 deletions were found in a minority of neoplastic cells in PLL. The finding of a 13q14 deletion preceding the development of the 11q-/ATM+/- in one patient (#1) with typical CLL that evolved into PLL provides further evidence for the role of 11q22-23 deletion in the progression of this lymphoproliferative disorder.
Interestingly, all three CLL patients showed deterioration of their clinical picture concomitant with the acquisition of the 11q deletion, due to disease progression with hyperleukocytosis and/or adenopathies, which proved resistant to alkylating agents in all cases and to fludarabine in one case.

The second aspect in this study deserving attention is the preferential association of the 11q- chromosome with CLL/PL and PLL. Whereas in the majority of cases of typical CLL, the presence of the 11q-/+ATM/+ is readily detected by interphase FISH at presentation (12), we found that a significant fraction (35.7%) of CLL/PL and PLL, studied sequentially in this study, acquired the 11q- anomaly as a secondary chromosome lesion. All three cases of CLL/PL with 11q- showed cytological transformation into a more aggressive disease, along with the recent demonstration of its secondary nature in aggressive mantle cell lymphomas9 argue in favor of a role for this chromosome lesion in the progression of CD5+ B-lymphoid neoplasia. The 11q-/+ATM/+ adds to a list of cytogenetic factors, including the 17p-/p53 deletion, the 9p21/p16 deletion,29-35 which notoriously play a role in histologic/clinical progression of low-to-intermediate grade lymphoid neoplasias.

Though the characteristics of this series (sample size, duration of follow up and heterogeneity of patient population and treatment) make the assessment of the prognostic correlations difficult, it is noteworthy that established prognostic variables (Table 4) were found to predict, as expected, a shorter survival. Thus, the finding that those patients who developed the 11q- as a secondary aberration had a worse prognosis may support the argument that this chromosome lesion identifies a subset of patients with CLL, CLL/PL and PLL having a more aggressive disease and possibly deserving innovative treatment.

The late appearance of the 11q22.3-23.1 deletion involving the ATM locus may represent a previously unrecognized cytogenetic event playing a role in the progression or transformation of a distinct minority of CLL and related disorders.

### Contributions and Acknowledgments

AC, RB GM R, MN and GC designed the study and drafted the article; RB, M GR, AB, FC, RM, CM, AT, MD, PA, and ET performed and interpreted the hematologic, cytogenetic and FISH studies; all authors discussed the significance of the experimental data, revised the manuscript for important intellectual content and approved the final version.

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### Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.
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**Late-appearing 11q- in CLL**

**PEER REVIEW OUTCOMES**

**What is already known on this topic**

Deletions of chromosome 11q23 involving the ataxia telangiectasia mutated gene are found in a variety of lymphoid disorders and in up to 20% of CLL cases where it has been shown to have a prognostic impact.

**What this study adds**

11q23 deletions may be acquired during the disease course in a few CLL cases and is associated with morphological transformation. This implies that at least in some cases 11q23 deletion is a secondary change.

**Manuscript processing**

This manuscript was peer-reviewed by two external referees and by Dr. Estella Matutes, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Matutes and the Editors. Manuscript received July 11, 2001; accepted October 6, 2001.

**Potential implications for clinical practice**

Sequential FISH studies investigating 11q23 deletion may be of value to detect the acquisition of this abnormality with prognostic implications in CLL.

Estella Matutes, Associate Editor