Chromosome 6 Abnormalities Associated with Prolymphocytic Acceleration in Chronic Lymphocytic Leukemia*†

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ABSTRACT

Chronic lymphocytic leukemia (CLL) is most characteristically associated with the cytogenetic abnormalities +12, 13q14, and 14q32. Recently abnormalities of chromosome 6 have been reported in patients with mantle zone lymphoma, CLL mixed type, and a CLL variant with larger prolymphocytoid cells in the peripheral blood. The purpose of this study was to review the cases of CLL karyotyped at the University of Texas M. D. Anderson Cancer Center (UTMDACC) and to determine the number and type of chromosome 6 abnormalities. Precisely 830 cases of CLL with karyotypes were reviewed. Among these, 257/830 (31 percent) had abnormal karyotypes, 56/257 (22 percent) had an abnormality of 6, 18/56 (32 percent) had translocations involving 6 and, in most instances, a different chromosome was involved, 37/56 (66 percent) had deletion 6 or loss of at least a portion of 6q, and 9/56 (16 percent) had an abnormality of 6p. The losses of 6q were in the q13 to q25 regions. Of these, 13/56 (23.2 percent) of patients with 6q abnormalities had ≥10 percent prolymphocytes (PL) in the bone marrow (BM) and/or peripheral blood (PB), 10/56 (17.9 percent) had ≥10 percent PL in the bone marrow, 8/56 (14.3 percent) had ≥10 percent PL in the peripheral blood, and 5/56 (9 percent) had ≥10 percent PL in both (see table I). The 201 CLL patients with chromosome abnormalities other than 6 contained 23 with excess PL (11.9 percent). A subset of karyotypic changes of 6 associated with increased PL is recognizable and may be useful in aiding in clinical diagnosis and therapy.

Introduction

There are a variety of lymphoid diseases that are derived from either B-cell or T-cell origin.

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B-lymphocytes. B-cell CLL is an extremely variable disease with a wide range of morphologic appearances and clinical outcomes further characterized by a prevalence of morphologically mature and biologically immature B-lymphocytes. B-cell CLL can also be characterized by clonal proliferation, which is indicated by the presence of the CD5 antigen.

Through clonal evolution, an expansion or proliferation of the B-cells, along with prolymphocytic transformation (>30 percent PL in peripheral blood) can eventually lead to a probable diagnosis of prolymphocytic leukemia (PLL). As reported by Juliusson and et al in 1990, the cytogenetic hallmark of CLL is trisomy 12 with other frequent aberrations found involving 13q14 and 14q32. Over the period of approximately 7 years, over 800 CLL patients were karyotyped at M. D. Anderson Cancer Center (MDACC). This present study was designed to investigate a subgroup of CLL patients with abnormalities of chromosome 6 together with prolymphocytes >10 percent in the bone marrow and or the peripheral blood.

Methods

Precisely 830 patients with CLL diagnosed by clinical and laboratory parameters were entered into a databank and evaluated. These patients were selected from the cytogenetic cases that were analyzed between 1990 and 1996 in the Cytogenetics Laboratory, Department of Laboratory Medicine at the University of Texas M. D. Anderson Cancer Center (UTMDACC) in Houston, TX. Parameters that were catalogued in our database were age, sex, race, karyotype, percent prolymphocytes in the bone marrow, and percent prolymphocytes in the peripheral blood.

Routine cytogenetic studies were performed on bone marrow specimens. These bone marrow specimens were processed either with a standard 24-hour incubation (unstimulated culture) or with a 72-hour incubation in media supplemented with a B-cell mitogen, lipopolysaccharide (LPS), 0.1 mg/dl (stimulated culture). The standard Giemsa and trypsin G-banding technique by Seabright was used for cytogenetic analysis.

Interest was focused on chromosome 6 as a possible marker for prolymphocytic expansion and/or transformation in CLL. Information regarding the percent of prolymphocytes was obtained from differentials performed on the same bone marrow specimens and peripheral blood which were sent for cytogenetic evaluation. Our experimental hypothesis (Hx) was tested against the null hypothesis (Ho) using the X² test for goodness of fit, in order to determine whether or not chromosome 6 abnormalities were statistically significantly associated with >10 percent prolymphocytes in the bone marrow or peripheral blood lymphocytes of CLL patients.

Results

Of 830 patients with the diagnosis of CLL, 257 had an abnormal karyotype. Exactly 56 of the 257 had a chromosome 6 abnormality; 13 of the 56 (23.2 percent) with a chromosome 6 abnormality had a prolymphocyte (PL) count of >10 percent in either the peripheral blood (PB) or bone marrow (BM) (table I). The CLL patients with karyotypic abnormalities tended to be older, more frequently male, and were significantly (p > 0.05 by the Chi-squared Goodness of Fit) more likely to have PL > 10 percent (Table I). Analysis of the karyotype revealed that the most frequent region for breaks associated with the chromosome 6 abnormalities was between 6q21 and 6q23 (figure 1). Other abnormalities associated with chromosome 6 and >30 percent PL included chromosomes 7q, 8q, 9q, -10, 14q and 17 (table II).

Discussion

The patient population of the MDACC clinical cytogenetic laboratory consists of 830 individuals seen over a period of 7 years who were initially diagnosed with CLL. Our population tends to fit that of other studies, with an
average age of 59 years, and approximately a 2:1 sex ratio of males to females. Racial distribution of the patients in this study indicates: Caucasian (89 percent), Black (5 percent), Hispanic (5 percent) and other (1 percent) (table III). This parallels the demographics of all admissions at MDACC with various diagnosed malignancies. Within this study group of 830 patients initially diagnosed with CLL, 573 of them presented with normal karyotypes upon analysis and 257 with abnormal karyotypes. It is unknown what percentage of patients with abnormal karyotypes received treatment prior to their admission to MDACC. Fully 257 of the 830 member population (31 percent) had chromosomal abnormalities, whereas in previous reports the average percentage for abnormal karyotypic presentation among a CLL population is 50 percent.1,2,4 Until the mid 1990s, MDACC was primarily a referral institution. Therefore, the lower percentage of patients with abnormal karyotypes may reflect prior treatment.

Fifty percent of all diagnosed CLL patients will eventually develop chromosome abnormalities. It is believed that these chromosome

### TABLE I
Prolymphocytic Expansion in Patients with Chronic Lymphocytic Leukemia Karyotyped at U. Texas M.D. Anderson from 1990–1996

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Number of Patients</th>
<th>PL &gt; 10% BM or PB</th>
<th>PL &gt; 10% in BM</th>
<th>PL &gt; 10% in PB</th>
<th>PL &gt; 10% in BM &amp; PB</th>
</tr>
</thead>
<tbody>
<tr>
<td>All CLL</td>
<td>830</td>
<td>45 (5.4%)</td>
<td>35 (4.2%)</td>
<td>26 (3.2%)</td>
<td>16 (1.9%)</td>
</tr>
<tr>
<td>CLL with chr 6 abnormality</td>
<td>56</td>
<td>13 (23.2%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10 (17.9%)</td>
<td>8 (14.3%)</td>
<td>5 (9.0%)</td>
</tr>
</tbody>
</table>

<sup>a</sup>p < 0.05 using Chi square goodness of fit.

CLL = chronic lymphocytic leukemia.

PL = prolymphocytes.

BM = peripheral blood.

### TABLE II
Chromosome Abnormalities Associated with Prolymphocytic Transformation in Chronic Lymphocytic Leukemia (> 30% PL)

<table>
<thead>
<tr>
<th>Chromosome Region</th>
<th>Number Observed in Patients with Chromosome 6 Abnormalities</th>
<th>Number Observed in All CLL/PL with Chromosome Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>6q21–qter</td>
<td>5/6 (83%)</td>
<td>5/16 (31%)</td>
</tr>
<tr>
<td>del(7)(q31–qter)</td>
<td>4/5 (67%)</td>
<td>4/16 (25%)</td>
</tr>
<tr>
<td>8q24</td>
<td>2/6 (33%)</td>
<td>5/16 (31%)</td>
</tr>
<tr>
<td>9q22–qter</td>
<td>2/6 (33%)</td>
<td>3/16 (19%)</td>
</tr>
<tr>
<td>−10</td>
<td>3/6 (50%)</td>
<td>4/16 (25%)</td>
</tr>
<tr>
<td>14q32</td>
<td>2/6 (33%)</td>
<td>5/16 (31%)</td>
</tr>
<tr>
<td>del(17p)/t(17p)/−17</td>
<td>4/6 (67%)</td>
<td>8/16 (50%)</td>
</tr>
</tbody>
</table>

CLL = chronic lymphocytic leukemia.

PL = prolymphocytes.
## TABLE III
Demographic Information on Chronic Lymphocytic Leukemia Patients Karyotyped at U. Texas M.D. Anderson from 1990–1996

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Number of Patients</th>
<th>Age Range Avg</th>
<th>Sex %M %F</th>
<th>Race %W %B %H % Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>All CLL</td>
<td>830</td>
<td>25–91 59</td>
<td>66 34</td>
<td>89 5 5 1</td>
</tr>
<tr>
<td>CLL with abnormal karyotype</td>
<td>257</td>
<td>25–91 67</td>
<td>72 28</td>
<td>90 5 4 1</td>
</tr>
<tr>
<td>CLL with chr. 6 abnormality</td>
<td>56</td>
<td>45–75 63</td>
<td>80 20</td>
<td>86 5 5 4</td>
</tr>
<tr>
<td>CLL with &gt;10% PL</td>
<td>45</td>
<td>42–76 62</td>
<td>78 22</td>
<td>87 2 2 7</td>
</tr>
</tbody>
</table>

CLL = chronic lymphocytic leukemia.
W = white.
B = black.
PL = prolymphocytes
H = hispanic.

![Figure 1](image-url)  
**Figure 1.** Patients with chromosome 6 abnormalities. The dots indicate translocation breakpoints and the lines represent regional or complete deletions of chromosome 6. The white dots and lines (○, ---) are abnormalities from patients with prolymphocytic expansion, while the solid dots and lines (●, —) are abnormalities from patients without >10 percent prolymphocytic expansion in either the bone marrow or the peripheral blood. Dotted line (-----) indicates one patient with extra copies of chromosome 6.
abnormalities play a key role in the malignant transformation and progression to more aggressive leukemias from CLL. If the patients with normal karyotypes were followed over time, and subsequent specimen dates utilized, it is probable that our percentage of abnormal vs. normal karyotypes would fall closer to the average, and a pattern of clonal evolution would be established.

Kay et al³ have demonstrated that chromosomal abnormalities present upon initial diagnosis indicates a more progressive disease stage; the more complex the abnormality, the more advanced the disease. Normal karyotypes, on the other hand, could be indicative of early or more indolent disease stages. Current treatment protocols could be utilized to prevent the karyotypic evolution that is considered to indicate a more aggressive disease.

This study focused on 56 of the 257 patients who presented with chromosome 6 abnormalities upon first specimen analysis. In our sample, 13 of 56 cases (23.2 percent) showed increased prolymphocytes (>10 percent) in conjunction with a chromosome 6 abnormality (table I). Sixty-six percent of our patients had a 6 deletion or at least a loss of a portion of 6q. Eleven of 13 cases (85 percent) of CLL with increased PL showed a common region of deletion which falls between 6q21 and 6q23.

In order to determine the critical region for this study, observed aberrations were mapped against an ideogram of chromosome 6. Translocations and deletions are represented, as well as whether or not the chromosome 6 event was found in conjunction with increased prolymphocytes (>10 percent) (figure 1).

Another study (Ofïit et al)⁸ using a much smaller population base concluded that the deletion of the genes at the 6q21-23 region could be representative of a subset of B-cell lymphoma that has increased prolymphocytes in the peripheral blood and the bone marrow. Those authors classify their population as being that of a non-Hodgkins lymphoma. This is the same critical region which was reported in the CLL patients of this study.

Nonrandom abnormalities of chromosomes other than chromosome 6 were observed in the patients who had >10 percent PL (chromosomes 7, 8, 9, 11, 12, 13, 14, and 17). The chromosome changes in those CLL patients with prolymphocytic transformation (≥30 percent) were also nonrandom but showed some different chromosomes. The chromosome regions involved in structural and numerical abnormalities include 6q21–q23, del(7)(q31-qter), 8q24, 9q22-qter, -10, 14q32, and del(17p)/t(17p)/−17 (table II). Our results indicate that while chromosome 6 abnormalities play a key role in the prolymphocytic expansion and/or transformation of patients with CLL, there is the suggestion that other chromosome changes are involved in this prolymphocytic expansion/transformation or leukemic acceleration process.

Conclusion

Chromosome 6 abnormalities are associated with prolymphocytes ≥ 10 percent (p < 0.05) in patients with CLL. The critical region for excess (≥10 percent) PL in CLL based on chromosome banding analysis is 6q21–q23 in this study. Eighty-five percent of patients in this group have abnormalities involving this 6q21–q23 region. Chromosomes 6, 7, 8, 9, 10, 14, and 17 are nonrandomly rearranged in karyotypes of patients with prolymphocytic transformation (≥30 percent PL). All patients with prolymphocytic transformation (≥30 percent PL) and chromosome 6 abnormalities also have either del(7)(q31-qter) or −17/del(17p)/t(17p) as an additional chromosome change.

Acknowledgments

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References