Cytogenetically Different Leukemic Clones at Relapse of Childhood Acute Lymphoblastic Leukemia

By Susana C. Raimondi, Ching-Hon Pui, David R. Head, Gaston K. Rivera, and Frederick G. Behm

Sequential analysis of blast cell chromosomes in 98 cases of acute lymphoblastic leukemia (ALL) disclosed entirely different karyotypes for nine patients at the time of relapse. The presenting clinical, immunophenotypic, and cytogenetic features of this subgroup were similar to those of the 89 patients without major karyotypic shifts. The median length of initial remissions in these nine patients, all of whom received intensive multiagent therapy, was 24 months (range, 6 to 35); responses to subsequent treatment have been uniformly poor. Prominent cytogenetic changes included a gain of modal chromosome numbers in five cases, a loss of chromosomes in two, and the acquisition of an 11q23 rearrangement in three. We propose several different mechanisms to account for these findings. In one, the presence of an entirely different ALL karyotype at relapse may represent induction of secondary leukemia analogous to the well-described entity of epipodophyllotoxin-related secondary acute myeloid leukemia (AML).

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**Table 1. Pertinent Findings in ALL With a Different Karyotype at Relapse**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>WBC Count (×10^9/L)</th>
<th>Age (yr)/Sex</th>
<th>Prior Chemotherapy</th>
<th>Initial Remission Duration (mo)</th>
<th>Immunophenotype</th>
<th>Markers at Relapse</th>
<th>Karyotype</th>
<th>Karyotype At Diagnosis</th>
<th>Karyotype At Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.1</td>
<td>5.7/F</td>
<td>PDN, VCR, DNR, ASP, 6MP, MTX, VM-26, CYT, VP-16, CTX</td>
<td>14</td>
<td>Pre-B</td>
<td>Early pre-B</td>
<td>clg</td>
<td>None</td>
<td>47,XX,+X, t(20;?)(q13;?), t(22;?)(p13;?)*</td>
</tr>
<tr>
<td>2</td>
<td>39.0</td>
<td>2.2/M</td>
<td>PDN, VCR, DNR, ASP, 6MP, MTX, VM-26, CYT, VP-16, CTX</td>
<td>32</td>
<td>Pre-B</td>
<td>Early pre-B</td>
<td>clg</td>
<td>CD33, CD13, CD11b</td>
<td>48,XY,+19,+22</td>
</tr>
<tr>
<td>3</td>
<td>1.7</td>
<td>10.5/M</td>
<td>PDN, VCR, ASP, 6MP, MTX, VM-26, CYT, VP-16, CTX</td>
<td>23</td>
<td>Pre-B</td>
<td>Transitional pre-B</td>
<td>None</td>
<td>TdT, slg</td>
<td>48,XY,+X,+18*</td>
</tr>
<tr>
<td>4</td>
<td>39.0</td>
<td>2.8/F</td>
<td>PDN, VCR, ASP, 6MP, MTX, VM-26, CYT</td>
<td>24</td>
<td>Common ALL</td>
<td>1st relapse:</td>
<td>CD10</td>
<td>None</td>
<td>45,XX, t(2;16)(p13;p13), t(4;11)(q21;q23)*</td>
</tr>
<tr>
<td>5</td>
<td>33.6</td>
<td>2.2/M</td>
<td>PDN, VCR, ASP, 6MP, MTX, VM-26, CYT</td>
<td>15</td>
<td>Early pre-B</td>
<td>Early pre-B</td>
<td>CD10</td>
<td>None</td>
<td>53,XY,+X,+6,+9,+15, +17,+21,+mar*</td>
</tr>
<tr>
<td>6</td>
<td>459.0</td>
<td>1.7/M</td>
<td>PDN, VCR, DNR ASP 6MP, MTX, VM-26, CYT, VP-16, CTX</td>
<td>6</td>
<td>T-cell</td>
<td>T-cell</td>
<td>CD34</td>
<td>CD8, CD11b</td>
<td>46,XY,del(6)(q21q23)*</td>
</tr>
<tr>
<td>7</td>
<td>207.0</td>
<td>12.2/M</td>
<td>PDN, VCR, ASP, 6MP, MTX, VM-26, CYT, VP-16, CTX, DOX, 6MP, MTX, VM-26, CYT</td>
<td>35</td>
<td>T-cell</td>
<td>T-cell</td>
<td>CD2</td>
<td>TdT, CD56</td>
<td>46,XY, inv(14)(q11q31)*</td>
</tr>
<tr>
<td>8</td>
<td>8.2</td>
<td>3.3/M</td>
<td>PDN, VCR, ASP, CTX, DOX, 6MP, MTX, VM-26, CYT</td>
<td>32</td>
<td>Early pre-B</td>
<td>Early pre-B</td>
<td>None</td>
<td>None</td>
<td>45,XY, t(1;9)(p21;p21), t(13;?)(q34;?), del(15)(q22), t(13;?) (t13;?)</td>
</tr>
<tr>
<td>9</td>
<td>26.3</td>
<td>5.8/M</td>
<td>PDN, VCR, DNR ASP, 6MP, MTX, VM-26, CYT, 6MP, MTX</td>
<td>35</td>
<td>Early pre-B</td>
<td>Early pre-B</td>
<td>None</td>
<td>None</td>
<td>46,XY, t(9;13)(p13-p21;q14-q21)*</td>
</tr>
</tbody>
</table>

Abbreviations: PDN, prednisone; VCR, vincristine; ASP, asparaginase; CTX, cyclophosphamide; DOX, doxorubicin, 6MP, 6-mercaptopurine; MTX, methotrexate; VM-26, teniposide; CYT, cytarabine; VF-16, etoposide; DNR, daunorubicin.

* Karyotype previously reported.

* clg not tested at diagnosis.
count of \(33.6 \times 10^9/L\) at diagnosis, not significantly different from findings in the group with similar karyotypic clones at diagnosis and relapse (analysis not shown). The leukemic cell morphology at diagnosis was predominantly L1; only two cases (no. 4 and 7) had L2 features. At relapse, the morphologic features were unremarkable, except that case no. 5 had two morphologically distinct populations of bone marrow blasts, one of which expressed myeloperoxidase at a low level (~2%).

Modal chromosome numbers at diagnosis were 46 in three cases, 45 and 48 in two cases each, and 47 and 53 in one case each. At relapse, seven cases had different modal numbers, with five gaining one to four chromosomes; new structural abnormalities were apparent in seven cases as well. The karyotypes in all nine cases were sufficiently changed at relapse to rule out clonal evolution as the underlying mechanism. It should be stressed that with the exception of cases no. 2 and 8, the karyotypes at relapse were not more complex than those studied at diagnosis. Interestingly, three cases (no. 4, 5, and 6) had 11q23 rearrangements at relapse, and another (no. 2) acquired multiple structural abnormalities, including a del(5q).

At diagnosis, seven cases were classified as CD10+ B-lineage ALL (no. 1 through 5, 8, and 9) and two as T-cell ALL (Table 1). Of the seven cases with changes in immunophenotypic markers, cases no. 1 and 2 lost clg, case no. 3 acquired sIg, and case no. 4 lost CD10 (first relapse) but then gained clg (second relapse). Immunophenotypic alterations in the remaining cases were either minor (no. 5 through 7) or absent altogether (no. 8 and 9). At diagnosis, the leukemic cells of patient no. 2 had the following markers: CD10 (73%), CD19 (78%), CD20 (18%), CD22 (78%), clg (30%), CD33 (6%), CD13 (6%), and CD11b (3%). At relapse, this case met the standard morphologic and cytochemical criteria for ALL, but showed increased expression of myeloid-associated antigens without loss of characteristic lymphoid antigens: CD10 (87%), CD19 (88%), CD20 (78%), CD22 (77%), clg (4%), CD33 (72%), CD13 (71%), and CD11b (50%). Although two-color immunofluorescence flow cytometry was not performed, it was clear from the overlapping percentages of lymphoid and myeloid markers that a majority of blasts were coexpressing markers of both lineages.

The median length of initial remissions in these patients was only 24 months (range, 6 to 35); treatment had been electively stopped in patients no. 2, 7 and 8 when their relapses occurred. Each child with a new leukemic cell line had received multiple antineoplastic drugs, including teniposide in nine cases and etoposide in five (Table 1). Additionally, four patients had received daunorubicin at a cumulative dose of 50 to 75 mg/m², and one had received doxorubicin at 180 mg/m². All three total therapy studies (X-XII) in the survey were represented by this unusual series of cases.

Of the nine patients who entered complete remission after retrieval therapy, only one survives (3+ months after allogeneic marrow transplantation). The durations of second remissions in these nine patients did not differ significantly from those of the 89 patients who lacked major karyotypic changes at relapse \((P = 0.9\) by the log-rank test; survival curves not shown).

**DISCUSSION**

In previous reports of sequential cytogenetic studies of leukemic lymphoblasts, clonal evolution with conservation of the original karyotype has been the most common finding at relapse. Detection of a completely different karyotype has been rare. In the study by Abshire et al, for example, only one of 116 cases possessed an ostensibly new leukemic stem line at relapse. By contrast, we present evidence for the emergence of a new leukemic clone in nine of 98 sequentially studied cases. One explanation for this apparent increase in frequency might lie in the high rate of detection of chromosomal abnormalities of cases at this center (>90%). A more likely reason would be the use of intensive multiagent chemotherapy for both higher- and lower-risk patients at our institution.

Intensified treatment could contribute to the development of cyogenetically different leukemic clones by any of several mechanisms. First, the stem line detected at relapse might have been present at diagnosis but at a level too low to be detected by standard methods. Effective treatment could then have eradicated the dominant line, allowing the minor (drug-resistant) clone to expand. In this regard, we have shown that ALL cases occasionally possess two cytogenetically independent blast cell populations at diagnosis, apparently derived from a common progenitor. Second, treatment might have abolished an overtly leukemic, cyogenetically abnormal clone but not a preleukemic stem cell, which eventually could have given rise to a new leukemic clone. This hypothesis is supported by studies of G6PD heterozygotes with AML in clonal remission. We have no compelling findings that would distinguish between these two alternatives.

A third possibility is the secondary induction of ALL by transformation of a normal lymphoid precursor. Thus, it may be important that the lymphoblasts in three of our cases studied at relapse (no. 4 through 6) were characterized by the acquisition of an 11q23 rearrangement, an abnormality often implicated in topoisomerase II inhibitor–induced secondary AML as well as secondary ALL arising after treatment for solid tumors. In this regard, patients no. 4 and 5 were treated with teniposide on a twice-weekly schedule that has been associated with a greatly increased risk of secondary AML. These observations suggest that DNA-topoisomerase II inhibitors have the potential to transform lymphoid, as well as myeloid progenitors.

The outcome of standard retrieval chemotherapy in this group of patients has been discouraging, indicating the need for effective alternatives (eg, bone marrow transplantation). Finally, routine study of ALL with sequential cytogenetic and molecular analyses is needed to pinpoint the mechanism(s) giving rise to new leukemic clones.

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