Nonrandom Chromosome Abnormalities in Acute Leukemia and Dysmyelopoietic Syndromes in Patients With Previously Treated Malignant Disease

By Janet D. Rowley, Harvey M. Golomb, and James W. Vardiman

Cytogenetic studies were performed on 26 patients who developed acute nonlymphocytic leukemia (ANLL) or a dysmyelopoietic syndrome after treatment of a primary malignancy. Fifteen patients had radiotherapy and chemotherapy, seven had only chemotherapy, and four had only radiotherapy. The median times from diagnosis of the initial disease to the development of bone marrow dysfunction for these treatment groups were 50, 46, and 49 mo, respectively. Twenty-five patients had an abnormal karyotype in myeloid cells. Loss of part or all of no. 5 and/or no. 7 was noted in 23 of 25 patients with aneuploidy. Loss of no. 5 was noted only in patients who previously had malignant lymphoma, whereas loss of no. 7 was seen in these patients as well as in those who had other malignancies. Abnormalities of both nos. 5 and 7 occurred in 53% of the patients treated with combined therapy and in only 27% of patients treated with either modality alone. Although these changes are distinctly different from those noted in lymphomas, they are similar to those seen in 25% of aneuploid patients with ANLL de novo.

MATERIALS AND METHODS

The 26 patients described here had a previously treated malignant disease, and they subsequently developed DMS or ANLL. Cytogenetic studies were performed in each case. The original diagnosis of malignancy in these patients was made between January 1965 and September 1976. Six of the patients were treated at other institutions in Chicago, and bone marrow material for morphological and cytogenetic evaluation was made available by the referring physicians cited in the acknowledgment.

Bone marrow and unstimulated peripheral blood samples were processed for chromosome analysis as previously described. Patients were classified as having an abnormal clone if at least two cells had had the same extra chromosome or the same structural rearrangement, or if three cells had loss of the same chromosome.

The type of leukemia was determined by review of peripheral blood, bone marrow aspirates, and bone core biopsies, and by review of cytochemical studies that were available for 17 of the 26 patients. Although an attempt was made to classify each case according to the French-American-British (FAB) criteria, the presence of marrow fibrosis in some of the patients prevented us from obtaining adequate marrow aspirates for the detailed cytologic and cytochemical studies required for delineation of the various subgroups of this classification.

If the case did not meet the criteria for ANLL, the alternate designation, DMS, was used provided the morphological features described by the FAB classification for such a diagnosis were met. If a patient was followed serially and evolved through a dysmyelopoietic phase into overt acute leukemia, the case was diagnosed...
<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Date of Dia. (mo/yr)</th>
<th>Radiation Therapy (rad)</th>
<th>Therapy*</th>
<th>First Day of BM Dysfunction (mo)</th>
<th>Pre-Leuk.</th>
<th>Time after Dia. (mo/yr)</th>
<th>Date of Leuk. Dia. (mo/yr)</th>
<th>Type of Leukemia</th>
<th>Leukemia Therapy</th>
<th>Response†</th>
<th>Date of Death (mo/yr)</th>
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<tbody>
<tr>
<td>1-10</td>
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<tr>
<td>11</td>
<td>11</td>
<td>M</td>
<td>ML, PDL, Nod</td>
<td>9/71</td>
<td>4400 TN</td>
<td>1971 CVP x 6</td>
<td>9/76            + 61</td>
<td>DMS</td>
<td>0</td>
<td>9/77</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>60</td>
<td>M</td>
<td>HD, MC, PS III A</td>
<td>6/72</td>
<td>0</td>
<td>MOPP x 2 mo CTX, Procarb — 18 mo</td>
<td>8/76            + 50</td>
<td>7/77</td>
<td>AML</td>
<td>Ara-C + TG</td>
<td>PR 11/77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>32</td>
<td>F</td>
<td>HD, NS, PS IV A</td>
<td>10/73</td>
<td>3500 Mantle</td>
<td>MOPP + Bleo x 6 until 4/74</td>
<td>7/77            + 45</td>
<td>11/77</td>
<td>EL</td>
<td>Ara-C + Daun</td>
<td>NR 4/78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>47</td>
<td>F</td>
<td>HD, MC, PS IV B</td>
<td>12/73</td>
<td>0</td>
<td>COPP x 9</td>
<td>10/77            + 46</td>
<td>1/79</td>
<td>AML</td>
<td>Ara-C + TG + Daun</td>
<td>NR 6/79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>55</td>
<td>M</td>
<td>HD, NS, PS III A</td>
<td>2/71</td>
<td>4000 TN</td>
<td>1971 COPP x 6</td>
<td>6/79            + 100</td>
<td>DMS</td>
<td>0</td>
<td>7/80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>34</td>
<td>M</td>
<td>HD, MC, PS III A</td>
<td>9/73</td>
<td>4000 Mantle</td>
<td>1974 MOPP x 3</td>
<td>7/77            + 46</td>
<td>2/79</td>
<td>AMLMdl</td>
<td>Hydroxyurea**</td>
<td>NR 2/79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>54</td>
<td>F</td>
<td>ML, PDL, Diffuse IVB</td>
<td>10/73</td>
<td>0</td>
<td>COPP x 9 thru 7/74 Maintenance COPP x 9 8/74-2/75</td>
<td>2/77            + 40</td>
<td>DMS</td>
<td>0</td>
<td>5/77</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>69</td>
<td>F</td>
<td>Multiple myeloma</td>
<td>4/73</td>
<td>0</td>
<td>Melphalan + Pred QD until 7/75</td>
<td>6/75            + 26</td>
<td>DMS</td>
<td>0</td>
<td>2/76</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>62</td>
<td>F</td>
<td>Multiple myeloma</td>
<td>9/72</td>
<td>RT Lower spine</td>
<td>1972 CTX + Pred — 2 yrs.</td>
<td>12/76            + 50</td>
<td>7/77</td>
<td>AML</td>
<td>Patient refused</td>
<td>8/77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>62</td>
<td>M</td>
<td>Multiple myeloma</td>
<td>1/72</td>
<td>3000 Sacrum</td>
<td>1973 CTX + Pred 3/7-74</td>
<td>7/78            + 67</td>
<td>7/78</td>
<td>EL</td>
<td>Ara-C + TG + Daun</td>
<td>Patient lost to follow up 9/78</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>25</td>
<td>46</td>
<td>F</td>
<td>Cervical Ca</td>
<td>4/69</td>
<td>Radium insertion x 2 1969</td>
<td>0</td>
<td>11/75            + 79</td>
<td>11/75</td>
<td>AML</td>
<td>Ara-C + TG + x 3</td>
<td>NR 3/76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>83</td>
<td>M</td>
<td>Squamous cell Ca of lung Stage III A</td>
<td>11/75</td>
<td>0</td>
<td>CAMP x 32 1975-79</td>
<td>6/79            + 43</td>
<td>DMS</td>
<td>0</td>
<td>11/79††</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Diagnosis: HD, Hodgkin disease; MC, mixed cell; NS, nodular sclerosing; ML, non-Hodgkin lymphoma; PDL, poorly differentiated lymphocytic; Nod, nodular. Stage: CS, clinical stage; PS, pathologic stage.

TN, total nodal; EM, extended mantle; MOPP, nitrogen mustard, Oncovin, procarbazine, prednisone; COPP, cyclophosphamide, Oncovin, procarbazine, prednisone; CPV, cyclophosphamide, vincristine, prednisone; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; VLB, vinblastine; Procarb, procarbazine; Pred, prednisone; CAMP, cyclophosphamide, Adriamycin, methotrexate, procarbazine.

AML, acute myelogenous leukemia; MPD, myeloproliferative disease; APL, acute promyelocytic leukemia; EL, erythroleukemia; AMLMdl, acute myelomonocytic leukemia; DMS, dysmyelopoietic syndrome.

†Ara-C, cytosine arabinoside; TG, thioguanine; Daun, daunorubicin; COAP, cyclophosphamide, Oncovin, Ara-C, prednisone.

Based on bone marrow biopsy only; asparate was dry tap.

Previously reported (see ref. 24).

**Partial response.

††Died with persistent thrombocytopenia; few blasts in peripheral blood.
RESULTS

Clinical

Of the 26 previously treated patients, 16 had Hodgkin’s disease (HD), 5 had poorly differentiated lymphocytic lymphoma (PDL), 3 had multiple myeloma (MM), 1 had cervical carcinoma, and 1 had squamous cell carcinoma of the lung (Table 1). Of the 16 HD patients, 9 had mixed-cell type and 7 had nodular sclerosis; 3 patients were in stage II, 10 in stage III, and 3 in stage IV. Of the five PDL patients, four had nodular histology and one had diffuse type; three patients were in stage III and two were in stage IV. The type of therapy and the time between the original diagnosis and the diagnosis of marrow dysfunction are summarized in Table 2.

Of the 26 patients, 20 could be documented as having a preleukemic phase. The morphological features of these patients during the preleukemia stage showed many similarities to the dysmyelopoietic syndrome, particularly refractory anemia with excess blasts as described in the FAB classification. The duration of the preleukemic phase ranged from 2 to 20 mo, with a median of 6 mo.

Of the 21 patients whose leukemia could be accurately classified, 16 had acute myelogenous leukemia (AML) (M1 or M2 by the FAB criteria); 1, acute hypergranular promyelocytic leukemia (APL); 3, erythroleukemia (EL); and 1, acute myelomonocytic leukemia (AMMoL). Five additional patients were classified as having DMS; these patients did not have sufficiently high blast counts to be considered as having overt acute leukemia. No relationship was observed between the type of leukemia, the type of therapy, and the initial disease.

Eleven patients who developed acute leukemia were treated with combination chemotherapy in an attempt to induce a remission. Ten of these did not respond at all; one patient had a partial response, but died 4 mo after the initiation of therapy.

Cytogenetic

None of the 26 patients had received therapy for leukemia at the time of the first chromosome analysis. Chromosomes were obtained from the bone marrow in 22 cases and from unstimulated blood cultured for 24–48 hr in the other 4. A chromosomally abnormal clone of cells was seen in the bone marrow of the patients except one (case 21). A consistent clonal abnormality could be identified in every aneuploid patient (Table 3). When a bone marrow sample was examined initially, the abnormal clone involved 60%–94% of the dividing cells in 10 patients and 100% of cells in 11 others. In unstimulated peripheral blood, the percentage of abnormal cells ranged from 16% to 93% in three patients and was 100% in a fourth. The modal chromosome number was 43–45 in 18 patients, 46 in 5 patients, only 1 of whom had a normal karyotype, and 47–51 in 3 patients.

One or both of two consistent chromosome changes were noted in 23 of 25 patients with aneuploidy. Eleven patients had loss of no. 5, and 3 others were lacking a part of the long arm of no. 5 (Sq–); no. 7 was missing from cells of 20 patients (Fig. 1). Eleven patients had a –7 as well as either –5 or Sq–. These changes were particularly noticeable, since losses of other chromosomes occurred at most in 2 patients (8%), and since gains of chromosomes were rarely seen except in ANLL secondary to MM. Structural rearrangements were observed most frequently in patients who had previously had malignant lymphoma; they involved deletions of 6q and translocations of 1p and 2q. No correlation between the karyotype and the type of leukemia was noted. The only two aneuploid patients whose cells had normal nos. 5 and 7 both had AML. The relationship of the chromosome pattern to prior disease and to the type of therapy is summarized in Tables 4 and 5, respectively.

Of 11 patients studied in the preleukemic phase, six were also studied in the leukemic phase. Additional chromosome changes were noted in five of the six patients; in each case, the evolution involved the original aneuploid clone. Of two patients who had –7 initially, one became +8 and the other –5; two patients had –5 or Sq, and both became –7; one other patient had –5 and –7 initially and developed other chromosome rearrangements. Five other patients who were first seen in the leukemic phase had two or three aneuploid clones; in one patient, evolution of a –7 clone involved loss of no. 5, and in a second patient, the evolution involved loss of no. 7. In both groups of patients, evolution of the karyotype tended to involve loss of chromosomes and thus a change to a lower modal chromosome number (6/10 patients).

The data relating survival to the presence of some

Table 2. Type of Therapy and Survival of Patients

<table>
<thead>
<tr>
<th>Therapy Type</th>
<th>No. of Malignant Lymphoma Patients</th>
<th>No. of Other Patients</th>
<th>Total No. of Patients</th>
<th>Median Time to Initial BM Dysfunction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation therapy and chemotherapy</td>
<td>13</td>
<td>2</td>
<td>15</td>
<td>50 mo</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>46 mo</td>
</tr>
<tr>
<td>Radiation therapy</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>49 mo</td>
</tr>
</tbody>
</table>
normal cells are very preliminary, but they suggest that patients with DMS who have a mixture of karyotypically normal and abnormal cells (AN) do something better than those who have only abnormal cells (AA). Of eleven patients studied in the preleukemic phase, four had only abnormal cells (AA) and seven had a mixture of normal and abnormal cells (AN). Two of the four AA patients developed leukemia in 2 and 7 mo (case 911 and case 22); the other two patients died of complications secondary to marrow dysfunc-

Table 3. Karyotype of Patients With Secondary ANLL

<table>
<thead>
<tr>
<th>Case No.</th>
<th>No. of This Sample/Total no. of Samples</th>
<th>Source*</th>
<th>Date</th>
<th>Total no. Cells†</th>
<th>Modal no.</th>
<th>Percent Abnormal Cells‡</th>
<th>Karyotypes§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with treated malignant lymphoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11††</td>
<td>1/4</td>
<td>BM</td>
<td>11-18-77</td>
<td>38(23)</td>
<td>45</td>
<td>80</td>
<td>45,XX, -7, -16, -21, +der(2;7)(cen;cen),del(2)(q33),del(11)(q22), +11p+, +14p+, +t(21;21),del(6)(q13),del(11)(q22),21q11q22,16p13</td>
</tr>
<tr>
<td>12</td>
<td>1/5</td>
<td>BM</td>
<td>3-28-77</td>
<td>30(13)</td>
<td>47</td>
<td>93</td>
<td>47,XY, +8 [same in all samples]</td>
</tr>
<tr>
<td>13</td>
<td>1/3</td>
<td>BM</td>
<td>11-4-77</td>
<td>33(21)</td>
<td>46</td>
<td>90</td>
<td>46,XX, -7, -16, -21, +der(2;7)(cen;cen),del(11)(q22), +11p+, +14p+, +t(21;21),del(6)(q13),del(11)(q22),21q11q22,16p13</td>
</tr>
<tr>
<td>14</td>
<td>1/4</td>
<td>BM</td>
<td>11-18-77</td>
<td>38(23)</td>
<td>45</td>
<td>80</td>
<td>45,XX, -7, -16, -17p+, +20q-(10 percent)/43,XY, -5, -7, -12, -16, 4q+, -1p+, -20q-, +mar(20 percent)/43,XYq+, same [30 percent]</td>
</tr>
<tr>
<td>15</td>
<td>1/2+</td>
<td>BM</td>
<td>6-12-79</td>
<td>32(13)</td>
<td>45</td>
<td>100</td>
<td>45,XY, -7</td>
</tr>
<tr>
<td>16</td>
<td>1/1+</td>
<td>BC</td>
<td>10-29-79</td>
<td>42(10)</td>
<td>43</td>
<td>60</td>
<td>44,XY, -7, -16, -17p+, +20q-(10 percent)/43,XY, -5, -7, -12, -16, 4q+, -1p+, -20q-, +mar(20 percent)/43,XYq+, same [30 percent]</td>
</tr>
<tr>
<td>17</td>
<td>1/1</td>
<td>BM</td>
<td>3-29-77</td>
<td>17(12)</td>
<td>45</td>
<td>100</td>
<td>45,XY, -5, -7,del(3)(p13), +der(5)(t5;17)(q11?q11)</td>
</tr>
<tr>
<td>18</td>
<td>1/1</td>
<td>BM</td>
<td>8-2-78</td>
<td>29(16)</td>
<td>45</td>
<td>94</td>
<td>45,XY, -7,del(5)(q13(?)[6 cells]/same with t(12;17)(q13;p12)?[7 cells]</td>
</tr>
<tr>
<td>19</td>
<td>1/1</td>
<td>BM</td>
<td>2-15-79</td>
<td>27(13)</td>
<td>45</td>
<td>100</td>
<td>45,XY, -7,del(3)(p13?),del(8)(q22),del(16)(p12)</td>
</tr>
<tr>
<td>20</td>
<td>1/1</td>
<td>BM</td>
<td>2-8-79</td>
<td>17(8)</td>
<td>43</td>
<td>88</td>
<td>44,XY, -2, -3, -6, -12, -15, -21, -22, +del(2)(p11),del(11)(p14),t(14;7)(q32;?),t(17;7)(p12;?),del(22)(q11), +5mar/43,same, -7</td>
</tr>
<tr>
<td>21</td>
<td>1/1</td>
<td>BM</td>
<td>2-28-77</td>
<td>6(6)</td>
<td>46</td>
<td>0?</td>
<td>46,XX?;one X and one 10 appear atypical</td>
</tr>
<tr>
<td>Patients with treated multiple myeloma</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>22†</td>
<td>1/4</td>
<td>BM</td>
<td>9-10-75</td>
<td>6(6)</td>
<td>45**</td>
<td>100</td>
<td>45,XX, -13, -14, +t(13;14)(p13;q11),**del(5)(q14?)</td>
</tr>
<tr>
<td>23†</td>
<td>1/1</td>
<td>PB24</td>
<td>7-29-77</td>
<td>52(22)</td>
<td>45</td>
<td>100</td>
<td>45,XX, -7,t(3;9)(q23;p21)(16 cells)/same 44, -X [6 cells]</td>
</tr>
<tr>
<td>24</td>
<td>1/1</td>
<td>BM</td>
<td>7-11-78</td>
<td>28(25)</td>
<td>51</td>
<td>100</td>
<td>51,XY, +1, +2, +6, -7, +8, -14, +15, +21, +mar</td>
</tr>
</tbody>
</table>

When only one or two samples are recorded, the karyotype in all subsequent samples is the same as the last one listed.

*Source: PB24 — unstimulated culture of peripheral blood for 24 hr; BM — bone marrow; BC — bone core.
†Number in parentheses indicates the number of cells analyzed with banding.
‡Percentage of all analyzed cells.
§Of abnormal cells; if no change in subsequent samples, these are not listed.
Case nos. 7 and 8 in Liang et al, Cancer 44:630-644, 1979.
Case no. 5 in reference 24.
**Patient had a constitutional chromosome abnormality consisting of fusion between nos. 13 and 14.
††Case no. 86 in reference 33.
Karyotype of a bone marrow cell obtained from patient no. 13. This broken cell has 46 chromosomes, is lacking one no. 7 and one no. 16, and has an extra abnormal no. 2 and two extra abnormal no. 11s. The first no. 2 appears to have part of the missing no. 7 replacing the short arm (t). The third no. 2 has a deletion of part of the long arm (q -). The first no. 11 has additional material added to the short arm (t); the last two have a deletion of the end of the long arm (q -). The first no. 14 has a small band added on the short arm. The first no. 21 appears to consist of a triplication of no. 21 involved in a translocation of the entire missing no. 16 (M). The loss of one no. 9 was a technical artifact. (Inset) G-banding (bottom) and C-banding (top) of chromosomes 16 and 21 from another cell. Note the persistence of the centromeric C band of chromosome no. 16 in the marker chromosome. The functional centromere is provided by the first no. 21.

Fig. 1. Karyotype of a bone marrow cell obtained from patient no. 13. This broken cell has 46 chromosomes, is lacking one no. 7 and one no. 16, and has an extra abnormal no. 2 and two extra abnormal no. 11s. The first no. 2 appears to have part of the missing no. 7 replacing the short arm (t). The third no. 2 has a deletion of part of the long arm (q -). The first no. 11 has additional material added to the short arm (t); the last two have a deletion of the end of the long arm (q -). The first no. 14 has a small band added on the short arm. The first no. 21 appears to consist of a triplication of no. 21 involved in a translocation of the entire missing no. 16 (M). The loss of one no. 9 was a technical artifact. (Inset) G-banding (bottom) and C-banding (top) of chromosomes 16 and 21 from another cell. Note the persistence of the centromeric C band of chromosome no. 16 in the marker chromosome. The functional centromere is provided by the first no. 21.

Table 5. Correlation of Karyotype With Therapy

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Number of Patients</th>
<th>Only 5/5q</th>
<th>Only 7</th>
<th>Both 5 and 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiotherapy and chemotherapy</td>
<td>15</td>
<td>1</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>7*</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

*One patient had a normal karyotype.

cells were early changes in the peripheral blood in this group. Erythroid hyperplasia with megaloblastoid features, a mild to moderate shift toward granulocytic immaturity with increased numbers of blasts, increased numbers of megakaryocytes with cytologic abnormalities, and increased amounts of reticulin were found in the bone marrow. As overt leukemia evolves, serial marrow studies usually show a gradual increase in the number of blasts and in the amount of reticulin, and a decrease in the number of megakaryocytes and erythroid precursors. Because of the presence of a slowly evolving preleukemic phase in most cases, lasting a median of 6 mo in our patients, aggressive combination chemotherapy is usually not recommended until the acute phase develops.

The chemotherapy of treatment-related leukemias has resulted in very poor responses. Of the 11 treated patients in our series, only one had a partial remission, and he lived only 4 mo after initiation of therapy. Casciato and Scott reported that more than 90% of the patients died within 6 mo and 68% died within 2 mo of the development of leukemia; only two patients in their review survived for more than 1 yr after the development of acute leukemia. Recently, Beltran and Stuckey reported complete remission in four of five patients with ANLL after malignant lymphomas which were treated with chemotherapy. Zarrabi and Rosner reported two complete remissions in 15 patients treated for ANLL following treatment for solid tumors. Further data are needed regarding the response to combination chemotherapy.

One of our initial questions referred to the prognostic importance of the presence of some normal metaphase cells (AN) during the preleukemic phase. Of 11 patients studied in this phase, 7 were AN; in 4 of these, this phase lasted longer than 10 mo, whereas only 1 of the 4 patients who were AA at the time of development of the preleukemic phase survived for more than 10 mo. The ability to predict the length of the preleukemic phase is important for the patient who wants to plan his future. Although the importance of AN or AA status remains to be established, the presence of a clonal cytogenetic abnormality in a patient with a previously treated malignancy who develops an unexplained cytopenia or pancytopenia without recurrence...
of the original tumor is diagnostic of a preleukemic phase.

Regardless of the failure to respond to treatment of the acute leukemia, the majority of patients developing secondary ANLL benefited from therapy of their initial malignancy with respect to both clinical remission and prolongation of life. However, since the risk of developing a second malignancy is substantially increased with both chemotherapy and radiotherapy and only moderately increased with either chemotherapy or radiotherapy alone, it is important to establish whether patients with some malignancies really need combined-modality therapy. In addition, certain classes of chemotherapeutic agents, particularly alkylating agents, might be more leukemogenic than others that are equally effective in the treatment of a specific disease. Valagussa et al. reported on the incidence of second malignancies in patients with Hodgkin's disease after they had received various forms of treatment. In patients who received nitrogen mustard, oncovin, prednisone, and procarbazine (MOPP) or a derived combination (234 patients), they observed an incidence of leukemia at 10 yr ranging from 3.8% to 5.4%. However, among 55 patients treated with adriamycin, bleomycin, velban, and dacarbazine (ABVD) there were no cases of ANLL at 5 yr or at 10 yr.

Cytogenetic

A consistent clonal chromosome abnormality was observed in bone marrow cells from 25 of our 26 patients, including the 10 patients whom we reported previously. Only six cells were obtained from the marrow of the patient who was classified as normal, and the banding pattern in these cells was of suboptimal quality. This patient might thus have had an abnormal clone that we failed to detect. Fourteen of these patients had loss of all of no. 5 or part of the long arm of no. 5, and 21 had loss of all no. 7. One or both of these changes were noted in 23 of the 25 aneuploid patients. These chromosome changes are remarkably consistent when one considers that, in ANLL de novo, the most frequent change, namely, an extra no. 8, occurs in only 25% of aneuploid patients. Moreover, these patients had received a variety of treatment regimens for their initial disease. The loss of no. 5 or 7 or both is associated with a high frequency of hypodiploid modal numbers. Thus, 18 of the 25 aneuploid patients had leukemic cells with less than 46 chromosomes, whereas only 3 patients had cells with a hyperdiploid number (Fig. 2).

Although the karyotype seen in secondary ANLL is distinctly different from that seen in the primary malignancy, the nature of the primary disease may influence the pattern of karyotypic changes seen in the leukemic cell (Table 4). This speculative notion is based on two observations. First, loss of no. 5 and/or no. 7 occurs with equal frequency in abnormal patients with ANLL secondary to treated malignant lymphoma. On the other hand, every patient with ANLL secondary to some other disease had a loss of part or all of no. 7, whereas an abnormality of no. 5 (a 5q− chromosome) was noted only in one case. Thus, loss of all of no. 5 was seen only in patients with treated malignant lymphoma. Two of our three patients with leukemia following multiple myeloma were the only ones whose leukemic cells had more than 48 chromosomes, with +1, +6, +8, +21; this constellation of abnormalities was not seen in any of our other patients.

Since most of the patients had received both radiotherapy and chemotherapy, it is difficult to determine whether specific chromosome changes are related more closely to one rather than the other of these types of treatment. It appears, however, that combined therapy is much more likely to result in abnormalities of both no. 5 and no. 7 (8 of 15 patients) than is either modality used alone (3 of 12) (Table 5).

Our data raise a number of important questions that cannot be answered at present; for example: (1) What was the karyotype of the myeloid cells prior to any therapy? (2) When did the aneuploid clone arise? (3) How many patients have aneuploid clones, but do not develop ANLL? The karyotype of the leukemic cells is distinctly different from that in malignant lymphoma, moreover, in an analysis of bone marrow samples obtained from more than 30 patients with lymphoma at the time of the initial clinical staging evaluation, we have observed no aneuploid clone (Rowley, unpub-
established observations). Kakati et al.\(^32\) have also reported primarily normal karyotypes in marrow samples from lymphoma patients; the abnormalities seen were similar to those noted in lymph nodes involved in lymphoma and they probably represented malignant lymphoid cells. We have begun a prospective study of our treated patients. Thus far, we have not found a single aneuploid clone in marrow aspirates from 25 patients who had been treated with radiotherapy and chemotherapy more than 3 yr before the sample was obtained (Rowley, unpublished observations). Since only a small percentage of treated patients develop ANLL, we hope that our report will encourage other investigators to undertake similar prospective studies and to obtain information from an adequate series of patients.

Results from the study of our treated patients can be compared with observations on our patients with ANLL de novo.\(^23\) The important differences are, first, the high incidence of an aneuploid clone, particularly of clones with a hypodiploid modal number (Fig. 2), and second, the increased involvement of no. 5 and/or no. 7 (Fig. 3). Each of these abnormalities occurred in 20%–25%, respectively, of our secondary ANLL patients.

In addition to the patients whom we have described, 31 others with secondary ANLL, studied with banding, have been reported.\(^13\) The prior disease was HD in 12, PDL in 5, other lymphomas in 5, MM in 3, carcinoma in 2, and malignant melanoma, acute lymphoblastic leukemia, chronic lymphocytic leukemia, rheumatoid arthritis, and postrenal transplant each in one patient. Six patients had received primarily radiotherapy, 10 had received only chemotherapy, and 12 had received a combination of radiotherapy and chemotherapy. A diagnosis of AML was made in 12 patients; 6 patients had EL and 3 had AMMol. Where adequate clinical data were provided, a preleukemic phase was seen in every patient. The median time from diagnosis of the initial disease to the start of the leukemic phase was 51 mo (range, 21–98 mo). As in our series, these patients failed to respond to treatment of the leukemic phase; the median survival was 2 mo (range, 1–9 mo).

Twenty-nine of the 31 patients had a clone of chromosomally abnormal cells comprising 50%–100% of the cells in division. Nineteen of the 29 aneuploid patients (61%) had clones with a hypodiploid modal number. Moreover, 22 of the 29 (76%) had loss of part or all of no. 5 and/or no. 7. These observations are similar to those that we have reported and to those reported for patients whose chromosomes were not studied with banding.\(^34\)

The cytogenetic hallmarks of secondary ANLL are, thus, an abnormal clone of cells, usually with a hypodiploid modal number, that is associated with the nonrandom loss of chromosomes 5 and/or 7. Our observations regarding the nonrandom abnormalities of nos. 5 and 7 are germane to the question whether the leukemic cells of patients with ANLL de novo contain specific karyotypic changes that allow one to distinguish between patients who have and those who have not been exposed to an environmental mutagen. Although this question cannot be answered at present, the evidence suggesting that the answer may be positive has recently been discussed in detail.\(^35\) First, the karyotypic pattern for ANLL de novo was distinctly different in children from that seen in adults. Second, as reported by Mitelman and his collaborators in Sweden and in Rome,\(^21\) patients with ANLL de novo whose occupations involved exposure to chemical solvents and insecticides had a much higher frequency of aneuploidy, particularly loss of no. 5 or no. 7, than did patients who were not so exposed. We recently completed an analysis of eight patients with smouldering acute leukemia, seven of whom had an abnormal clone.\(^36\) Five of the seven had a loss of no. 5, a 5q–, or a loss of no. 7. At least three of the seven patients had had some exposure to potentially mutagenic agents. In addition, one of the two patients described by Van Den Berghe et al., with a history of long exposure to benzene, had a clone of cells lacking a no. 7.\(^37\) The medical progress that has allowed patients with previously untreatable malignant diseases to live at least 5 yr and to develop a second malignancy, usually ANLL, may help us to determine the etiology in a subset of patients with ANLL de novo. The importance of various factors, such as environmental exposure and the genetic background of the patient, in the karyotypic pattern of the leukemic cells remains to be

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**Fig. 3.** Histogram comparing the frequency of various chromosome changes in 44 aneuploid patients with ANLL de novo among a total of 88 patients with 25 aneuploid patients among the 26 with secondary ANLL or DMS.
determined. Of more practical concern at the moment, however, is the development of treatment protocols that are equally effective in the treatment of malignant lymphomas and of other diseases, but that may be less leukemogenic than some of those currently in use.

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