Rearrangements of the human trithorax gene (MLL, HRX, Htrx-1, ALL-1) were studied by Southern blotting in blast cells stored at presentation from 65 adults with de novo acute myelomonocytic (AML-M4) and acute monocytic leukemia (AML-M5). MLL rearrangements were demonstrated in 15 (23%) cases, including eight patients in whom karyotype analysis had failed to detect abnormalities of chromosome band 11q23. The patients with MLL rearrangements did not differ significantly from those with germline configurations in terms of the sex and age of the patients, the presence of lymphadenopathy, hepatosplenomegaly, or central nervous system involvement, and the absolute blast count at diagnosis. Kaplan-Meier analysis of the treated patients demonstrated no difference in survival for patients with MLL rearrangements compared with those without rearrangements. Therefore, in contrast to infantile acute leukemia, in adults with AML-M4 and AML-M5, MLL rearrangements do not identify a subgroup of patients with different clinical features or prognosis. © 1994 by The American Society of Hematology.

MATERIALS AND METHODS

In the last 10 years 343 adult patients with de novo AML have been referred to the ICRF Department of Medical Oncology at St Bartholomew’s Hospital, including 60 patients with AML-M4 and 28 patients with AML-M5. Bone marrow or peripheral blood samples were stored at presentation from 65 patients (40 AML-M4 and 25 AML-M5). The clinical details are summarized in Table 1. Cytogenetic analysis was performed on fresh aspirates of bone marrow or peripheral blood samples using standard methods. DNA was extracted from frozen samples, digested with restriction enzymes EcoRI and BamHI, and Southern blots were prepared according to standard techniques. A 3-kb fragment of MLL cDNA, which includes exons 5-13 was radiolabeled using the Prime-it kit (Stratagene, La Jolla, CA). This probe spans the 8.5-kb BamHI genomic fragment, which contained all 51 breakpoints in a series of leukemias with 11q23 abnormalities. Filters were hybridized with the labeled probe at 65°C in 0.5 mol/L phosphate buffer (pH 7.2) and 7% sodium dodecyl/sulfate (SDS) in a Hybrid rotating oven (Teddington, UK). Following washing with 0.1% SDS, 1 x saline sodium citrate at 65°C, filters were exposed to Kodak X-omat film (Eastman-Kodak, Rochester, NY) for up to 7 days at -70°C. Only patients who showed clear abberant bands with both enzyme digests were scored as rearranged (Fig 1).

RESULTS

Rearrangements of the human trithorax gene were demonstrated in blasts from 15 of 65 patients (23%) by Southern

Prevalence and Clinical Correlations of MLL Gene Rearrangements in AML-M4/5

By M. Bower, P. Parry, M. Carter, D.M. Lillington, J. Amess, T.A. Lister, G. Evans, and B.D. Young

The human trithorax gene (MLL, HRX, Htrx-1, ALL-1) located at chromosome band 11q23 is fused by chromosomal translocation to a number of different partner genes in acute leukemias.1-4 This results in the expression of fusion transcripts from the derivative chromosomes. Rearrangements of MLL have been shown by Southern blotting in many leukemias with translocations involving 11q23 and at least 15 partner loci.5 These rearrangements are found in both acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML), in both infants and adults, and in both de novo and secondary leukemias.6

Cytogenetic studies suggest that abnormalities of 11q23 are found in about 75% of infants with acute leukemia, but only 5% of adults.7,8 In adults, translocations involving band 11q23 occur more frequently in ALL (7%) than in AML (1%).9 However, when 11q23 translocations are present in AML, the majority of both primary and therapy-related AML show monocytic differentiation and belong to the acute myelomonocytic leukemia (AML-4) or acute monocytic leukemia (AML-M5) subtypes of the French-American-British (FAB) classification.10 Furthermore, in these leukemias the t(6;11)(q27;q23) and t(9;11)(p21;q23) are the most common translocations involving the MLL gene, while t(4;11) (q21;q23) and t(11;19)(q23;pl3) are the most frequent 11q23 translocations in ALL. Translocations of 11q23 in infantile ALL are associated with a high circulating blast cell count at presentation, organomegaly, CNS involvement, and a poor prognosis.11-14

In one report MLL rearrangements were detected by Southern blotting in 21 of 30 cases of infantile ALL.15 The presence of this genetic rearrangement correlated significantly with adverse prognostic factors including age less than 6 months, high blast counts, CD10 negative immunophenotype, and early treatment failure. The event-free survival at a median follow-up of 46 months was 15% for the infants with MLL rearrangements compared with 80% for infants with germline 11q23 configurations.16 A similar study of 15 infants with acute leukemia detected the presence of MLL rearrangements by Southern blotting in 12, including five with apparently normal karyotypes.17 In this study, the presence of the rearrangement was also associated with a number of adverse clinical features and was a poor prognostic indicator.16 MLL rearrangements have been detected by Southern blotting in leukemias where no 11q23 translocation was demonstrated by conventional cytogenetics.15-19 These studies confirm the clinical importance of MLL gene rearrangements detected by Southern blotting in the management of infantile acute leukemia. The present study was undertaken to determine whether rearrangements of this locus carried similar importance in adults with AML-M4 and AML-M5.

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Confined cytogenetic analysis was successfully performed at the time of diagnosis on 50 of 65 (31 of 40 AML-M4 and 19 of 25 AML-M5) patients. Abnormalities of chromosome 11q23 were observed in 6 of 50 patients (4 of 31 AML-M4 and 2 of 19 AML-M5). All six patients with 11q23 abnormalities showed rearrangements by Southern blot analysis. Successful karyotype analysis was performed on cells from 8 of 9 remaining patients with MLL rearrangements. In six of the eight patients, no abnormal clone was detected by cytogenetic analysis. In the single enzyme rearrangements, no patients had BamHI rearrangements without EcoRI rearrangements, but two patients had EcoRI rearrangements without BamHI rearrangements (polymorphisms). One patient with AML-M4 had an inversion of chromosome 16 and an additional chromosome 22, and one patient with AML-M5 had an extremely complex karyotype (Table 2).

The clinical features at presentation for this cohort of patients were reviewed, including the sex and age of the patients, the presence of lymphadenopathy, hepatosplenomegaly, or CNS involvement, and the absolute blast count at diagnosis. The clinical features of the patients with MLL rearrangements did not differ significantly from those with germline MLL configurations. In particular, the MLL rearrangements were not associated with younger age or higher absolute blast count at presentation. In addition, the overall survival of the patients in whom blast cells were stored at

---

**Table 1. Clinical Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>AML-M4 Germline MLL</th>
<th>AML-M4 Rearranged MLL</th>
<th>AML-M5 Germline MLL</th>
<th>AML-M5 Rearranged MLL</th>
<th>AML-M4 &amp; AML-M5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>40</td>
<td>29</td>
<td>11</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>26:14</td>
<td>18:11</td>
<td>8:3</td>
<td>14:11</td>
<td>12:9</td>
</tr>
<tr>
<td>Median age (yr) (range)</td>
<td>52 (20-83)</td>
<td>54 (29-83)</td>
<td>51 (20-73)</td>
<td>43 (15-69)</td>
<td>43 (15-69)</td>
</tr>
<tr>
<td>Absolute blast count (x109/L)</td>
<td>15 (0.5-270)</td>
<td>6 (0.7-140)</td>
<td>14 (0.235)</td>
<td>11 (0-235)</td>
<td>71 (1.8-118)</td>
</tr>
<tr>
<td>Clinical outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>5 (12%)</td>
<td>4 (14%)</td>
<td>1 (9%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Remission</td>
<td>20 (57%)</td>
<td>14 (56%)</td>
<td>6 (60%)</td>
<td>13 (52%)</td>
<td>11 (52%)</td>
</tr>
<tr>
<td>Failed</td>
<td>8 (23%)</td>
<td>5 (20%)</td>
<td>3 (30%)</td>
<td>3 (12%)</td>
<td>3 (14%)</td>
</tr>
<tr>
<td>Early death</td>
<td>7 (20%)</td>
<td>6 (24%)</td>
<td>1 (10%)</td>
<td>8 (32%)</td>
<td>7 (33%)</td>
</tr>
<tr>
<td>Unassessable</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (4%)</td>
<td>0</td>
</tr>
</tbody>
</table>

---

**Fig 1. Analysis of genomic rearrangement of MLL by Southern blot hybridization.** Ten micrograms of DNA from each patient's bone marrow or peripheral blood at presentation was digested to completion with restriction enzymes EcoRI and BamHI. After transfer to Oncor membranes, hybridization was with a radiolabeled MLL partial cDNA probe. The figure shows the resulting autoradiographs from a number of AML-M4 patients. Rearranged bands are marked with an arrowhead.
diagnosis and who are included in this study was compared with the patients with the same diagnoses who presented in the last 10 years, but who did not have cells stored. No significant differences were found in the survival of AML-M4 and/or AML-M5 with and without stored cells.

Four of 50 patients with germline MLL and 1 of 15 patients with rearrangements received supportive treatment only for their leukemias because of their age or infirmity, and one patient with AML-M5 and an MLL rearrangement was unassessable for response, as he died of an unknown cause at home after one cycle of chemotherapy, and postmortem examination was refused. Fifty-nine assessable patients received chemotherapy with curative intent. The chemotherapy regimens used were a short-term therapy until 1988, and more recently, myeloablative therapy with autologous bone marrow transplantation as consolidation of first remission. The outcome regimes used were a short-term therapy until 1988, and more recently, myeloablative therapy with autologous bone marrow transplantation as consolidation of first remission. The chemotherapy was continued for response, as he died of an unknown cause at home after one cycle of chemotherapy, and postmortem examination was refused. Fifty-nine assessable patients received chemotherapy with curative intent. The chemotherapy regimens used were a short-term therapy until 1988, and more recently, myeloablative therapy with autologous bone marrow transplantation as consolidation of first remission.

Southern blotting has revealed MLL rearrangements in the absence of 11q23 abnormalities at the cytogenetic level. The presence of rearrangements in two separate restriction enzyme digestions reduces the possibility that the rearrangements are a consequence of polymorphisms or partial degradation of the DNA. The greater sensitivity of molecular analysis of the MLL gene compared with cytogenetic examination has been noted previously. The lower sensitivity of karyotype analysis may be due to submicroscopic abnormalities of 11q23, or because only a small percentage of the cells studied carry abnormalities. Furthermore, the accurate diagnosis of 11q23 translocations by conventional cytogenetics may be difficult.

The high prevalence of MLL rearrangements in FAB subtypes with monocytic differentiation is reminiscent of the association of specific molecular abnormalities with particular leukemia phenotypes. Chromosomal translocations found in AML that have been characterized at the molecular level and that are associated with specific phenotypes include the PMI/ RARA gene fusion [t(15;17)(q22;q21)] in AML-M3, the AMLIETO gene fusion [t(8;21)(q22;q22)] in AML-M2 and the MYH11/CBFB gene fusion [inv(16) (p13;q22)] in AML-M4 with eosinophilia. Each of these three genetic rearrangements yields chimeric transcripts similar to those detected with 11q23 translocations in which MLL is fused to a number of partner genes that have been identified including AF-4/FEL (on 4q21), AF-6 (on 6q27), AF-9/MLLT3 (on 9p21), and TEL (on 10p12) (Chaplin et al., manu-

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>FAB Subtype</th>
<th>Blast Count (x10^4/L)</th>
<th>Karyotype</th>
<th>Clinical Outcome</th>
<th>Survival (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>54</td>
<td>AML-M4</td>
<td>2.6</td>
<td>46,XX;16;11(q27;23) [10]</td>
<td>Early death</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>52</td>
<td>AML-M4</td>
<td>92</td>
<td>46,XY;16;11(q27;23) [10]</td>
<td>CR, relapsed, died</td>
<td>8.5</td>
</tr>
<tr>
<td>3 (e)</td>
<td>M</td>
<td>45</td>
<td>AML-M4</td>
<td>140</td>
<td>46,XY,inv(6)(11)(q27;13q23) [10]</td>
<td>CR, relapsed, died</td>
<td>13</td>
</tr>
<tr>
<td>4 (a)</td>
<td>M</td>
<td>61</td>
<td>AML-M4</td>
<td>2.0</td>
<td>46,XY (22);46,XY,del(11)(1q23;225)</td>
<td>CR, relapsed, died</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>51</td>
<td>AML-M4</td>
<td>0.92</td>
<td>46,XY [15]</td>
<td>Failed, died</td>
<td>0.5</td>
</tr>
<tr>
<td>6 (c)</td>
<td>M</td>
<td>54</td>
<td>AML-M4</td>
<td>6.0</td>
<td>46,XY [25]</td>
<td>CR, relapsed, died</td>
<td>12</td>
</tr>
<tr>
<td>7 (g)</td>
<td>M</td>
<td>73</td>
<td>AML-M4</td>
<td>5.5</td>
<td>46,XY [10]</td>
<td>Failed, died</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>66</td>
<td>AML-M4</td>
<td>0.74</td>
<td>46,XX [15]</td>
<td>Untreated</td>
<td>1</td>
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<tr>
<td>10</td>
<td>F</td>
<td>36</td>
<td>AML-M4</td>
<td>34</td>
<td>47,XX,inv(16)(p13q22),+22 [10]</td>
<td>CR, relapsed, died</td>
<td>31</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>93</td>
<td>AML-M4</td>
<td>12</td>
<td>Failed</td>
<td>Failed, died</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>68</td>
<td>AML-M5</td>
<td>46,XY,16;11(q22;23) [10]</td>
<td>Unassessable</td>
<td>Unassessable</td>
<td>0.5</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>28</td>
<td>AML-M5</td>
<td>62</td>
<td>46,XY <a href="10;11">34;46,XY</a>(p12;q23)</td>
<td>CR, relapsed, died</td>
<td>13</td>
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<tr>
<td>14</td>
<td>F</td>
<td>22</td>
<td>AML-M5</td>
<td>1.8</td>
<td>46,XX [23]</td>
<td>Early death</td>
<td>0.5</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>43</td>
<td>AML-M5</td>
<td>118</td>
<td>51,XX,46,XY,del(11)(q23;225)</td>
<td>CR, relapsed, died</td>
<td>13</td>
</tr>
</tbody>
</table>

The letters in parentheses after the patient numbers correspond to the lanes in Fig 2. The karyotypes are written according to the current ISCN recommendations. Abbreviations: CR, complete remission; ABMT, autologous bone marrow transplantation.

**DISCUSSION**

Conventional karyotype banding studies have suggested that the prevalence of 11q23 abnormalities in adults with AML is approximately 1%. These abnormalities are more common in the AML-M4 and AML-M5 subgroups, which constitute 35% of AML. Southern blot analysis of cells from 145 patients of all ages with acute leukemia showed MLL rearrangements in nine, including 3 of 30 cases of AML-M5, but not in patients with AML-M4. In this study, a high frequency of MLL rearrangement was detected in adult AML patients with these two subgroups of leukemia. The overall rate of MLL rearrangement in all cases of AML at our institution over the last 10 years exceeds 15 of 343 (4.4%), as rearrangements have also been found in other FAB subtypes with 11q23 translocations.

Southern blotting has revealed MLL rearrangements in the absence of 11q23 abnormalities at the cytogenetic level. The presence of rearrangements in two separate restriction enzyme digestions reduces the possibility that the rearrangements are a consequence of polymorphisms or partial degradation of the DNA. The greater sensitivity of molecular analysis of the MLL gene compared with cytogenetic examination has been noted previously. The lower sensitivity of karyotype analysis may be due to submicroscopic abnormalities of 11q23, or because only a small percentage of the cells studied carry abnormalities. Furthermore, the accurate diagnosis of 11q23 translocations by conventional cytogenetics may be difficult.

The high prevalence of MLL rearrangements in FAB subtypes of AML with monocytic differentiation is reminiscent of the association of specific molecular abnormalities with particular leukemia phenotypes. Chromosomal translocations found in AML that have been characterized at the molecular level and that are associated with specific phenotypes include the PMI/RARA gene fusion [t(15;17)(q22;q21)] in AML-M3, the AMLIETO gene fusion [t(8;21)(q22;q22)] in AML-M2 and the MYH11/CBFB gene fusion [inv(16) (p13;q22)] in AML-M4 with eosinophilia. Each of these three genetic rearrangements yields chimeric transcripts similar to those detected with 11q23 translocations in which MLL is fused to a number of partner genes that have been identified including AF-4/FEL (on 4q21), AF-6 (on 6q27), AF-9/MLLT3 (on 9p21), and TEL (on 10p12) (Chaplin et al., manu-
Further molecular analysis is necessary to ascertain the nature and consequence of MLL rearrangements when no translocations are detected by cytogenetics to determine whether these are cryptic gene fusions or deletions. The identification of the full range of partner genes will enable the detection of MLL gene rearrangements by reverse transcriptase-polymerase chain reaction (RT-PCR), which has been reported for the t(4;11)(q21;q23) translocation. This approach is more sensitive than Southern blotting and has potential clinical application to the monitoring of patients in hematologic remission.

In infantile acute leukemia, the presence of the MLL rearrangement identifies a large group of patients with unique clinical features and a poor prognosis, and many physicians recommend allogeneic bone marrow transplantation in first remission for these patients. This study, in contrast, suggests that in adults with AML-M4 and AML-M5, the presence of the rearrangement does not identify a subgroup of patients with different clinical features, nor does it carry prognostic significance.

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REFERENCES


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