The Translocation t(1;22)(p13;q13) Is a Nonrandom Marker Specifically Associated With Acute Megakaryocytic Leukemia in Young Children

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We present the nonrandom occurrence, frequency, and degree of immunophenotype association of the t(1;22)(p13;q13) in children with acute nonlymphocytic leukemia (ANLL). This karyotype anomaly occurred in leukemia cells from five of 445 (1.1%) children with newly diagnosed ANLL who were successfully studied by cytogenetic analysis at four European centers between January 1987 and January 1992. The occurrence of the t(1;22) was restricted to the French-American-British classification (FAB) subtype M7. The overall incidence in children with acute megakaryocytic leukemia (AMKL) was 27.6% (5/18 cases) versus 6.7% (4/6 cases). Three of the patients carrying this anomaly had a diploid karyotype, whereas in two cases a hyperdiploid karyotype was found. However, in all five patients, the t(1;22) was the only translocation event present at diagnosis. All patients received aggressive chemotherapy for acute myelogenous leukemia (AML). Two patients died within 15 months of diagnosis without entering remission. One of three patients who entered remission died 7 months after diagnosis, most likely from intramedullar hemorrhage. Only two of the five children with the t(1;22) who received autologous bone marrow transplantation (BMT) are alive and in complete remission (CR) 23 and 40 months after diagnosis, respectively. At the time of diagnosis, the age of the oldest child carrying the t(1;22) was 18 months. The cases with this chromosome anomaly were compared with an age-matched group of five children with AMKL lacking this translocation. The patients with the t(1;22) had a lower median value of the peripheral white blood cell (WBC) count and a higher median hemoglobin level than the patients from the matched group. In the latter cases, normocellular or hypercellular bone marrow (BM) was detected at diagnosis. In contrast, all children with the t(1;22) in our series had a hypocellular BM. Histological BM analyses were available in three of these patients and showed marked fibrosis. Other clinical and laboratory parameters showed no obvious differences between the matched groups. Despite intensive chemotherapy, AMKL in children appears to be associated with a poor prognosis. The clinical courses of the children with AMKL and the t(1;22) presented may be indicative of a beneficial effect of autologous BMT in this subset of patients.

A VARIETY OF CONSISTENT chromosome abnormalities, particularly translocations, have been identified in patients with acute nonlymphocytic leukemia (ANLL) as being specific for particular French-American-British classification (FAB) subtypes of ANLL. However, for acute megakaryocytic leukemia (AMKL), the FAB subtype M7, no characteristic chromosome anomaly has as yet been firmly established. This could be attributed to the formerly encountered difficulties in distinguishing megakaryocytic leukemia from other forms of acute leukemia and myelodysplastic syndromes, and to the fact that the diagnostic criteria for this subtype of ANLL have relatively recently been defined by the FAB Cooperative Group. Therefore, appropriate cytogenetic evaluation has been available only from a limited number of well-defined cases.

AMKL comprises a heterogeneous group of disorders characterized by the appearance of morphologically undifferentiated blasts of megakaryocytic lineage, which usually represent greater than 30% of bone marrow (BM) cells. Frequently observed clinical and hematologic findings include BM fibrosis, hepatosplenomegaly, and pancytopenia, although the platelet count may be normal or even increased. Until recently, detection and intracellular localization of platelet peroxidase (PPO) activity by electron microscopy provided the only means for reliable identification of megakaryocytic lineage. Immunological phenotyping with monoclonal antibodies (MoAbs) recognizing platelet glycoprotein (GP) complexes represents an alternative technique for identification of megakaryoblasts in routine diagnosis.

Based on recent data, the incidence of subtype M7 in ANLL appears to be 2% to 8% in adults, 4% in children, and approximately 20% in infants. In adults, AMKL has more frequently been observed as secondary leukemia after chemotherapy for a solid tumor, after leukemic transformation of either osteomyelofibrosis (OMF) or myelodysplastic syndrome (MDS), and during blast crisis of chronic myelogenous leukemia (CML). In children, AMKL generally appears de novo. Moreover, a strong association of AMKL with Down's syndrome has been reported.

Despite recent improvements in the immunological and ultrastructural definition of AMKL, the cytogenetic findings remain heterogeneous. A small number of infant patients with AMKL whose blast cells displayed the translocation t(1;22) are described. The patients, who generally appear de novo, have had leukemic transformation of either osteomyelofibrosis or myelodysplastic syndrome. In contrast to the adult cases, the initial presentation was often manifesting as an acute leukemia with BM fibrosis. Other clinical and laboratory parameters showed no obvious differences between the matched groups. Despite intensive chemotherapy, AMKL in children appears to be associated with a poor prognosis. The clinical courses of the children with AMKL and the t(1;22) presented may be indicative of a beneficial effect of autologous BMT in this subset of patients.

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cocation t(1;22)(p13;q13) have been observed by different investigators. In this report, we provide evidence that the t(1;22)(p13;q13) is a nonrandom cytogenetic anomaly that appears to be specific for ANLL-M7 in young children. Moreover, we compare the clinical and biological features that appears to be specific for ANLL-M7 in young children. MATERIALS AND METHODS

Patients. Specimens of leukemic cells (usually BM) from children with newly diagnosed ANLL were generally transferred to reference laboratories for immunologic marker studies and for cytogenetic characterization. A subgroup of 18 children, which included six infants, were diagnosed with AMKL. Three of these cases (no. 1, 6, and 7) were reported previously. Generally, the diagnosis of AMKL was based on (1) blast cell morphology suggestive of megakaryoblasts (see below), (2) absence of myeloperoxidase or Sudan black expression by the leukemic cells, and (3) expression of one or more platelet-associated markers (CD4la, CD42b, CD61) on at least 30% of the leukemic blasts. In one of the patients from whom very little material was available (no. 3), only a few immunologic markers were determined (CD10, 0%; CD13, 0%; HLA-DR, 14%), and these were diagnostically inconclusive. In this case, the diagnosis was based on the typical morphology of the blasts and was supported by cytochemistry studies (see Table 2). The absence of the common acute lymphocytic leukemia antigen (CALLA) and the monomyeloid-associated marker CD13 on the blast cells was compatible with the diagnosis of AMKL.

In the present study, children with AMKL carrying the t(1;22) whose ages at diagnosis ranged from 1 week to 18 months were compared with a matched group of children lacking this translocation.

Blast cell morphology. The blast cells of the patients presented were two to three times larger than normal lymphocytes and morphologically resembled undifferentiated myeloblasts with a high nucleocytoplasmic ratio. No granules were present in the cytoplasm. The blasts displayed cytoplasmic blebs and budding of platelets. The nuclei were round or polymorphic with finely reticulated or, in some instances, with slightly more condensed nuclear chromatin, and only rarely contained nucleoli.

Immunologic characterization. The patients were classified using standard immunofluorescence technology essentially as described elsewhere. Chromosome analysis. Cytogenetic studies were performed according to standard procedures as described previously. Chromosomes were either G-banded with trypsin7,18 or R- and C-banded with chromomycin A3, distamycin A, and 4'-6-diamino-2-phenylindole (DAPI). In two cases (no. 1 and 3), karyotyping was performed with a Genevision 121 chromosome analysis system (Applied Imaging, Santa Clara, CA).

RESULTS

In this study, 445 children with ANLL including 65 infants (<12 months at diagnosis) were investigated. The overall incidence of AMKL in this series was 4% (18/445 cases). In the infants studied, the incidence was 9.2% (6/65 cases). The t(1;22) was associated exclusively with the FAB subtype M7 and was present in 27.8% (5/18) children with AMKL. The incidence of this translocation among infants with AMKL was 66.7% (4/6 cases).

Clinical data and laboratory findings at presentation for each of the patients with the t(1;22) and the matched group of children lacking this translocation are shown in Table 1. Four of five patients with the t(1;22) (group A) and two of five patients without the translocation (group B) were girls. All patients had enlarged livers and spleens (Table 1). The patients' white blood cell (WBC) counts ranged from 3.0 to 27.2 x 10^9/L within group A (median, 13.0 x 10^9/L) and from 6.2 to 81.9 x 10^9/L within group B (median, 33.1 x 10^9/L), the hemoglobin levels from 6.9 to 9.5 g/dL within group A (median, 8.0 g/dL) and from 4.8 to 11.6 g/dL within group B (median, 5.1 g/dL), and the platelet counts from 4.0 to 30.0 x 10^9/L within group A (median, 18.0 x 10^9/L) and from 9.0 to 44.0 x 10^9/L within group B (median, 23 x 10^9/L). Histological analysis of BM biopsies showed myelofibrosis in three of five cases with the t(1;22) (patients 1, 2, and 4). In patients 3 and 5, BM histology was not

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex/Age (mo)</th>
<th>Hb (g/dL)</th>
<th>WBC (x 10^9/L)</th>
<th>Platelets (x 10^9/L)</th>
<th>Blast Cells in BM/PB (%)</th>
<th>Liver (cm)</th>
<th>Spleen (cm)</th>
<th>Cellularity* (BM)</th>
<th>Clinical Status (months after diagnosis)</th>
</tr>
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<tbody>
<tr>
<td>Group A</td>
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<tr>
<td>1</td>
<td>M/0.3</td>
<td>9.5</td>
<td>27.2</td>
<td>19</td>
<td>25/38</td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>CR (40)</td>
</tr>
<tr>
<td>2</td>
<td>F/2</td>
<td>6.9</td>
<td>13.0</td>
<td>18</td>
<td>32/27</td>
<td>10</td>
<td>8</td>
<td>1</td>
<td>Dead (16)</td>
</tr>
<tr>
<td>3</td>
<td>F/18</td>
<td>8.0</td>
<td>6.2</td>
<td>4</td>
<td>41/NA</td>
<td>10.5</td>
<td>7.5</td>
<td>1</td>
<td>Dead (3)</td>
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<tr>
<td>4</td>
<td>F/7</td>
<td>8.9</td>
<td>3.0</td>
<td>10</td>
<td>46/1</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>CR (23)</td>
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<tr>
<td>5</td>
<td>F/12</td>
<td>8.3</td>
<td>15.5</td>
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<td>44/52</td>
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<td>7</td>
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<tr>
<td>6</td>
<td>M/8</td>
<td>11.6</td>
<td>81.9</td>
<td>44</td>
<td>79/40</td>
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<td>7</td>
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<td>86/35</td>
<td>5</td>
<td>9</td>
<td>4</td>
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<tr>
<td>8</td>
<td>M/18</td>
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<td>4</td>
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<td>9</td>
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<td>35.8</td>
<td>16</td>
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<td>3</td>
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<tr>
<td>10</td>
<td>M/13</td>
<td>8.4</td>
<td>33.1</td>
<td>9</td>
<td>44/10</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>Ind (1)</td>
</tr>
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Group A: patients 1 through 5, AMKL cases with the t(1;22); group B: patients 6 through 10, AMKL cases without the t(1;22).
Abbreviations: Hb, hemoglobin; PB, peripheral blood; CR, complete remission; Ind, induction chemotherapy; NA, not available.

*Bone marrow cellularity: 1, very low; 2, low; 3, normal; 4, high; 5, very high.
available; however, hypocellularity was detected in BM aspirates. In the patients from group B, the BM was normocellular or hypercellular at diagnosis (Table 1).

Blast cells of all infants with AMKL investigated displayed morphological features typical of megakaryoblasts. All of these cases were myeloperoxidase- or Sudan black B-negative (Table 2). With the exception of patient 3, the expression of markers for megakaryocytic lineage, including antiplatelet GP Ib/IIa, antiplatelet GPIb, or antiplatelet GPIIIa, were detected on the surface of blast cells by the monoclonal antibodies CD41a, CD42b or CD61, respectively, thus confirming the diagnosis of AMKL. Selected results of the cell-surface phenotyping are summarized in Table 3. In addition to immunologic marker studies, in patient 2 the diagnosis was further assessed by the detection of intracellular PPO activity using electron microscopy.

The characteristic clonal abnormality present in patients 1 through 5 was an acquired balanced translocation t(1;22)(p13;q13) (Figs 1 and 2). In patients 1, 2, and 4, this translocation was the only chromosome abnormality detected. In two other patients (no. 3 and 5), the t(1;22) was part of a hyperdiploid karyotype (Table 4). Moreover, in the latter two patients, the product of the translocated chromosome 1 was duplicated (Fig 2). In addition to these clonal abnormalities, we also found a varying number of normal metaphases in all patients, which most probably represent normal hematopoietic cell populations.

The presence of a masked Ph-chromosome has been reported in a case of acute myelomonocytic leukemia with a t(1;22)(q12;q11).23 It was therefore intriguing to rule out the presence of a masked Ph-chromosome in our patients carrying the t(1;22). Material for molecular studies was available from one of the patients presented. Analysis by Southern blot and by PCR showed no involvement of the genes typically participating in the formation of the Ph-chromosome, the abl-oncogene and the breakpoint cluster regions of the BCR gene (Mbcr;mbcr) (data not shown).

All patients received aggressive multiagent chemotherapy according to the BFM 1987 or a similar protocol for ANLL.24,25

### Table 3. Immunological Phenotypes of Nine Young Children With AMKL

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>CD41</th>
<th>CD42</th>
<th>CD61</th>
<th>CD33</th>
<th>HLA-DR</th>
<th>CD34</th>
<th>CD13</th>
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<td>1</td>
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<td>50</td>
<td>50</td>
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<td>80</td>
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<td>2</td>
<td>72</td>
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<td>90</td>
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<td>7</td>
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<td>0</td>
<td>10</td>
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<td>10</td>
<td>90</td>
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</table>

Selected immunologic markers of AMKL patients with (group A) and without (group B) the t(1;22) are shown. In patient 3, immunological investigation of megakaryocytic markers was not available. The numbers are expressed as percent positive BM blasts.

Of the cases with AMKL and the t(1;22), patients 1, 4, and 5 achieved complete remission (CR). The latter patient died of respiratory arrest as a result of intramedullar hemorrhage or leukemic infiltration 7 months after diagnosis. In absence of adequate donors, patients 1 and 4

![Fig 1. Partial R-, C-, and G-banded karyotypes of normal and translocated chromosomes 1 and 22 from four young children with AMKL.](image-url)
received autologous bone marrow transplantation (BMT) in first CR and are still in remission 40 and 23 months after diagnosis (24 and 8 months after BMT), respectively. Patient 2 showed partial response to the chemotherapeutic treatment. However, progressive renal failure occurred and the child died at the age of 15 months. Patient 3 developed severe pancytopenia during the induction treatment. Despite dose reduction, the child died of intracranial hemorrhage shortly after the onset of therapy.

Of the patients with AMKL from group B, patients 6, 7, and 9 failed to achieve remission and died of septicemia within 20 months after diagnosis. Patient 8 achieved CR and still remains in this status 20 months after diagnosis. Patient 10 has only recently been diagnosed with AMKL and is currently receiving induction chemotherapy.

**DISCUSSION**

In this report, we present five children with AMKL (FAB M7) in whom a translocation t(1;22)(p13;q13) was detected and compare the clinical and laboratory findings with a matched group of five children with AMKL whose blast cells displayed different chromosome anomalies. The t(1;22) has been previously described in a small number of infants with megakaryocytic leukemia. In our series, this anomaly was present in four of six infants with AMKL investigated. One of the children carrying the t(1;22) was 18 months old at diagnosis (no. 5). However, this patient presented with anemia, thrombocytopenia, and hypocellular BM several months before the diagnosis of leukemia was established. This case may therefore be in accordance with the previous observation that the occurrence of this chromosome anomaly is restricted to infants with AMKL. Hence, our data may be compatible with the notion that this translocation represents a cytogenetic marker specific for AMKL in infants.

In two of our patients with the t(1;22), duplication of the chromosome 1 involved in the translocation has been observed. In a recent report on AMKL patients carrying the t(1;22), the cytogenetic findings in two cases also indicate the presence of a duplicated abnormal chromosome. A similar situation is encountered in leukemias with a t(9;22) in which duplication of the derivative chromosome 22, the
Ph-chromosome, can be part of the clonal karyotype evolution. In Ph-positive leukemias, the pathogenetically important gene rearrangement is located on chromosome 22. In accordance with these findings, we suggest that in the cases with a t(1;22) described, the critical genetic event occurs on chromosome 1.

Trisomy 19 was the only numeric change common to our two patients and two recently reported patients with the t(1;19) and a hyperdiploid karyotype. It may therefore represent a nonrandom secondary abnormality.

Involvement of chromosomes 1 and 22 has been observed in a number of patients with various hematologic malignancies, where one of the breakpoints was located within the same region of either chromosome 1 or 22. Although these observations were made in different types of leukemia, it was tempting to suspect that genes involved in malignant transformation, possibly oncogenes, may map to these breakpoints. Indeed, the regions on chromosomes 1 and 22 implicated in the translocation contain the NRAS (1p13), the YESP (22q11-12), and the SIS (22q12-13) loci, respectively.

The sis-oncogene, which encodes the platelet-derived growth factor-β (PDGF-β) has been described to play a causative role in the occurrence of myelofibrosis. The lack of material did not allow us to investigate the expression of c-sis in our cases; however, BM fibrosis was found in three of five patients carrying the t(1;22)(p13;q13). In the remaining two cases, histological analysis was not available. Nevertheless, in these patients, hypocellular BM was detected at diagnosis. The difference in BM cellularity between the matched groups of children with AMKL (Table 1) may allow speculations about a c-sis involvement in the cases with the t(1;22). In accordance with this notion, a recent study disclosed c-sis gene expression in noncultured cells and in an established cell line from a child with AMKL and Down’s syndrome. Moreover, amplification of a variant sis-oncogene has recently been reported in a case of atypical malignant lymphoma with a t(1;22)(q42;11.2).

Comparison between the two groups of young children with AMKL carrying or lacking the t(1;22) investigated in this study and pediatric AMKL cases described in the literature, did not show any obvious differences in clinical and laboratory findings. We are aware of nine previously reported cases of pediatric AMKL with the t(1;22) which indicate that this subset of patients may have a poor prognosis despite intensive ANLL-directed chemothera-

NOTE ADDED IN PROOF

Carrol et al and Baruchel et al were published after the submission of this manuscript.
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q13) is nonrandom and restricted to infants with acute megakaryoblastic leukemia: A Pediatric Oncology Group Study. Blood 78:748, 1991


23. Suciu S, Marinca E, Gadauleanu V: Masked Philadelphia chromosome due to the translocation (1q-;22q+) and a small chromosome 19 in a case of acute leukemia. Neoplasma 31:573, 1984


33. Tanaka T, Takahashi H, Miyashita Y, Imamura S: Chromosomal translocation t(1;22) and ss oncogene variant with gene amplification in a case of atypical malignant lymphoma. Cancer Invest 8:613, 1990
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