t(3;21)(q26;q22): A Recurring Chromosomal Abnormality in Therapy-Related Myelodysplastic Syndrome and Acute Myeloid Leukemia

By Charles M. Rubin, Richard A. Larson, John Anastasi, Jane N. Winter, Maya Thangavelu, James W. Vardiman, Janet D. Rowley, and Michelle M. Le Beau

We have identified an identical reciprocal translocation between the long arms of chromosomes 3 and 21 with breakpoints at bands 3q26 and 21q22, \(t(3;21)(q26;q22)\), in the malignant cells from five adult patients with therapy-related myelodysplastic syndrome (t-MDS) or acute myeloid leukemia (t-AML). Primary diagnoses were Hodgkin's disease in two patients and ovarian carcinoma, breast cancer, and polycythemia vera in one patient each. Patients had been treated with chemotherapy including an alkylating agent for their primary disease 1 to 18 years before the development of t-MDS or t-AML. We have not observed the \(t(3;21)\) in over 1,500 patients with a myelodysplastic syndrome or acute myeloid leukemia arising \textit{de novo} or in over 1,000 patients with lymphoid malignancies. We have previously reported that the \(t(3;21)\) occurs in Philadelphia chromosome-positive chronic myelogenous leukemia (CML). Thus, the \(t(3;21)\) appears to be limited to t-MDS/t-AML and CML, both of which represent malignant disorders of an early hematopoietic precursor cell. These results provide a new focus for the study of therapy-related leukemia at the molecular level.

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cisplatin intravenously every 3 weeks followed by eight additional cycles of cyclophosphamide as a single agent.

In May 1984 at age 71 years, she was clinically free of ovarian carcinoma, but was found to have unexplained pancytopenia. Chemotherapy was discontinued and tamoxifen was prescribed. Bone marrow examinations performed in July 1984 and October 1984 showed myelodysplasia. Cytogenetic studies of the bone marrow were performed in October 1984. An adnexal mass representing ovarian carcinoma was detected at that time, but no further therapy was given. In December 1984 she died of cardiac arrest; the precise cause of the arrest was not determined. No specific treatment for the t-MDS had been given.

Patient 2. This woman was diagnosed with carcinoma of the breast in 1969 at age 48 years. At that time she underwent a radical mastectomy followed by three injections of thiopeta. In January 1985 she developed a malignant mass in the abdomen, which, based on aspiration cytology, was believed to be metastatic breast cancer. From January 1985 to September 1986 she received chemotherapy including doxorubicin, vincristine, fluoroxymesterone, tamoxifen, and dibromodulcitol; a complete response was obtained. In January 1987, at age 65 years, a bone marrow examination and cytogenetic studies were performed to evaluate unexplained pancytopenia; myelodysplastic syndrome was diagnosed. A 21-day trial of low-dose subcutaneous cytosine arabinoside was administered; a complete hematologic and cytogenetic response was documented in March 1987. In July 1987 the hematologic disease recurred and was refractory to low-dose cytosine arabinoside. By October 1987 there

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### Table 1. Clinical and Hematologic Features at Diagnosis, Treatment, and Outcome for Five Patients With t-MDS or t-AML and the t(3;21)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Primary Disease</th>
<th>Age at onset (yrs)</th>
<th>Type</th>
<th>Therapy</th>
<th>Age at onset (yrs)</th>
<th>Secondary Disease</th>
<th>Interval since first chemotherapy (mo)</th>
<th>Type</th>
<th>Peripheral Blood</th>
<th>Bone Marrow</th>
<th>Therapy</th>
<th>Initial response</th>
<th>Progression to t-AML</th>
<th>Survival (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>Ovarian carcinoma</td>
<td>70</td>
<td>t-MDS</td>
<td>Chemotherapy</td>
<td>71</td>
<td>No</td>
<td>12</td>
<td>t-MDS</td>
<td>Hemoglobin (g/dL)</td>
<td>10</td>
<td>0.10</td>
<td>-</td>
<td>Complete</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>Breast carcinoma</td>
<td>48</td>
<td>t-MDS</td>
<td>Yes</td>
<td>65</td>
<td>No</td>
<td>210</td>
<td>t-MDS</td>
<td>Platelets (x 10^9/L)</td>
<td>10</td>
<td>0.13</td>
<td>-</td>
<td>Partial</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>Hodgkin’s disease</td>
<td>29</td>
<td>t-MDS</td>
<td>Yes</td>
<td>32</td>
<td>No</td>
<td>36</td>
<td>t-MDS</td>
<td>White blood cells (x 10^9/L)</td>
<td>5</td>
<td>0.05</td>
<td>-</td>
<td>Partial</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>Hodgkin’s disease</td>
<td>49</td>
<td>t-MDS</td>
<td>Yes</td>
<td>52</td>
<td>No</td>
<td>35</td>
<td>t-AML</td>
<td>Blasts (%)</td>
<td>45</td>
<td>0.01</td>
<td>-</td>
<td>Partial</td>
<td>45</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>Polycythemia vera</td>
<td>54</td>
<td>t-AML</td>
<td>Yes</td>
<td>63</td>
<td>Yes</td>
<td>47</td>
<td>t-AML</td>
<td>Cellularity (%)</td>
<td>100</td>
<td>0.01</td>
<td>-</td>
<td>Marrow hypoplasia</td>
<td>-</td>
</tr>
</tbody>
</table>

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### Table 2. Cytogenetic Studies at Diagnosis of Five Patients With t-MDS or t-AML and the t(3;21)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Source</th>
<th>No. of Metaphase Cells Examined</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bone marrow</td>
<td>22</td>
<td>46,XX (32%)/46,XX,t(3;21)(q26;q22) (68%)</td>
</tr>
<tr>
<td>2</td>
<td>Bone marrow</td>
<td>31</td>
<td>46,XX (3%)/46,XX,t(3;21)(q26;q22) (97%)</td>
</tr>
<tr>
<td>3</td>
<td>Peripheral blood</td>
<td>29</td>
<td>46,XY (31%)/46,XY,del(5)(q13q34),t(3;21)(q26;q22) (7%)/46,XY,-5,13t(21)(q26;q22),+mar (62%)</td>
</tr>
<tr>
<td>4</td>
<td>Bone marrow</td>
<td>31</td>
<td>46,XY (23%)/46,XY,-7,t(3;21)(q26;q22),+der(7)t(7?)(q21;?) (77%)</td>
</tr>
<tr>
<td>5</td>
<td>Bone marrow core</td>
<td>20</td>
<td>46,XX,-12,-13,t(3;21)(q26;q22),+der(1)t(1;21)(p11;q21),+der(12)t(6;12)(q22;q21),+mar (100%)</td>
</tr>
</tbody>
</table>
was progression to acute myeloid leukemia. No response was observed following treatment with a 5-day course of mitoxantrone. She died from progressive leukemia and infection in March 1988.

**Patient 3.** This man was diagnosed with stage IV Hodgkin’s disease at age 29 years. From June 1985 to May 1986 he received alternating courses of MOPP (nitrogen mustard, vincristine, procarbazine, prednisone) and ABVD (doxorubicin, bleomycin, vincristine, dacarbazine) for a total of six courses each; a complete response was obtained. In June 1988 at age 32 years, he became severely pancytopenic; bone marrow examination revealed marked hypoplasia and myelodysplastic syndrome. There was no evidence of Hodgkin’s disease. Cytogenetic studies were performed. In December 1988 he began receiving granulocyte-macrophage colony-stimulating factor (GM-CSF) and had a marked rise in granulocyte count, as well as improvement in red blood cell and platelet counts, but the karyotype remained abnormal. The hematologic disease progressed to acute leukemia in October 1989 and did not regress after discontinuation of the GM-CSF therapy. He died from Aspergillus pneumonia in February 1990 after intensive remission induction chemotherapy.

**Patient 4.** This man was diagnosed with stage IV Hodgkin’s disease with involvement of the thoracic spine in May 1986 at age 49 years. He received 40 Gy of radiation to the thoracic spine in June 1986. Shortly thereafter, he received two cycles of MOPP without response and one cycle of ABVD. In February 1987 he received 40 Gy of radiation to the hip for a recurrence. From June 1987 to February 1989 he received courses of lomustine (CCNU), vinblastine and dexamethasone alternating with doxorubicin and etoposide (VP-16).

In April 1989 at age 52 years he developed severe anemia and thrombocytopenia; bone marrow examination revealed a myelodysplastic syndrome. Cytogenetic studies were performed. Initial therapy consisted of high-dose cytosine arabinoside; he achieved a partial remission, but died 3 months later with t-MDS.

**Patient 5.** This woman was diagnosed with myelofibrosis in 1976 at age 54 years. No treatment was given until April 1982 when leukocytosis, thrombocytosis and splenic enlargement prompted initiation of busulfan therapy. In March 1984 she was noted to have a hemoglobin of 18.5 g/dL, and during the following year underwent several phlebotomy procedures. At this point her diagnosis was considered to be polycythemia vera.

In March 1986 at age 63 years, while continuing on busulfan therapy, she was noted to have increasing splenomegaly; hematologic evaluation revealed acute myeloid leukemia. Cytogenetic studies were performed. Treatment with high-dose cytosine arabinoside resulted in marked marrow hypoplasia. While pancytopenic, she developed pneumonia and died of respiratory failure.

**Morphologic Findings at Diagnosis of Therapy-Related Hematologic Disease**

Morphologic features in all five cases were similar to those reported previously in t-MDS and t-AML. Findings are summarized in Table 1. In patients 1, 2, and 3, findings were consistent with a diagnosis of refractory anemia with excess blasts. In each of these cases the peripheral smear showed marked thrombocytopenia, aniso-poikilocytosis of red blood cells with macroovalocytosis, and mild-to-moderate granulocytic dysplasia as evidenced by decreased cytoplasmic granulation and/or nuclear atypia. Circulating blasts were not identified. Bone marrow biopsies and aspirates demonstrated that marrow cellularity was substantially decreased, ranging between 5% and 10%. Megakaryocytes were severely decreased in number and were usually too few to evaluate for dysplasia. Granulocytic maturation in all of these cases was left shifted and dysplastic, and blasts were only moderately elevated to 7% to 13%. The erythroid series had megaloblastic changes and dysplasia in the form of nuclear budding. Additionally, each of these cases had increased reticulin fibrosis at least focally, and increased iron stores. The disease in patient 4 had features similar to those noted above and likewise could be classified as refractory anemia with excess blasts. The cellularity in this case, however, was increased because of a marked marrow eosinophilia (60% eosinophils). The eosinophils appeared dysplastic. The fifth patient had a hypercellular bone marrow and sufficient number of blasts and monocytes to warrant a diagnosis of acute myelomonocytic leukemia. The marked decrease in megakaryocytes, and the findings of left-shifted and dysplastic granulocytic elements, moderately dysplastic erythroid precursors, and focal fibrosis were similar to those in the other cases.

**Cytogenetic Findings at Diagnosis of Therapy-Related Hematologic Disease**

An identical reciprocal chromosomal translocation between the long arms of chromosomes 3 and 21 with breakpoints at bands 3q26 and 21q22 [t(3;21)(q26;q22)] was observed in each of the five patients (Fig 1). The abnormality was clonal and was present in 68% to 100% of metaphase cells analyzed. In two patients (patients 1 and 2), the t(3;21) was the only cytogenetic abnormality. In the remaining three patients, all abnormal cells contained the t(3;21); however, additional chromosome changes were also present in these cells. Patient 3 had an interstitial deletion of the long arm of chromosome 5 with loss of material from bands 5q13 to 5q34 [del(5)(q13q34)] in the primary abnormal clone; in a secondary clone, the entire chromosome 5 was lost, and an unidentified marker chromosome was present. Patient 4 had an unbalanced translocation that resulted in loss of material from the long arm of chromosome 7 distal to band 7q21 [–7, +der(7)(7qh;?)]. Patient 5 had multiple abnormalities including unbalanced translocations involving chromosomes 1 and 21 [–21, +der(1)(1;21)(p11;q21)] and chromosomes 6 and 12 [–6,–12, +der(12)(6;12)(q22;q21)], and an unidentifiable marker chromosome. The 1;21 translocation results in trisomy for the long arm of chromosome 1.

**DISCUSSION**

We identified an identical t(3;21) in four patients with t-MDS following therapy for solid tumors and in one patient with t-AML following a long history of polycythemia vera. In
In therapy-related MDS and AML

Fig 1. Partial karyotypes of metaphase cells demonstrating the t(3;21). Each horizontal row includes chromosomes 3 and 21 from one metaphase cell from each patient. Numbers on the left correspond to the patient identification numbers in Tables 1 and 2. Rearranged chromosomes are located on the right of each pair of homologs and are identified with arrows. Leukemia cells in patient 5 lacked a normal chromosome 21.

the latter individual, it is not known whether the acute leukemia represents evolution of the primary hematologic condition or a new neoplasm; in either case, therapeutic exposure may have played a causative role in the acute process. All five patients had received chemotherapy including an alkylating agent for their primary disease, and one patient also had radiotherapy. The t(3;21) abnormality appears to represent a relatively early event in the malignant process, because in two patients it was the sole cytogenetic abnormality, and in every patient it was present in all chromosomally abnormal cells. Two patients had associated chromosomal abnormalities highly characteristic of t-MDS/t-AML, namely loss of material from the long arm of chromosomes 5 or 7. This observation suggests that the hematologic processes in the patients with the t(3;21) are closely related to those observed in other patients with therapy-related myeloid disease.

Two other patients with apparent secondary myeloid hematologic processes in association with a 3;21 translocation have been reported. A 60-year-old woman, who was treated 3 years earlier with surgery, radiation therapy, and cyclophosphamide for breast cancer, developed acute megakaryoblastic leukemia; cytogenetic studies revealed the t(3;21)(q26;q22) and a duplication of the long arm of chromosome 1. A 57-year-old man developed acute myeloid leukemia 10 years after the diagnosis of polycythemia vera; cytogenetic studies demonstrated the t(3;21)(q26;q22) as the only abnormality in the primary clone. This patient had received radioactive phosphorus isotope, two alkylating agents, and hydroxyurea at various times for control of the polycythemia vera.

Although the frequency of abnormalities of chromosomes 5 and 7 is high in t-MDS and t-AML, recent studies suggest that there may be other subgroups of this disease, such as therapy-related monoblastic leukemia with a t(9;11)(p21-22; q23). Patients with the t(3;21) may represent another new subgroup of t-MDS/t-AML. We have not identified clinical or morphologic features that distinguish patients with the t(3;21); however, experience with a larger number of patients is needed.

The t(3;21) is not specific for t-MDS/t-AML. We first described the same rearrangement in patients with Philadelphia chromosome-positive CML; this has been confirmed by other investigators. In our patients, this abnormality was limited to treated CML patients who had evidence of disease progression, suggesting the possibility that the therapy played a role in the genesis of the t(3;21). However, the t(3;21) has been observed in a few patients in the chronic phase of CML without prior treatment. We have not observed the t(3;21) in 1,516 patients with de novo myelodysplastic syndrome or acute myeloid leukemia, or in 1,101 patients with a lymphoid malignancy.

The breakpoints of the t(3;21) are of interest with respect to previously identified cytogenetic abnormalities in leukemia. The breakpoint in 3q26 corresponds to one of the breakpoints in the t(3;3)(q21;q26), and the inv(3)(q21q26), both associated with acute myeloid leukemia and abnormal megakaryocytopenia. The high platelet counts and numerous micromegakaryocytes typically observed in the t(3;3) and inv(3) cases, were not features of the t(3;21) cases reported here, nor of most of the CML patients with the t(3;21). The breakpoint in the t(3;21) at band 21q22 also corresponds to one breakpoint in another specific abnormality found in AML de novo, namely the t(8;21)(q22q22).

By analogy to other recurring chromosomal rearrangements in human leukemia that have been studied at the molecular level, the consistency of the breakpoints in the t(3;21) implies that specific genes in bands 3q26 and 21q22 participate in this rearrangement. It is possible the abnormality results in a specific genetic mutation critical to the malignant process. Candidate genes on chromosome 3 near band 3q26 include the transferrin receptor gene (TFRC) and the homolog of the Friend murine leukemia virus...
integration site 3 (FIM3). Candidate genes on chromosome 21 near band 21q22 include a homolog of the avian erythroblastoisis virus E26 oncogene (ETS2), another human gene related to the oncogene of E26 (ERG), and the genes encoding the receptors for interferon α (IFNAR) and β (IFNBR). Thus, the identification of this recurring abnormality in t-MDS and t-AML provides a new focus for molecular biologists to study these diseases.

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REFERENCES

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