Clinical and Molecular Characterization of a Rare Syndrome of Acute Promyelocytic Leukemia Associated With Translocation (11;17)


Analysis of a variant translocation t(11;17) in a case of acute promyelocytic leukemia (APL) led to discovery of a novel zinc finger gene, PLZF, fused to the retinoic acid receptor-α (RARα) gene. We reviewed the clinical and molecular features of five additional patients with t(11;17)-associated APL. The clinical course of three patients was characterized by early death and three experienced disseminated intravascular coagulation. Morphologically all of the patients fell in an unusual morphologic spectrum of APL, with features intermediate between M2 and M3 AML. All six patients had PLZF-RARα gene fusion as detected by reverse transcription/polymerase chain reaction assay, Southern blotting, or pulsed-field gel electrophoresis. Five of the six patients failed to achieve complete remission after initial chemotherapy or differentiation therapy with all-trans retinoic acid (ATRA). A sixth patient responded to initial chemotherapy, but on relapse failed to respond to ATRA. When tested in vitro, cultured cells from three of the patients failed to differentiate in response to ATRA. APL associated with t(11;17) and fusion of the PLZF and RARα genes is a discrete clino-pathologic syndrome with a distinctly worse prognosis than t(15;17) APL.

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Molecular Analysis of chromosomal translocations associated with cancer have identified genes involved in the pathogenesis of neoplasia, many of which encode DNA binding transcription factors. Chromosomal rearrangements may lead to aberrant expression of these factors, as in the case of Burkitt’s lymphoma associated with t(8;14) and the fusion of the myc oncogene with the Ig heavy chain promoter. Alternatively, chromosomal translocations may yield fusion genes encoding chimeric transcription factors with novel DNA binding or transcriptional effector properties. Acute promyelocytic leukemia (APL) is associated with the consistent chromosomal translocation t(15;17) fusing the promyelocytic leukemia (PML) and retinoic acid receptor-α (RARα) genes. Cyto genetic or molecular evidence of this translocation is detected in 90% to 100% of patients with morphologically diagnosed APL. This disease is uniquely sensitive to treatment with all-trans retinoic acid (ATRA) yielding complete remission (CR) rates of 75% to 95%. APL is also highly responsive to conventional chemotherapy. Although the disease is associated with higher rates of early mortality, largely caused by coagulopathy, the long-term prognosis for APL is more favorable than other forms of acute myelogenous leukemia, with 5-year survival rates of 35% to 45%

The molecular pathology of t(15;17) APL may be largely explained by the chimeric PML-RARα transcription factor generated by the t(15;17) translocation. The PML-RARα fusion protein interferes with retinoic acid (RA)- and RARα-mediated transcription, known to be important for normal myeloid differentiation. When transduced into the model leukemia cell lines HL60 or U937, the PML-RARα fusion protein prevents differentiation of these cells in the presence of physiologic levels of ATRA (10⁻¹⁰ mol/L) and actually enhances differentiation in the presence of pharmacologic doses of ATRA (10⁻⁶ mol/L). This finding parallels the clinical observation that t(15;17) APL can be treated to CR in a high number of cases with ATRA alone. These remissions are not caused by the elimination of the malignant clone, but are rather caused by the induction of differentiation in the clinical setting. Peripheral blood granulocytes from patients with APL treated with ATRA still harbor the t(15;17) translocation and resulting PML-RARα fusion RNA, suggesting that the malignant clone persists. This finding may explain the high rate of relapse of these patients when treated with ATRA alone without subsequent chemotherapy.

Recently, in a survey of Chinese patients with morphologically diagnosed APL (French-American-British [FAB] M3), a novel translocation t(11;17) (q23;q21) was identified. In a manner analogous to t(15;17) APL, t(11;17) creates novel fusion genes by linking the gene encoding RARα to a gene encoding a protein with 9 zinc finger motifs named PLZF (promyelocytic leukemia zinc finger). The index patient was treated with ATRA, but failed to respond to therapy. Subsequently, we have identified five other leukemic patients with the t(11;17) and fusion of the RARα and PLZF genes. Four of these additional patients were diagnosed with APL by morphology and one was identified from among a group of patients with leukemia bearing features of both myeloid and natural killer cells. Upon review, all of the cases were...
found to have cytologic features intermediate between M2 and classical M3 acute myeloid leukemia (AML). In contrast with the more typical t(15;17)-associated APL, five of six patients with t(11;17) APL did not respond to initial conventional or ATRA therapy. The sixth patient responded to initial chemotherapy, but had a short disease-free interval and failed to respond to ATRA upon relapse. Together, these data indicate that (11;17) APL with PLZF-RARα fusion constitutes a distinct clinical syndrome whose pathophysiology and prognosis are significantly worse than that of classical t(15;17) APL with PML-RARα fusion.

MATERIALS AND METHODS

Case Reports

Case no. 1 (Shanghai, China). The index case, who has been previously reported, was a 67-year-old Chinese man from Shanghai who presented with a white blood cell count (WBC) of 4.1 × 10^9/L. Bone marrow (BM) aspirate results showed a hypercellular marrow with 69% promyelocytes and the diagnosis of M3 AML was made (Fig 1A). Cytogenetic analysis showed 46 XY t(11;17) (q23;q17) (q23;q21). The patient was treated with ATRA at a dose of 60 mg/day for 19 days. In response, he developed leukocytosis, with the WBC increasing to 131 × 10^9/L with minimal evidence of differentiation. The patient died of respiratory failure on day 20 of treatment.

Case no. 2 (Paris, France). A 68-year-old Belgian man, also described elsewhere, presented in April 1990 with a hemoglobin level (HGB) of 100 g/L and a WBC of 10.6 × 10^9/L. BM aspirate results showed 70% abnormal promyelocytes, many with Auer Rods (Fig 1B). The diagnosis of M3 AML was made. Cytogenetic analysis showed t(11;17) (q23;q21). The patient was initially treated with daunorubicin (30 mg/m^2/d on days 1 through 3) and cytosine arabinoside (Ara-C; 100 mg/m^2/d on days 1 through 7). BM aspiration in May 1990 showed 50% promyelocytes. In June 1990, ATRA was administered orally at a dose of 45 mg/m^2/d for 22 days. Subsequently, the percentage of promyelocytes in the BM declined from 35% to 25%. ATRA was discontinued because of a drug shortage and was restarted in September 1990, at which time the patient had 65% promyelocytes in the BM with t(11;17) in 9 of 10 metaphases. ATRA was administered for 90 days and was followed by a decline in marrow promyelocytes from 65% to 1% to 2%. However, histologic and cytogenetic remissions were not achieved. Therapy was withdrawn in December 1990, with a rapid rebound of the marrow promyelocytes to 65%. High-dose Ara-C and mitoxantrone were administered, resulting in a brief CR. However, the patient died in relapse 4 months later in septic shock.

Case no. 3 (New York, NY). An 81-year-old man with a history of prior deep venous thrombosis treated with coumadin presented in early November 1992 with fatigue, dyspnea, fever, and bone pain. On admission to the hospital, the WBC was 7.6 × 10^9/L, with 14% neutrophils, 18% bands, and 7% promyelocytes. The hematocrit level (HCT) was 30%, with a mean corpuscular volume of 85 μL, and the platelets were 103 × 10^9/L. The prothrombin time (PT) was 17.6 seconds; however, the patient received 2.5 mg of coumadin only once per week. The fibrinogen level was depressed at 700 mg/L and the amounts of the fibrin split products were elevated. A BM biopsy was performed, leading to bleeding requiring 30 minutes of direct pressure to resolve. The BM aspirate results showed 90% cellularity and 90% of the cells were promyelocytes. Faggot cells and Auer rods were present, but most cells lacked primary granules and the cytoplasm of the cells was salmon colored, suggesting the presence of abnormal primary granules. The diagnosis of micromyelocytic leukemia (M3v-FAB) complicated by disseminated intravascular coagulation (DIC) was made (Fig 1C). Cytogenetic analysis showed 46XY t(11;17) (q23;q21). The patient was treated with 500 U/h of heparin and oral ciprofloxin and received oral ATRA at 45 mg/m^2/d for a planned 30-day course. The WBC increased to 25 × 10^9/L, with a decrease in the percentage of promyelocytes in peripheral smears. DIC improved so that there was only a slight elevation of fibrin split products and a near normal fibrinogen level by the time of death. The administration of ATRA led to lip chapping and headache. The patient also developed respiratory distress, which was initially attributed to pneumonia and treated with intravenous cefazidime. After respiratory distress persisted, the retinoic acid/pulmonary syndrome and pulmonary edema were considered as etiologies but a ventilation/perfusion scan showed multiple perfusion deficits consistent with pulmonary emboli. An inferior vena cava filter was then placed. Despite a platelet count greater than 50 × 10^9/L, low levels of fibrin split products, and a normal fibrinogen level, the patient developed a spontaneous brain stem hemorrhage, diagnosed by computed tomography, and died on day 18 of therapy.

Case no. 4 (Albuquerque, NM). A 37-year-old American black woman presented in April 1991 with an HGB of 69 g/L, an HCT of 20%, a platelet count of 30 × 10^9/L, and a WBC of 45.2 × 10^9/L (64% myelocytes, 16% segmented forms, 11% lymph, 4% monocytes, and 1% basophils). Pretreatment BM evaluation showed a myeloid/erythroid ratio of 50:1 with 47% blasts, 10% promyelocytes, 9% metamyelocytes, 3% bands, 5% lymphocytes, and 2% erythroid precursors (Fig 1D). The blasts stained positive for Sudan Black B and negative for α-naphthyl butyrate and α-naphthyl acetate. The patient was initially classified as M2 by the FAB criteria, although the peripheral blood showed leukocytosis with an arrest at the myelocyte stage reminiscent of a myeloproliferative disorder. Cytogenetic studies showed 46, XX t(11;17) (q23;q21). As determined by immunophenotyping, the leukemic blasts were CD33+, CD13+, CD56+, CD16+. The patient was treated with daunorubicin (45 mg/m^2, days 1 through 5) and Ara-C (200 mg/m^2, days 1 through 7) according to Southwest Oncology Group Protocol 8601, with complications of vaginal bleeding and gram-negative sepsis. After marrow recovery, BM examination in May 1991 showed 2% promyelocytes, 55% myelocytes, 13% metamyelocytes, and 18% segmented forms but no myeloblasts. Cytogenetic analysis showed the persistence of t(11;17) and no Philadelphia chromosome was detected. The patient was then treated with α-interferon (1 × 10^8 U/d) without effect. In August 1991, she presented with fever, bone pain, and myeloblasts in the BM aspirate. She was treated with mitoxantrone (12 mg/m^2, days 1 through 5) and etoposide (100 mg/m^2, days 1 through 5), which induced a remission. In November 1991, she had an HCT of 55% and a WBC of 4.0 × 10^9/L, with 1% myelocytes in the differential count and no blasts seen. The patient was lost to further medical follow-up and died in March 1992. No autopsy was performed.

Case no. 5 (Paris, France). A 34-year-old French man presented with bone pain and neutropenia. The initial WBC was 2.4 × 10^9/L, the platelets level was 168 × 10^9/L, and the HGB was 127 g/L. There were 23% blasts in the peripheral blood. BM examination showed 63% myeloblasts with Auer rods and typical promyelocytic morphology (Fig 1E). Cytogenetic analysis showed a t(11;17) and reverse transcription/polymerase chain reaction (RT/PCR) indicated the presence of a fusion between the PLZF and RARα genes. The patient was randomized to a protocol arm containing 31 days of oral ATRA (45 mg/m^2 on days 1 through 31), followed by daunorubicin (60 mg/m^2 on days 3 through 5) and Ara-C (200 mg/m^2 on days 3 through 9). During the first 5 days of treatment, the WBC count remained stable, but the percentage of blasts in the peripheral blood increased to 59%. By day 8, peripheral blasts disappeared secondary to myeloablative therapy. At diagnosis, mild DIC was present, without complication of skin or mucosal bleeding. The PT was 17.5 seconds (11.6 seconds for the control) and fibrin split products were elevated at a dilution of between 1/64 and 1/1024. D-dimers were detected at a 1/32 dilution and the fibrinogen level was 800 mg/L.
The patient received low-dose heparin at 100 mg/kg/d on days 4 through 26 of treatment. DIC persisted in the absence of peripheral blasts and marrow examination at day 11 showed 95% blasts. BM examination on day 17 showed the persistence of blasts. DIC also persisted, with a fibrinogen level of 1,200 mg/L and a PT of 15 seconds (11.6 seconds for the control); fibrin split products were detected at a dilution of 1/256 and D-dimers at a 1/16 dilution. The patient was then treated with amsacrine, (225 mg/m² on days 1 through 3) and high-dose Ara-C (1 g/m² every 12 hours on days 1 through 3). After 4 days, DIC resolved and normal hematopoietic
recovery was noted by 21 days after the second induction therapy. Marrow examination indicated a CR, which has persisted for 4 months.

**Case no. 6 (Houston, TX).** A 53-year-old man presented in May 1989 after a 1-month history of easy bruising. At diagnosis, the WBC was 15.3 x 10^9/L with a differential count including 16% blasts and 27% promyelocytes, an HGB of 105 g/L, and a platelet count of 120 x 10^9/L. BM aspiration results showed 90% cellularity with 45% promyelocytes and 3% blasts and the diagnosis of APL was made (Fig 1F). The karyotype was 46XY + (3) + (13) (q34), t(11;17)(q23;q21). The fibrinogen level was 1.300 mg/L and the fibrin split products were detected at a dilution of 1/160, indicating the presence of DIC. The patient received daunorubicin and Ara-C and achieved a CR after two treatment cycles. Maintenance therapy was administered using the same drugs, but the patient relapsed in April 1990, 7 months after the completion of therapy. A second remission was obtained with daunorubicin, Ara-C, and granulocyte-macrophage colony-stimulating factor (GM-CSF). A second relapse occurred in April 1991 and a third remission was induced with etoposide and mitoxantrone. Maintenance therapy was administered consisting of monthly cycles of prednisone, vincristine, 6-mercapto-purine, and methotrexate, but in August 1991 a third relapse was noted. At that time, the WBC was 2.9 x 10^9/L and the BM aspirate results showed 90% cellularity with 25% promyelocytes. The karyotype remained unchanged from that of the initial diagnosis. The patient received ATRA at a dose of 50 mg/m^2/d and there was a steady increase in the WBC such that by day 10 of therapy the WBC was 29 x 10^9/L. With 36% promyelocytes and 5% blasts. Repeat BM aspirate results then showed 74% promyelocytes. Idarubicin was administered at 12 mg/m^2/d for 4 days, leading to only a transient decrease in the peripheral and marrow promyelocyte count. The patient then received fludarabine and Ara-C, with complications of congestive heart failure, DIC, and acute renal failure leading to the patient’s death in December 1991.

**Southern Blot Analysis**

After receiving informed consent, BM aspirations were obtained from the patients and DNA was extracted as described. The genomic DNA was digested with restriction enzymes as noted, electrophoretically separated through a 0.7% agarose gel, and transferred to nitrocellulose. A 5.5-kb EcoRI genomic fragment of the RARx gene or a 2.3-kb BamHI/Bgl II genomic fragment of the PLZF gene was labeled by the random priming method with α-32P dCTP and allowed to hybridize to the filters in a solution of 0.5X SSPE, 0.1% SDS, 10°C for 1 to 5 days. All the hybridizations and washings were performed in a hybridization oven (Robbins Scientific Inc, Sunnyvale, CA), as described previously, with the final wash performed at 42°C. The blots were analyzed using a Phosphorimager and ImageQuant software (version 3.3; Molecular Dynamics, Sunnyvale, CA) and exposed with an intensifying screen at -80°C.

**PCR for Detection of the PLZF-RARα Fusion mRNA**

RNA was extracted from BM specimens using RNAzol (Chemed Biotech, Friendship, TX). Approximately 4 μg of RNA from each leukemic sample was hybridized to a primer ( Primer-RT; Fig 3B) (5’ TGGATGCTGGCGCAAGAGACCCCATC (primer-1) or a 4.5-kb BamHI/Hind I genomic RARα fragment and a primer complimentary to the B region of the RARα gene and reversed transcribed into cDNA by incubation with 200 U of Moloney murine leukemia virus (MMLV) reverse transcriptase (BRL, Bethesda, MD) at 37°C for 45 minutes. One tenth of the cDNA product was subsequently amplified by PCR using Taq polymerase (Promega, Madison, WI) with a nested primer complimentary to the B region of RARα (5’ GGCACATCTATCCTCTCAGC (primer-2; Fig 3B) or a primer complimentary to the B region of PLZF (5’ CTGTCCTCATGG-AATGAC (primer-3; Fig 3B) or a primer complimentary to sequences more 5’ in PLZF (5’ GAGAGACCTCCTCCTGG (primer-1; Fig 3B) as described. One tenth (10 μL) of the PCR products were electrophoretically separated through a 14% agarose gel and visualized by ethidium bromide staining of the gel. To further confirm the nature of the PCR products, a portion of each of the products was electrophoretically separated through an agarose gel, purified from the gel using glass beads (Gene Clean; Bio 101, La Jolla, CA), and subcloned into the TA cloning vector (Invitrogen, San Diego, CA). The subcloned fragments were sequenced on both strands using the dyeodeoxy chain termination method (Sequenase; US Biochemicals, Cleveland, OH).

**In Vitro Differentiation Assay**

Cryopreserved leukemic blasts from case no. 5 and from a patient with classical M3, t(15;17) APL were thawed at 37°C in defrosting media (RPMI 1640, 20% fetal calf serum), centrifuged at 1,106 rpm for 5 minutes, and resuspended in the same media. Cells were cultured in the presence or absence of 10^-7 mol/L ATRA (Sigma) for 7 days. Aliquots of cells were removed at time 0, 2, 5, and 7 days after initiation of culture for cytologic evaluation. Maturation was assessed by increase in the cytoplasmic/nuclear ratio, gain of nuclear segmentation, and loss of promyelocytic granules.

**Graphics**

 Autoradiographs and photographs were imaged using a Silverscan flatbed digital scanner (La Cie, Ltd, Beaverton, OR). Ektachrome slides were imaged using a 35-mm Rapid Film Scanner (Eastman Kodak, Rochester, NY). Image processing was performed on a Macintosh Quadra 800 (Apple, Cupertino, CA) using Adobe Photoshop (Adobe, Mountain, CA) and Aldus Persuasion (Aldus, Seattle, WA) software. Figures were printed using an XL7700 Digital Continuous Tone Printer (Eastman Kodak).
RESULTS AND DISCUSSION

Molecular Characterization of t(11;17) Cases

Table 1 summarizes the clinical features, molecular and cytogenetic findings, and clinical courses of the six patients. All six patients had the t(11;17) translocation on initial cytogenetic evaluation. Leukemic blasts from cases no. 1 and 2 were found to contain rearrangement and fusion of the RARα gene and PLZF gene as demonstrated by Southern blotting. For the other cases, more precise localization of the RARα breakpoint was not determined.

**Table 1. Clinical and Molecular Features of Six Patients With t(11;17)-Associated Leukemia and Fusion of the RARα and PLZF Genes**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age/ Sex</th>
<th>Initial BM WBC (x10^9/L)</th>
<th>BM Pathology</th>
<th>Cytogenetics</th>
<th>Molecular Pathology</th>
<th>DIC</th>
<th>Initial Therapy</th>
<th>Response to ATRA</th>
<th>Response to Chemotherapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67/M</td>
<td>4.1</td>
<td>69% promyelocytes</td>
<td>(t11;17)</td>
<td>2ZF (PZF-RARα)</td>
<td>No</td>
<td>ATRA</td>
<td>Increase in WBC</td>
<td>No response to Daun/ AraC, transient CR with AraC/Mitoxantrone</td>
<td>Died resp failure d 20</td>
</tr>
<tr>
<td>2</td>
<td>66/M</td>
<td>10.6</td>
<td>70% promyelocytes</td>
<td>(t11;17)</td>
<td>2ZF (PZL/PML-RARα)</td>
<td>No</td>
<td>Daun/ AraC</td>
<td>Promyelocytes decreased but CR not achieved</td>
<td>Died of septic shock in relapse at 11 mo</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>81/M</td>
<td>7.6</td>
<td>90% promyelocytes</td>
<td>(t11;17)</td>
<td>3ZF (PZL/PML-RARα)</td>
<td>Yes</td>
<td>ATRA</td>
<td>Decrease in promyelocytes-No CR</td>
<td>Died of brainstem hemorrhage d 18</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>37/F</td>
<td>45.2</td>
<td>47% myeloblasts, 10% promyelocytes</td>
<td>(t11;17)</td>
<td>2ZF (PZL/PML-RARα)</td>
<td>No</td>
<td>Daun/ AraC</td>
<td>NA</td>
<td>No response to Daun/ AraC, responded to Mitoxantrone/etoposide</td>
<td>Died at 11 mo, disease status unknown</td>
</tr>
<tr>
<td>5</td>
<td>34/M</td>
<td>2.4</td>
<td>63% promyelocytes</td>
<td>(t11;17)</td>
<td>2ZF (PZL/RARα)</td>
<td>Yes</td>
<td>Daun/ AraC + ATRA</td>
<td>No response</td>
<td>Alive in CR at 4 mo on maintenance therapy</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>53/M</td>
<td>15.3</td>
<td>47% promyelocytes, 3% blasts</td>
<td>(t11;17)</td>
<td>2ZF (PZL/PML-RARα)</td>
<td>Yes</td>
<td>Daun/ AraC</td>
<td>Increase in WBC, no differentiation</td>
<td>Died at 31 mo in relapse</td>
<td></td>
</tr>
</tbody>
</table>

| Abbreviations: ZF, zinc finger; Daun, Daunorubicin; RARα(B-F), RARα B through F domains; NA, not applicable. |

**PFGE**

Despite the variable location of the t(15;17) breakpoint in t(15;17) APL, PFGE can detect the PML-RARα fusion gene in 100% of these cases using one restriction enzyme digestion and a single genomic DNA probe. We extended this approach to the analysis of t(11;17) translocation (Fig 5). High molecular weight DNA from BM cells of a t(11;17) APL (case no. 3), from three non-APL, AML patients, and from the t(15;17) NB4 cell line were digested with Sal I. The resulting fragments were separated by PFGE, transferred to nitrocellulose, and hybridized to PLZF (Fig 5A) and RARα (Fig 5B) genomic probes. A 90-kb Sal I fragment cohybridized to PLZF and RARα probe (lane 1, Fig 5A and B), illustrating the t(11;17) translocation and confirming the presence of the PLZF-RARα fusion gene detected by RT/PCR analysis (Fig 3A). In AML patients without t(11;17), this rearranged fragment was not detected with either probe (lanes 2 through 4, Fig 5). In DNA from NB4 cells, the RARα probe detected a 70-kb rearranged fragment (lane 5, Fig 5B), whereas the PLZF probe did not (compare lanes 1 and 5, Fig 5A). As expected, the rearranged RARα fragment from the t(15;17) NB4 cells did cohybridize to a PML genomic probe (data not shown). In all lanes some extra high molecular weight fragments were noted, which is related to the incomplete digestion of genomic DNA by the methylation-sensitive Sal I enzyme. These data show that the DNA-based PFGE technique can be used to diagnose t(11;17) APL when RNA cannot be extracted from the specimen. This technique is not subject to false-positives caused by contamination, unlike PCR-based approaches to diagnosis.
PFGE can also be used to identify novel rearrangements of the RARα gene when the identity of the fusion partner gene is not known.

Clinical and Pathologic Characterization

Clinically, most of the patients presented with features initially indistinguishable from classical APL. In general, the patients had a preponderance of promyelocytes in the initial BM aspirate and a relatively low peripheral WBC count (Table 1). Cases no. 3, 5, and 6 presented with clinical and laboratory evidence of DIC. Case no. 4, although satisfying diagnostic criteria for AML, had a variety of early myeloid forms in the peripheral circulation, a feature associated with myeloproliferative disorders.

Case no. 4 was particularly interesting because it was found by immunophenotypic and molecular analysis to also be a member of a newly identified group of natural killer (NK)/myeloid leukemia.31 None of these patients had t(15;17) and only 1 patient of 20 (case no. 4) had t(11;17) and PLZF-RARα fusion. Morphologically, the leukemic blasts of these patients exhibit few granules and a peculiar deep nuclear invagination. Typically, these cells have the immunophenotype CD33+, CD13+, CD56−, CD16−, HLA-Dr−, CD34+. Expression of myeloid markers (CD33 and CD13) in conjunction with the absence of CD34 and HLA-Dr expression is similar to the pattern seen in M3-AML. However, the leukemic blasts of case no. 4 and other cases of NK/myeloid leukemia also expressed an NK-associated marker, CD56 (N-CAM), and exhibited functional NK cell-mediated cytotoxicity. CD33+, CD13+, CD56+, CD16− cells can be identified in the peripheral blood of normal individuals. It therefore appears that NK/myeloid leukemia results from the malignant transformation of a primitive precursor cell common to both the myeloid and NK cell lineages.31,41

In five of six cases, the initial diagnosis by the referring pathologist was M3 AML. All of the morphologic material was reviewed by a single hematopathologist (D.R.H.) and compared with a series of 71 documented t(15;17) APL cases. All six t(11;17) cases, including case no. 4, consisted of a proliferation of myeloblasts more granular than the usual M2 AML, but less granular than the usual M3h (hypergranular) AML (Fig 1A through F). The t(11;17) leukemic cells lacked the usual reddish color shift of M3 granules and obscured nuclear outline of M3h. The specimens reviewed lacked Faggot cells and did not have the bilobed grooved nucleus and fine granules of M3v (microgranular variant) AML. Only one case (case no. 5, Fig 1E) had morphologic features approaching classical FAB M3 APL. None of the cases, including the CD56− case no. 4, had the nuclear invagination usually seen in CD56− NK/myeloid leukemia.31 We tentatively place the t(11;17) cases in a small morphologic spectrum with features intermediate between M2 and classical M3 AML. The molecular pathology of the intermediate morphology may be more diverse, because several other cases with this distinctive phenotype were identified without t(11;17), but none had t(15;17) (D. Head, personal communication). The degree of overlap of the t(11;17) APL syndrome with NK/myeloid leukemia appears small, because only 1 of 20 NK/myeloid patients had the PLZF-RARα fusion gene. It would be useful to perform immunophenotypic analysis on all future t(11;17) cases to determine whether these cells express CD56. However, it would appear that in both diseases myeloid differentiation is blocked at a more primitive stage than classical M3 AML. Together, the morphologic data support the concept that t(11;17) APL is a clinicopathologic entity distinct from t(15;17) APL.

In Vitro Differentiation

In vitro differentiation studies were performed on mononuclear cells from patients no. 2, 4, and 5. In all three cases
APL ASSOCIATED WITH t(11;17)

leukemic blasts failed to differentiate in the presence of $10^{-7}$ mol/L retinoic acid, whereas concomitant controls of promyelocytes from patients with t(15;17) APL did\(^3\) (Fig 6C and D). It was previously shown that optimal differentiation of fresh leukemic samples could be achieved at $10^{-7}$ mol/L ATRA.\(^2\) Incubation of cells from cases no. 2 and 5 with a 10-fold higher concentration of ATRA ($10^{-6}$ mol/L) still did not induce differentiation. The failure of leukemic cells from t(11;17) patients to differentiate in vitro correlated with the inability of ATRA to induce a clinical response in these patients. These data support the utility of the in vitro differentiation assay as predictor of clinical response to ATRA.

**Response to Treatment**

Despite the superficial morphologic similarity of the t(15;17) and t(11;17) patients, the latter group with fusion of the PLZF and RAR\(\alpha\) genes generally responded poorly to treatment. One of the six patients is currently in CR after salvage therapy and a second died at 31 months after multiple short remissions and relapses. In contrast, classical t(15;17) APL is characterized by a 60% to 80% response rate to conventional chemotherapy, a similar 75% to 95% CR rate with ATRA therapy, and a 5-year survival rate of 35% to 45%.\(^1\) Five of the six patients with t(11;17) APL received ATRA at some time during their clinical course, but none had a clinically significant response to the agent (Table 1). Cases no. 1 and 3 were initially treated with ATRA, whereas case no. 2 received this agent after failing to respond to induction therapy with daunorubicin and Ara-C. Case no. 1 died early in the clinical course of respiratory failure with a highly elevated leukocyte count. In retrospect, this patient may have suffered from the retinoic acid syndrome\(^1\) characterized by a proliferation of leukemic cells with resulting pulmonary leukostasis. In t(15;17) patients, this hyperproliferative effect may precede the clinical response characterized by the induction of differentiation of the leukemic promyelocytes. Case no. 2 also had evidence of some response to ATRA because there was a decline in the number of promyelocytes in the BM while on therapy, but, unlike patients with t(15;17), this patient did not achieve morphologic or cytogenetic remission. Because blasts from this patient did not differentiate when cultured in the presence of ATRA in vitro,\(^2\) the clinical response to ATRA may have been antiproliferative in nature. Similarly, in case no. 3, administration of ATRA was associated with a decline in the promyelocyte count and improvement of biochemical parameters indicative of DIC. However, this patient died early in the clinical course of a bleeding complication. Case no. 4 was treated only with chemotherapy and did not respond to initial therapy with daunorubicin and Ara-C, but achieved a brief remission with second-line therapy with mitoxantrone and etoposide and appeared to convert to a chronic myeloid leukemia (CML)-like syndrome. Case no. 5 received combination chemotherapy and ATRA therapy on a randomized protocol with no evidence of response. After initiation of ATRA therapy, case no. 6 experienced an increase in the promyelocyte count, but this was not followed by evidence of differentiation. Case no. 6 was identified among a series of patients with APL in relapse after initial treatment with chemotherapy.\(^3\) Although 14 of 17 (82%) of these patients completely responded to ATRA and idarubicin as a second-line therapy, case no. 6 was among the 3 that did not. We conclude that APL morphology

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**Fig 3.** (A) A novel PLZF-RAR\(\alpha\) fusion transcript detected by RT-PCR. Total RNA (4 \(\mu\)g) from case no. 3 (lane 1) or the index patient, case no. 1 (lane 2), was reverse transcribed into cDNA and subjected to amplification with an RAR\(\alpha\)/PLZF primer pair. The PCR products were electrophoretically separated and visualized by ethidium bromide staining. Molecular weight standards are shown in basepairs. (B) Schematic representation of the PLZF-RAR\(\alpha\) fusion transcripts detected in cases no. 1 and 3. The junction of the PLZF and RAR\(\alpha\) transcripts is indicated by a bold arrowhead. The DNA and amino acid sequences spanning the junction of the PLZF and RAR\(\alpha\) genes (in bold and normal characters, respectively) are shown below. Arrows indicate the approximate position of oligonucleotide primers relative to the PLZF-RAR\(\alpha\) fusion mRNA. RT, Reverse transcription primer complimentary to RAR\(\alpha\); 3, PCR primer complimentary to RAR\(\alpha\) sequences; 1 and 2, PCR primers complimentary to PLZF sequences.
**Breakpoints in the PLZF and RARα Genes in 6 Cases of t(11;17)**

![Diagram showing breakpoints in PLZF and RARα genes](image)

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**The Role of RARα, PML, and PLZF Genes in the APL Syndromes**

It is evident from this series that both t(11;17) and t(15;17) translocations are associated with similar APL morphology. The common factor in these two syndromes is disruption of one of the RARα alleles in the leukemic clone. RARα plays an important role in normal myeloid differentiation and, experimentally, expression of a dominant negative form of RARα in a multipotent cell line causes a block in myeloid differentiation at the promyelocyte stage. Preliminary evidence indicates that the PLZF-RARα fusion protein shares many properties with the PML-RARα fusion protein, including the ability to bind to RA response elements.
elements, the ability to act as an RA-dependent transcription factor, and the ability to antagonize the function of the wild-type RARα. The ability of the PML-RARα and PLZF-RARα fusion proteins to antagonize wild-type RARα function may explain why the two syndromes have the same cytologic morphology.

Treatment of t(11;7) leukemic cells in vitro with 10 times more ATRA (10⁻⁶ mol/L) than required for optimal induction of t(15;17) cells failed to induce differentiation. However, the PLZF-RARα and PML-RARα fusion proteins mediate similar amounts of transcription in response to 10⁻⁶ mol/L ATRA, which is reduced compared with wild-type RARα (R. Shaknovich, M. English, J. Licht, manuscript in preparation). Therefore, it is difficult to explain why only the disease associated with t(15;17) and PML-RARα fusion responds well to ATRA. The key to this problem is to understand the role of the PML and PLZF genes in myeloid development and how the functions of these genes are affected by fusion to the RARα gene.

In this regard, it must be stressed that there are considerable differences between the PML and PLZF genes. PML is a ubiquitously expressed gene that encodes a member of an enlarging family of proteins with an unusual cysteine-rich cluster termed the “ring” finger. In at least some cases this motif is used for binding to nucleic acids. However, PML has an unusual nuclear localization in an average of 10 small concentric structure called nuclear bodies or PML oncogenic domains (PODs). These bodies do not appear to colocalize with sites of RNA transcription or splicing and their function is unknown. In t(15;17) APL cells, the PML protein is abnormally localized in hundreds of small domains in the nucleus, apparently because of the action of the PML-RARα fusion protein. Upon addition of ATRA to APL cells the PML protein relocates into the wild-type nuclear body configuration. PML also appears to have tumor suppressor activity. PML overexpression in t(15;17)-containing NB4 cells reduces the clonogenicity of these cells in soft agar and their tumorigenicity in nude mice. In addition, PML suppresses oncogenic transformation of rat embryo fibroblast cells cotransfected with the ras and myc oncogenes. These data suggest that PML acts as a governor of normal cell proliferation and that the function of PML is disrupted by fusion to the RARα protein, perhaps by the aberrant localization of PML away from the nuclear body structure. According to this hypothesis, ATRA treatment restores the normal localization of PML in t(15;17) APL cells and leads to the cessation of cell growth and onset of differentiation. This notion is supported by the discovery of an ATRA-resistant cell line that fails to relocalize PML and PML-RARα protein upon treatment with ATRA. In t(15;17) APL, a reciprocal fusion protein RARα-PML is also generated and is found to be localized to the nucleus. However, no clear role for this fusion protein has been assigned to this protein in leukemogenesis.

In contrast to the PML gene, the PLZF gene is expressed in a tissue-specific manner, particularly in the myeloid lineage. The PLZF protein is a member of the large family of cysteine- and histidine-containing zinc finger transcription factors typified by the Drosophila Krüppel protein and encoded by human oncogenes such as Gli and tumor suppressors genes such as WT1. Preliminary data indicate that the PLZF protein, although localized to the nucleus, is not found in nuclear body structures (R. Shaknovich and J. Licht, manuscript in preparation). Furthermore, it appears that the PLZF protein has specific DNA binding and transcriptional effector activities (J. Li, M. English, and J. Licht, manuscript in preparation). Therefore, PLZF is likely to be a transcription factor that regulates genes required for white
blood cell development. The expression of the wild-type PLZF gene decreases during ATRA-mediated myeloid differentiation. Hence, the genes targeted by the PLZF transcription factor would be expected to be differentially regulated during differentiation. In t(11;17) and fusion of the PLZF and RARα genes, a PLZF-RARα fusion protein is generated linking two or three zinc fingers of the PLZF protein to the RARα. This protein, in addition to acting as an RA-responsive transcription factor, could also bind to DNA targets through the PLZF zinc fingers. Perhaps more importantly, the reciprocal fusion protein generated in t(11;17) links DNA encoding the last 7 zinc fingers of PLZF to the RARα gene, including the RARα promoter. The resulting RARα-PLZF protein contains the last 7 of the 9 zinc fingers of the PLZF protein and may bind the same target genes as the wild-type protein. The RARα-PLZF fusion gene would be expected to be constitutively expressed during myeloid differentiation, paralleling the pattern of expression of the wild-type RARα and could act as a dominant negative inhibitor of wild-type PLZF. This effect could even be enhanced by ATRA therapy because the RARα promoter is activated by ATRA treatment of myeloid and other cells. This hypothesis predicts that disruption of the wild-type PLZF gene rather than interference with RARα function may be critical for the most important clinical feature of t(11;17). It appears that rearrangement of the RARα locus with genes resulting RARα-PLZF protein contains the last 7 zinc fingers of the PLZF protein and may bind the same target genes as the wild-type protein. The RARα-PLZF fusion gene would be expected to be constitutively expressed during myeloid differentiation, paralleling the pattern of expression of the wild-type RARα and could act as a dominant negative inhibitor of wild-type PLZF. This effect could even be enhanced by ATRA therapy because the RARα promoter is activated by ATRA treatment of myeloid and other cells.

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Clinical and molecular characterization of a rare syndrome of acute promyelocytic leukemia associated with translocation (11;17)

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