Complete Remission in Acute Promyelocytic Leukemia Despite Persistence of Abnormal Bone Marrow Promyelocytes During Induction Therapy: Experience in 34 Patients


Thirty-four patients with acute promyelocytic leukemia (APL) (median age 37 years, range 20 to 69 years) received induction treatment between 1974 and 1985 with cytosine arabinoside (ara-C) and an anthracycline. Bone marrow hypercellularity was present at the time of diagnosis in all patients, although the median peripheral leukocyte count was 2,600/µL. A second course of induction therapy consisting of further ara-C and anthracycline was initiated 15 days after the start of treatment if bone marrow hypocellularity could not be documented. Karyotypic analysis of bone marrow blasts was performed on 15 of 34 patients; 11 of 15 had abnormalities in chromosomes 15 and/or 17. Twenty-nine of 34 (85%) patients had laboratory evidence of disseminated intravascular coagulopathy. Of the 29 patients surviving 14 days, 24 (83%) received a second course of induction therapy. Complete remission was achieved in 25 of 34 (74%) patients, with four of 25 (16%) requiring one course of induction chemotherapy and 21 of 25 (84%) receiving two courses. Bone marrow specimens obtained 15 days after the start of therapy from the 25 patients who eventually attained complete remission showed the continued presence of dysplastic promyelocytes in 21 cases; three specimens were technically inadequate and only one was truly devoid of promyelocytes. Seventeen of 25 (68%) patients still had persistence of abnormal bone marrow promyelocytes seven or more days after the second course of therapy. Patients in complete remission received various forms of postremission therapy. Ten of the 25 (40%) completely responding patients remain alive in continuous complete remission. Neither the absence of bone marrow hypocellularity nor the persistence of dysplastic promyelocytes during induction exerted any influence on the probability for survival. These findings confirm and extend prior reports that complete remission in APL, in contrast to other subtypes of acute nonlymphocytic leukemia (ANLL), can frequently be achieved without bone marrow aplasia. Whether this observation signifies that complete remission in APL is due to leukemic cell differentiation or selective cytotoxicity is unknown. The absence of therapy-induced bone marrow hypoplasia in APL is not an absolute indication of induction failure or a poor ultimate prognosis.

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USE OF INDUCTION regimens that include cytosine arabinoside (ara-C) and an anthracycline has led to complete remission rates of 65% to 75% in previously untreated adults with acute nonlymphocytic leukemia.1,4 Such remissions are believed to result from the elimination of detectable, malignant-appearing cells, generally coincident with marrow hypoplasia at a time when both the malignant clone and normal hematopoietic elements are suppressed. The persistence of abnormal-appearing cells and/or the absence of marrow hypoplasia is associated with a poor prognosis and frequently signifies an indication for further chemotherapy.4 The continued presence of abnormal-appearing cells following additional therapy is generally considered to indicate treatment failure and the need to introduce alternative forms of treatment.

Acute promyelocytic leukemia (APL) represents 5% to 15% of cases of acute nonlymphocytic leukemia (ANLL).5-8 This entity has several well-recognized features, including a distinctive morphology categorized as the M-3 subtype within the French-American-British (FAB) classification system,8 an associated coagulopathy believed to be due to the release of thromboplastin activating substances in the granules that characterize malignant promyelocytes.10,11 and a unique cytogenetic abnormality often typified by a balanced translocation between the long arm of chromosome 15 and the long arm of chromosome 17.12-15 The hemorrhagic diathesis associated with APL once led to the impression that patients with this disorder had a lower probability of achieving complete remission than did patients having other subtypes of ANLL.6,16 An appreciation of the existence of the associated coagulopathy, however, coupled with its management through the use of intensive blood product support with10,16-22 or without23 heparin as well as the prompt initiation of effective chemotherapy has led to complete remission rates comparable to those in other forms of ANLL.

We recently observed several patients with APL in whom dysplastic-appearing promyelocytes remained in the bone marrow and for whom hypoplasia was difficult to achieve throughout the period of induction therapy; nonetheless, such patients subsequently achieved complete remission. Because these patients had been classified as treatment failures by experienced morphologists, we performed a retrospective review of our population of patients with APL to determine the frequency of this observation and its prognostic implications.
MATERIALS AND METHODS

Patient population. Thirty-four consecutive patients with APL underwent diagnosis and treatment at the Dana-Farber Cancer Institute (12 patients), the Brigham and Women's Hospital (15 patients), and the Beth Israel Hospital (7 patients) between January 1974 and January 1985. The morphological designation was based in all cases on an examination of a bone marrow aspiration and/or biopsy that showed a predominance of characteristically abnormal promyelocytes and myeloblasts consistent with a FAB M-3 histologic pattern. Dysplastic promyelocytes were defined as cells with large coarse granules having an immature chromatin pattern. Cyto genetic studies using quinacrine-banding techniques were carried out in 15 of the 34 patients, and immunophenotyping using monoclonal antibody reagents was performed on the cells of seven individuals.

Chemotherapy. Patients with APL were managed with three successive treatment regimens during this 11-year period (Table 1). The first four patients received COD,24 which included ara-C [2 mg/kg/day by bolus intravenous (IV) administration for three to six days], vincristine (1 mg/M2/day on the first treatment day), and daunorubicin (1 mg/kg/day by IV bolus administration for three to six days). Nine subsequent patients were treated with VAPA,2 which included: vincristine (1 mg/M2/day by bolus IV administration on days 1 and 8), ara-C (100 mg/M2/day by continuous IV infusion for seven days), methylprednisolone (20 mg/M2 every 12 hours by bolus IV administration on days 1 through 5), and doxorubicin (30 mg/M2/day by bolus IV administration on days 1 through 3). Twenty-one additional patients have been treated with VAPA,2 which included: daunorubicin (45 mg/M2/day by bolus IV administration on days 1 through 3) and ara-C (100 to 200 mg/M2/day by continuous IV infusion for seven days). A bone marrow aspiration and/or biopsy was performed on the 15th day after the start of induction to determine if a second course of therapy should be initiated at that time. Such additional treatment was to be given if the bone marrow revealed the persistence of malignant-appearing cells or if hypoplasia (cellularity < 10%) had not been achieved. The second course of treatment generally included two further administrations of an anthracycline and five additional days of infusional ara-C. Periodic bone marrow examinations were subsequently carried out until a definitive response to induction treatment could be clearly determined. The median time to bone marrow examination following the second induction was 20 days (range: nine to 32 days). Various postremission consolidation and/or maintenance regimens were used thereafter.24 All patients gave written informed consent as required by Institutional Review Boards for the administration of chemotherapy as well as the performance of bone marrow aspirations and biopsies.

Supportive care. Patients were treated in private rooms on regular hospital floors without the use of laminar air flow facilities, special diets, or prophylactic antibiotics. Broad-spectrum antibiotics were administered if patients had fever and granulocytopenia, and an antifungal agent was added either in the presence of prolonged fever or when a fungal infection was documented. Granulocyte transfusions were not used. Transfusions with platelet concentrates, packed RBCs, and fresh frozen plasma were given for thrombocytopenia (platelet count < 20,000/mL), anemia, clinical bleeding, or if there was evidence of a disseminated coagulopathy.23 Heparin was neither used routinely for the treatment of the coagulopathy nor was it given prophylactically.23

Statistical methods. Complete remission was defined according to the criteria of Cancer and Leukemia Group B,2 which include a bone marrow aspiration revealing <5% blasts with evidence of maturation of all hematopoietic cell lines and restoration of normal peripheral blood counts. Survival was measured from the date of the start of treatment until death; remission duration was computed from the date of remission to the date of documented relapse. Survival and remission estimates were made by the technique of Kaplan and Meier.25

RESULTS

Patient characteristics. The 34 patients who comprise this study ranged in age from 20 to 69 years (median, 37 years). Despite bone marrow replacement with promyelocytes at the time of presentation, the median peripheral WBC count was only 2,600/mL, (range, 300 to 41,000/mL). The median platelet count was 20,000/mL (range, 5,000 to 171,000/mL), with 31 of 35 (89%) patients having an initial platelet count <50,000/mL (Table 2). Of the 15 patients whose bone marrow cells were subjected to karyotypic analysis, 12 had abnormal cytogenetic patterns. One of these 12 patients appeared to have random, nonspecific chromosomal changes, two showed a deletion of the long arm of chromosome 17, and the remaining nine had the characteristic reciprocal translocation between chromosomes 15 and 17. Three of these latter nine patients had additional cytogenetic abnormalities, including two with trisomy 8. Cytogenetic analyses were performed on bone marrow cells from three of these 12 patients at the time of remission, revealing a normal karyotype in each case.
The malignant promyelocytes from all seven patients whose cells underwent immunophenotyping lacked the presence of the Ia antigen on their cell surface. This differentiation antigen is found on the cell surface in most other forms of ANLL and is present on myeloid precursors throughout granulocytic maturation, except at the promyelocyte stage.27

Coagulopathy. Twenty-nine of 34 (85%) patients had laboratory evidence for a coagulopathy at the time of diagnosis or shortly after initiation of induction chemotherapy. Although routine use of heparin has been advocated by many investigators as a means of managing the coagulopathy associated with APL, only six of our 34 patients received anticoagulation therapy, and whether such treatment was actually beneficial is unclear.23

Response. Complete remission was achieved in 25 of 34 (74%) of our patients (Table 3). Five patients, all of whom were treated before 1979, died of intracranial hemorrhage within ten days of their diagnosis. Of the remaining 29 patients who remained alive on the 14th day after start of induction therapy and underwent a bone marrow examination, 25 (86%) subsequently achieved complete remission. Two of the four patients who survived beyond 15 days but failed to enter complete remission had a hypercellular marrow 15 days after the initiation of chemotherapy. Three of these four patients died within 14 days after the initiation of a second course of induction therapy; the fourth refused a second induction course and died of persistent leukemia 6 months later.

Dysplastic promyelocytes remained in the bone marrow 15 days after the start of induction therapy in 21 of 25 (84%) patients who eventually entered complete remission. Only one patient had a bone marrow specimen truly devoid of promyelocytes; the marrow samples in three others were technically inadequate. A review of the day 15 bone marrow revealed that 17 patients had hypercellular samples, five were hypocellular, and three were technically inadequate. Based on these observations, 21 of 25 (84%) patients received a second course of induction therapy (Table 4). A subsequent bone marrow examination performed seven or more days after the start of the second course of induction therapy, corresponding to 3 weeks after the initiation of chemotherapy treatment, continued to demonstrate the persistence of dysplastic bone marrow promyelocytes in 17 of the 25 complete responding patients. Hypocellularity was noted in the bone marrow biopsies of eight of 17 patients with persistent dysplastic promyelocytes and five of eight patients in whom the promyelocytes had disappeared. Malignant-appearing cells were frequently observed in the bone marrow during the sixth and seventh weeks after the initiation of treatment. Despite concerns that these patients had failed to respond to induction treatment, the return of adequate numbers of normal-appearing circulating blood cells in association with bone marrow megakaryocytes heralded a complete hematologic remission. Examples of the morphological pattern observed in serial bone marrow aspirations and biopsies in two of our patients are shown in Figs 1 and 2.

Survival. After a median follow-up time of 84 months, ten of our 34 (29%) patients with APL, representing 40% of the 25 complete responding individuals, remain alive and in continuous remission. The median survival time for the entire 34 patient cohort is 20 months (Fig 3), whereas that for the 25 patients who entered complete remission is 34

Table 3. Induction Therapy: Outcome

<table>
<thead>
<tr>
<th>Outcome</th>
<th>No. of Courses of Chemotherapy</th>
<th>Total</th>
<th>One</th>
<th>Two</th>
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</thead>
<tbody>
<tr>
<td>Complete responders</td>
<td>25</td>
<td>4</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Induction failure</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Early death (all due to major hemorrhage)</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>10</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Persistence of Dysplastic Bone Marrow Promyelocytes in 25 Complete Responding Patients

<table>
<thead>
<tr>
<th>Day after Start of Induction Therapy</th>
<th>Persistent Promyelocytes</th>
<th>No Promyelocytes</th>
<th>Marrow Technically Inadequate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 15</td>
<td>21*</td>
<td>1</td>
<td>3†</td>
</tr>
<tr>
<td>Day 22 or after</td>
<td>17</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

*Twenty patients received second induction course.
†One patient received second induction course.

Fig 1. (A) Low power (100×) and (B) high power (1,000×) views of bone marrow aspirations obtained from a 30-year-old male with APL on the day of diagnosis, 2 weeks after diagnosis (the start of a second course of induction therapy) and 4 weeks after diagnosis. Although hypocellularity was present 2 weeks after the start of the second course of treatment, dysplastic promyelocytes persisted.
after the start of induction therapy did not appear to affect disease-free survival adversely (Fig 4). Seven of the 17 (41%) patients whose bone marrows continued to demonstrate promyelocytes remain alive in complete remission as compared with three survivors among the eight patients whose promyelocytes were totally eliminated during induction treatment.

DISCUSSION

This study represents a retrospective analysis of 34 consecutive patients with APL who exhibited the typical and distinctive features that make this leukemic subtype a unique entity. These features include a characteristic morphological appearance of the bone marrow, leukopenia at the time of presentation,5,6,22,28,29 a coagulopathy,8,16,18,20,30 cytogenetic abnormalities involving chromosomes 15 and 17,12,15 and lack of the Ia antigen on the surface of the malignant cells.27 We have confirmed several recent reports that patients with APL have a complete remission rate comparable to that observed in other subtypes of ANLL9,22 and have further shown that these remissions are durable in ~40% of patients. More importantly, we have significantly extended the observations of other researchers7,29,33 that patients with APL may enter remission without demonstrating bone marrow hypoplasia and that the continued presence of dysplastic promyelocytes in the bone marrow during induction therapy is a frequent occurrence and is not associated with a poor ultimate prognosis.

The mechanism by which patients with APL achieve complete remission despite the continued presence of dysplastic marrow promyelocytes is unclear. One possible explanation for this observation is selective cytotoxicity of the chemotherapy for the unique hematopoietic clone that gives rise to the malignant-appearing promyelocytes. Such a clone may be able to differentiate partially through the promyelocyte stage. Although chemotherapy might successfully ablate the true malignant precursors, such treatment would not necessarily eradicate nonclonogenic progeny, which could still be observed in the form of dysplastic promyelocytes.

Alternatively, the progressive marrow evolution that char-
characterizes the appearance of remission in patients with APL could represent a process of leukemic cell differentiation. The HL-60 cell line, originally derived from a patient with APL,\(^2\) has served as the prototype for experiments studying the effects of putative differentiation agents on human leukemic cells. The maturation of HL-60 cells, as defined by morphologic and histochemical changes as well as the appearance of cell surface and functional markers consistent with granulocytic or monocytic differentiation, has been reported following exposure to such chemotherapeutic agents as ara-C,\(^3\) 6-thioguanine,\(^4\) and 5-azacytidine,\(^5\) chemicals such as dimethylsulfoxide,\(^6\) butyric acid,\(^7\) retinoic acid,\(^8\) and phorbol esters,\(^9\) γ interferon,\(^10\) and 1,25 dihydroxy-vitamin D\(_3\).\(^11\) The molecular events involved in the differentiation of HL-60 cells, both in terms of alterations in cell membranes and gene expression, are the subject of current investigative efforts. Whether the in vitro phenomenon of cell differentiation observed with HL-60 cells can be applied in vivo to the treatment of patients with APL remains unclear. Several case reports have suggested that cells from patients with APL have been induced to differentiate by exposure to phorbol esters.\(^37\)\(^42\)\(^44\) and there have been occasional instances where the use of retinoic acid has been beneficial in producing bone marrow maturation in patients with this disease.\(^55\)\(^66\) Remissions in the absence of bone marrow hypoplasia have been achieved in some patients with myelodysplastic syndromes and ANLL using agents that cause differentiation of HL-60 cells.\(^47\)\(^49\) The mechanism responsible for the long-term remissions observed in these patients is uncertain.

Nonetheless, no definitive data at present support the notion that differentiation is the mechanism by which cytotoxic chemotherapeutic agents induce remission without hypoplasia in either myelodysplastic syndromes or ANLL. Despite the initially encouraging reports suggesting the existence of such in vivo differentiation, published observations remain anecdotal, and neither ara-C in low doses\(^50\)\(^51\) nor retinoic acid\(^52\)\(^53\) has been sufficiently effective in patients with myelodysplastic syndromes to permit adequate assessment of the proposal. An optimal test of such a concept would require a cellular marker present in both dividing and nondividing mature myeloid cells. Unlike standard diploid metaphase cytogenetic analysis, either serial immunophenotyping\(^26\)\(^27\) or restriction fragment length polymorphism analysis\(^28\) would be able to assess nondividing cells and thus prove useful in distinguishing differentiation and cytotoxicity.

The continued presence of dysplastic promyelocytes in a persistently cellular bone marrow poses a dilemma for clinicians managing patients with APL. Although many such patients may enter complete remission without further chemotherapy, the ominous appearance of the bone marrow sample 15 days after initiation of therapy frequently leads to administration of a second course of possibly unnecessary induction treatment, thereby prolonging the duration of hospitalization, pancytopenia, and risk for infection and bleeding. Indeed, most (21 of 25) of our patients who entered complete remission received a second course of induction therapy. Correspondingly, our high complete response rate and the encouraging remission duration may be partially related to this additional treatment. In the absence of any morphologic guidelines, it would be most useful in future studies to perform serial cytogenetic analyses of bone marrow obtained at various points during the induction period to correlate the time at which the t(15;17) cytogenetic abnormality disappears with the cellularity of the bone marrow and the persistence of dysplastic promyelocytes.

In summary, we have shown that complete responses leading to durable remissions and extended survival can be achieved in most patients with APL. The continued presence of dysplastic promyelocytes in bone marrow and/or the absence of therapy-induced marrow hypoplasia occurs frequently, does not signify induction failure, and does not predict a poor ultimate prognosis. A better understanding of this presently unexplained phenomenon may lead to important knowledge concerning the pathogenesis of acute leukemia.

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Complete remission in acute promyelocytic leukemia despite persistence of abnormal bone marrow promyelocytes during induction therapy: experience in 34 patients

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