RAPID COMMUNICATION

Effect of All Transretinoic Acid in Newly Diagnosed Acute Promyelocytic Leukemia. Results of a Multicenter Randomized Trial

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We designed a multicenter randomized trial comparing chemotherapy with daunorubicin-Ara C (chemotherapy group) and all transretinoic acid (ATRA) combined to the same chemotherapy (ATRA group) in newly diagnosed APL patients aged 65 years or less. The major endpoint of the study was event-free survival (EFS) (“events” being defined as failure to achieve complete remission [CR], occurrence of relapse, or death in CR). Early termination of the trial was decided after the first interim analysis, as EFS was significantly higher in the ATRA group. At the time, 101 patients had been randomized (54 in the ATRA group and 47 in the chemotherapy group). In the ATRA group, 49 (91%) patients achieved CR, 5 (9%) had early death, and 0 had resistant leukemia, compared with 38 (81%), 4 (8%), and 5 (10%) patients, respectively, in the chemotherapy group. The difference in CR rate between the two groups was not significant. The duration of coagulopathy was significantly reduced in the ATRA group, compared with the chemotherapy group. In the ATRA group, six patients relapsed after 7 to 15.5 months. In the chemotherapy group, 12 patients relapsed after 1 to 16 months, and 2 died in CR. Kaplan-Meier EFS was estimated at 79% ± 7% and 50% ± 9% at 12 months, respectively, in the ATRA and the chemotherapy group (P = .001). Kaplan-Meier estimate of relapse was 19% ± 8% and 40% ± 12% at 12 months (P = .005). In conclusion, ATRA followed by chemotherapy increases EFS in newly diagnosed APL. These results strongly suggest that ATRA should be incorporated in the front line therapy of newly diagnosed APL.

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ACUTE PROMYELOCYTIC leukemia (APL) is characterized by the morphology of blast cells (M0),1,2 by t(15;17) translocation,3 which fuses the PML gene to the retinoic acid receptor (RAR) α gene,4,5 and by a coagulopathy combining disseminated intravascular coagulation (DIC), fibrinolysis, and proteolysis.6,7 Anthracycline-cytosine arabinoside (Ara C) intensive chemotherapy yields a complete remission (CR) in 50% to 80% of newly diagnosed APL patients.8-17 Failure to achieve CR generally results from fatal bleeding, due to worsening of coagulopathy by chemotherapy, or from fatal sepsis during the phase of aplasia, rather than leukemic resistance, which seems to be less frequent in APL than in other acute myeloid leukemia (AML).8-17 Fifty percent to 65% of the patients who achieve CR subsequently relapse, two thirds of them within 1 year of CR. Thus, event-free survival (EFS) (where “events” are defined by failure to achieve CR, occurrence or relapse, or death in CR) is estimated at 25% to 55% 1 year after diagnosis.8-17

All transretinoic acid (ATRA) selectively differentiates abnormal promyelocytes into mature granulocytes in APL, both in vitro18,19 and in vivo20-32 and induced CR in 80% to 90% of newly diagnosed18,20,23-26 and first-relapsing APL cases.37 ATRA rapidly improved coagulopathy and induced no aplasia, suggesting that it could reduce the incidence of fatal bleeding and sepsis in APL. However, in 25% to 30% of the patients, treatment with ATRA was associated with a rapid increase in leukocytes and signs of “ATRA syndrome,”25,26 which could have a fatal outcome unless rapid decrease in leukocyte (WBC) counts or reversal of symptoms by intravenous dexamethasone was obtained.25,26 In addition, most of the patients who achieved CR with ATRA and were maintained on ATRA alone or relatively mild chemotherapy rapidly relapsed.18,20-24 Thus, we performed a pilot study combining ATRA and intensive chemotherapy in newly diagnosed APL. Intensive chemotherapy was administered either after CR had been obtained with ATRA alone to prevent relapse, or was added to ATRA in patients with rapidly increasing WBC counts to prevent the ATRA syndrome. Twenty-five of the 26 patients (96%) included in this pilot study achieved CR and, with a minimum follow-up of 18 months, only three had relapsed,28 suggesting that the combination of ATRA and chemotherapy increased the CR rate, but perhaps more importantly reduced the relapse rate, compared with chemotherapy alone.

To confirm these findings in newly diagnosed APL, we designed a randomized clinical trial comparing ATRA plus chemotherapy and chemotherapy alone, using EFS as major endpoint.

PATIENTS AND METHODS

Eligibility Criteria

Between March 1, 1991 and December 1, 1992, 101 patients with newly diagnosed APL from 46 European centers were included in a
clinical trial randomizing ATRA followed by chemotherapy versus chemotherapy alone (APL 91 trial). This trial had been approved by the ethical committee of the University Hospital of Lille for French centers (according to French law) and by local ethical committees in other countries. Inclusion criteria were: (1) diagnosis of APL, based on French-American-British (FAB) group morphology criteria; (2) age 65 years or less; and (3) informed consent given. Diagnosis had to be subsequently confirmed by presence of t(15;17) translocation or PML-RAR α gene rearrangement (detected by previously published methods). In the absence of t(15;17) or in case of cytogenetic failure and if the analysis of the rearrangement could not be made, review of initial marrow slides by an independent morphologist (M.T.D.) was mandatory. Randomization was performed through a centralized assignment procedure.

Protocol Design

Patients allocated to chemotherapy alone (chemotherapy group) received two successive courses of daunorubicin (DNR) 60 mg/m²/d for 3 days and Ara C 200 mg/m²/d for 7 days (courses I and II) (Table 1). Those who achieved CR after the first course received a third, final consolidation course of DNR 45 mg/m²/d for 3 days and Ara C 1 g/m² every 12 hours for 4 days (course III). Patients who had leukemic resistance after course I received, after course III, an additional consolidation course with DNR 45 mg/m²/d for 2 days and Ara C 1 g/m² every 12 hours for 4 days (course IV). Patients who had resistant leukemia after courses I and II were considered failures and were administered ATRA, as in the ATRA group. However, after chemotherapy, in APL, a slow disappearance of marrow blasts is encountered in some patients who will, however, subsequently achieve CR without further treatment. Thus, if after courses I and II, the day 20 marrow aspirate showed greater than 50% blasts, sequential bone marrow (BM) aspirates and coagulation studies were performed before concluding to resistant leukemia.

Patients allocated to ATRA plus chemotherapy (ATRA group) received ATRA 45 mg/m²/d orally until CR, or for a maximum of 90 days. After CR achievement, they received the three same chemotherapy courses as in the chemotherapy group (courses I, II, and III). However, course I was added to ATRA on day 1 of treatment if initial WBC counts were greater than 5 x 10⁹/L, or rapidly started if WBC increased to above 6 x 10⁹/L, 10 x 10⁹/L, or 15 x 10⁹/L by day 5, 10, or 15 of ATRA treatment, respectively, because, from our experience, patients were at risk of ATRA syndrome above those thresholds.

In this treatment group, leukemic resistance was defined by either: (1) absence of CR after 90 days of treatment with ATRA (whether or not chemotherapy course I had been added, due to increasing WBC counts), or (2) absolute resistance to ATRA, defined by the absence of significant improvement of cytopenias and coagulopathy, and the presence of greater than 50% of APL blasts in the BM without any morphologic sign of differentiation on day 30 of treatment. Those patients were scheduled to receive the same chemotherapy as in the chemotherapy group (courses I, II, and III if they had not received any chemotherapy; courses II, III, and IV if they had already received course I).

In both treatment groups, amsacrine (90 mg/m²/d) was substituted for DNR in courses III and IV if the left ventricle ejection fraction was below normal values before these courses. Allogeneic BM transplantation (BMT) in first CR was recommended in patients ages less than 40 years with an HLA-identical sibling only in cases presenting with leukocytes greater than 10,000/mm³ or who had resistant leukemia after chemotherapy course I or after ATRA. For the analysis of relapse and EFS, those patients were censored at the time of their transplant.

Coagulopathy

The hemostatic disorder (DIC and/or fibrinolysis), was considered significant if the fibrinogen level was less than 1.5 g/L or if at least two of the following criteria were fulfilled: (1) fibrinogen between 1.5 g/L and 2 g/L; (2) fibrinogen degradation products (FDP) or D dimers at least twice the maximum value; (3) prothrombin time at least 3 seconds longer than control value, or factor V less than 60%; (4) partial thromboplastin time at least 9 seconds greater than control value. Because DIC and fibrinolysis are interlinked in APL, no attempt at separating the two syndromes was made and the term “coagulopathy” was used.

Treatment of coagulopathy, in the chemotherapy group, was based on intensive platelet support to maintain the platelet count above 50 x 10⁹/L until disappearance of significant coagulopathy. The use of heparin, tranexamic acid, fresh frozen plasma, and fibrinogen transfusions was optional. In the ATRA group, the same approach was recommended in patients who also received chemotherapy. In patients receiving ATRA alone, platelet transfusions were recommended only if platelet counts were below 30,000/mm³, and the use of other treatments of coagulopathy was also optional.

Endpoints

EFS, where failure to achieve CR (defined below), relapse, and death in CR were considered as “events,” was the major endpoint

<table>
<thead>
<tr>
<th>Table 1. APL91 Trial: Treatment Schedule</th>
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<tbody>
<tr>
<td><strong>ATRA Group</strong></td>
</tr>
<tr>
<td>ATRA 45 mg/m²/d until CR</td>
</tr>
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<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Induction Phase</strong></td>
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<tr>
<td><strong>Course I</strong></td>
</tr>
<tr>
<td><strong>Course III</strong></td>
</tr>
</tbody>
</table>

In the ATRA group, course I was added on day 1 of ATRA treatment if WBC >5 x 10⁹/L at diagnosis, and rapidly added if WBC >6 x 10⁹/L, >10 x 10⁹/L, >15 x 10⁹/L by days 5, 10, and 15 of ATRA treatment, respectively. In the chemotherapy group, patients who had resistant leukemia after course I received course II. Patients who only obtained CR after course II received, after course III, an additional DNR 45 mg/m²/d d₁,₃ and Ara C 1 g/m²/12 d₁,₄ course (course IV).
of the study. EFS was calculated from the date of randomization and analyzed as censored data. Findings at a reference date were used, as recommended in survival studies.

The CR rate, relapse-free survival, and overall survival were considered as secondary endpoints. CR was defined by the disappearance of APL blasts in the BM, the normalization of blood count (neutrophils >1,500/mm³, stable hemoglobin level >10 g/dL, platelets >100,000/mm³) and the absence of significant coagulopathy, as defined above. Disappearance of t(15;17) was not a mandatory criterion for CR. Failures were classified as resistant leukemia and early death. Early death was defined as death during chemotherapy or ATRA, or during the period of aplasia after chemotherapy, without evidence of resistant leukemia. Resistant leukemia was defined above. Relapse-free survival was calculated from the day of CR achievement. Overall survival was calculated from the date of randomization. The reference date of February 1, 1993, was used for all the censored data.

Sample Size

Estimation of sample size was based on the method described by George and Desu with an anticipated annual accrual of 100 patients, a type I error of α = 0.05, a type II error of β = 0.10 for a one-sided test, and an assumption of treatment benefit given by an increase in 1-year EFS from 50% in the chemotherapy group to 70% in the ATRA group. It was computed that 215 patients had to be randomized in 2.15 years to observe the required number of 78 events. A maximum of five interim analyses were planned, using a level of 0.016 to maintain an overall level of α = 0.05.33

Statistical Analysis

Analysis was made on an intention-to-treat basis. Censored criteria (EFS, relapse-free survival, and overall survival) were analyzed with the Kaplan-Meier estimate,34 the log-rank test,35 and the Cox’s regression model.36 After results of the first interim analysis performed at the reference date of December 1, 1992, it was decided to discontinue patient accrual and to plan a new statistical analysis including evaluation of induction response for all randomized patients that requires a 2-month delay. Findings at the reference date of February 1, 1993, used in this report, corresponded to results of this second interim analysis.

For each endpoint, treatment comparison was adjusted on a predetermined subset of prognostic parameters using the appropriate regression models, either logistic regression or Cox’s model.36 These parameters, derived from previous APL studies were age, WBC count, platelet count, absolute number of circulating blasts, M4 subtype (“classical” M4 versus microgranular variant M3), presence or absence of significant coagulopathy, and fibrinogen level. They were introduced as binary covariates using a threshold for defining poor subgroups.

RESULTS

Early Termination of the Trial

A first interim analysis was performed at the reference date of December 1, 1992, on the 84 patients randomized prior to September 1, 1992. A significantly better EFS was found in the ATRA group (8 events in the ATRA group, 19 events in the chemotherapy group, P = .002 by the two-sided log rank test), and the decision to stop patient accrual was made on December 1, 1992. At the time, 102 patients had been randomized.

Table 2. Initial Characteristics of the Patients Included in APL 91

<table>
<thead>
<tr>
<th>Trial</th>
<th>ATRA Group</th>
<th>Chemotherapy Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>54</td>
<td>47</td>
</tr>
<tr>
<td>Male</td>
<td>30 (56%)</td>
<td>23 (49%)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>40 ± 13</td>
<td>40 ± 13</td>
</tr>
<tr>
<td>Median (range)</td>
<td>41.5 (6-63)</td>
<td>40 (17-67)</td>
</tr>
<tr>
<td>Fever</td>
<td>24 (44%)</td>
<td>17 (37%)</td>
</tr>
<tr>
<td>Infection</td>
<td>25 (46%)</td>
<td>18 (39%)</td>
</tr>
<tr>
<td>Bleeding</td>
<td>37 (69%)</td>
<td>37 (79%)</td>
</tr>
<tr>
<td>(CNS bleeding)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>WBC (10⁹/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>12 ± 19</td>
<td>12 ± 20</td>
</tr>
<tr>
<td>Median</td>
<td>1.9</td>
<td>3</td>
</tr>
<tr>
<td>&lt;5</td>
<td>35 (65%)</td>
<td>28 (60%)</td>
</tr>
<tr>
<td>5-10</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>&gt;10</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Platelet count (10⁹/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>44 ± 41</td>
<td>49 ± 57</td>
</tr>
<tr>
<td>Median</td>
<td>28.5</td>
<td>31</td>
</tr>
<tr>
<td>&lt;20</td>
<td>18 (30%)</td>
<td>16 (34%)</td>
</tr>
<tr>
<td>Circulating blasts (10⁹/L)</td>
<td></td>
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</tr>
<tr>
<td>Mean ± SD</td>
<td>9.8 ± 16.4</td>
<td>10.6 ± 18.6</td>
</tr>
<tr>
<td>Median</td>
<td>1.6</td>
<td>2.3</td>
</tr>
<tr>
<td>&gt;5</td>
<td>19 (36%)</td>
<td>17 (37%)</td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classical M₄</td>
<td>50</td>
<td>44</td>
</tr>
<tr>
<td>Micromgranular variant M₃</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Coagulopathy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant coagulopathy</td>
<td>41 (76%)</td>
<td>40 (85%)</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.5 ± 1.0</td>
<td>1.4 ± 1.2</td>
</tr>
<tr>
<td>&lt;1</td>
<td>19 (35%)</td>
<td>22 (47%)</td>
</tr>
<tr>
<td>Karyotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(15;17)</td>
<td>40</td>
<td>35</td>
</tr>
<tr>
<td>No t(15;17)</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Not performed or technical failure</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

Initial Characteristics of the Patient Population

Of the 102 patients randomized in the study, one was considered ineligible as he eventually proved to have M₂ AML, according to FAB classification, with a t(8;21) translocation (Table 2). Median age of the 101 eligible patients was 40 years (range 6 to 67). One child aged less than 15 years and 1 patient older than 65 years were included. The 26 patients where no t(15;17) was found included 11 patients where cytogenetic analysis was not performed or was a failure, and 15 patients for whom the karyotype was considered normal (13 cases) or showed clonal abnormalities without t(15;17) (2 cases). In 7 of those 26 patients (5 in the ATRA group and 2 in the chemotherapy group) blast cell RNA from diagnosis was available. In every case, the RNA showed a PML RAR rearrangement after RT-PCR analysis. In the remaining 19 patients who had no t(15;17) and for whom no RNA from diagnosis was available, diagnosis of APL was confirmed on morphologic grounds after review of the slides (M.T.D.). Fifty-four patients were allocated to the ATRA group and 47 to the chemotherapy
group. The two randomized groups were well balanced for all initial characteristics (Table 2).

Complete Remission Induction

**ATRA group.** Of the 54 patients allocated to the ATRA group, 49 (91%) achieved CR, and five (9%) had early death. No patient had resistant leukemia. Fourteen patients achieved CR with ATRA alone and 35 with ATRA plus chemotherapy. In the latter 35 patients, chemotherapy course I was added to ATRA on day 1 or 2 of treatment because WBC were greater than \(5 \times 10^9/L\) at diagnosis (15 patients) or later on, between days 3 and 15 of treatment (median 7 days), because WBC increased above \(6 \times 10^9/L\) by day 5 or \(10 \times 10^9/L\) by day 10 or \(15 \times 10^9/L\) by day 15, as scheduled (20 patients). Median duration of treatment with ATRA was 38 days (range 21 to 90). ATRA was continued until CR, in all but one of the patients who achieved CR (in the remaining patient, chemotherapy was started after 13 days, and ATRA was stopped after 21 days). The only patient in the ATRA group who presented with central nervous system (CNS) bleeding achieved CR, and had complete neurologic recovery.

Cytogenetic analysis was performed after hematologic CR had been obtained in 20 of the patients with detectable t(15;17) at diagnosis. Among them, the three patients who reached CR with ATRA alone had a normal karyotype at the time of CR. Of the 17 patients who reached CR after the addition of chemotherapy, 16 had a normal karyotype at the time of CR, and 1 still had t(15;17) in two of the 20 mitoses analyzed, but the karyotype was found normal after chemotherapy course II. Of the 14 patients without detectable t(15;17), 3 had early death and eleven achieved CR (one of them relapsed).

**Chemotherapy group.** Thirty-eight of the 47 patients (81%) reached CR, and four (8%) had early death. Thirty-two patients achieved CR after course I, and six after course II. Five patients (10%) had resistant leukemia after courses I and II of chemotherapy and were thus considered as failure. In all 5 patients, marrow blasts were greater than 50% after the two courses, 46 to 60 days after onset of chemotherapy. Two of them had persisting DIC and two others still had t(15;17) translocation. Four of them were salvaged by ATRA, and the remaining patient by additional chemotherapy with mitoxantrone, VP-16, and intermediate-dose Ara C. Those 5 patients then received consolidation chemotherapy (4 cases) or allogeneic BMT (1 case). The last patient died during the procedure. Of the 4 other patients, 1 relapsed after 9 months, and 3 remained in CR after 3, 3, and 10 months.

Fifteen of the patients with detectable t(15;17) at diagnosis were analyzed cytogenetically at the time of hematologic CR. In all 15 patients, this karyotype was normal. The 12 patients without detectable t(15;17) achieved CR (five of them relapsed).

The difference in CR rate between the two treatment groups (91% v 81%) was not significant \((P = .25\) by the Fisher's test), including after adjusted treatment comparison by the logistic regression on the subset of seven prognostic parameters \((P = .09)\).

**Outcome of Patients Who Achieved CR**

**ATRA group.** Median follow-up was 11.5 months. One patient was allografted from an HLA-identical sibling in first CR. The remaining patients received the three scheduled chemotherapy courses, except one patient (aged 67) who received only two consolidation courses. Amsacrine was substituted for DNR during course III in four patients. Six patients have relapsed, after 7, 7.5, 8, 9, 12, and 15.5 months, respectively. The Kaplan-Meier estimate of relapse was 0% at 6 months and 19% ± 8% at 12 months (Fig 1). Three of the patients who relapsed were salvaged by ATRA (administered alone in one case, and followed by chemotherapy in two patients who developed high WBC counts with ATRA), two patients were salvaged by chemotherapy alone, and the last relapsing patient did not respond to chemotherapy alone but entered CR with a combination of ATRA and chemotherapy. Two of the six patients had a second relapse after 2 and 6 months (and one of them died), and four remained in second CR after 2 to 9 months.

All the other 43 patients remained in first CR, 1 to 20 months after CR achievement. No death occurred in first CR.

**Chemotherapy group.** Median follow-up was 11 months. One patient was allografted from an HLA-identical sibling in first CR. In one patient who had experienced severe CNS bleeding during course I and had persistent massive hemiplegia, all treatment was stopped. In another patient, also with persistent hemiplegia after an ischemic stroke during course I, less intensive consolidation chemotherapy was administered. The remaining 33 patients received the scheduled consolidation courses.

Twelve patients relapsed, after 1 to 16 months, including the two patients in whom treatment had been stopped or reduced in intensity. The Kaplan-Meier estimate of relapse was 10% ± 5% after 6 months, and 40% ± 12% after 12 months (Fig 1). The difference with the ATRA group was significant \((P = .005\) by the two-sided log rank test), including after adjustment on the prognostic covariates through the Cox model \((P = .02\) by the score test). One patient obtained a second CR with chemotherapy, seven with ATRA (followed by chemotherapy in one of them, who developed high WBC counts), and two patients, who had high WBC counts at relapse, with ATRA combined to chemotherapy. The result of treatment for relapse was not evaluable at the reference date in the remaining two cases. Two of the 10 patients who obtained a second CR had a second relapse after 4 and 7 months, three died in second CR after an autograft, an allograft and consolidation chemotherapy, respectively, and five remained in second CR, after 4 to 9.5 months.

Two patients died in first CR, after the last consolidation course, from septic shock and from disseminated candidiasis, respectively. Twenty-four patients remained in CR, after 1.5 to 17 months.

**EFS**

The Kaplan-Meier estimate of EFS was 91% ± 4% at 6 months and 79% ± 7% at 12 months in the ATRA group,
versus 76% ± 6% and 50% ± 9%, respectively, in the chemotherapy group (Fig 2). The difference was significant ($P = .001$ by the two-sided log rank test). A Cox’s model was performed to adjust treatment comparison; the $P$ value of the score test was .002.

**Early Death**

The main characteristics of the patients who had an early death are summarized in Table 3. In the ATRA group, early death occurred in five patients. Three of them (patients 1 through 3) with high WBC counts, received ATRA and chemotherapy from the outset, but died between days 2 and 4. The two remaining patients presented with leukopenia and died later after diagnosis: in patient 4, WBC slowly increased to 7,600/mm$^3$ on day 16, while coagulopathy rapidly disappeared. On day 16, she had sudden onset of atrial fibrillation, requiring several cardioversion attempts. She died the next day from massive pulmonary bleeding despite absent coagulopathy and of a platelet count at 45,000/mm$^3$. No autopsy could be performed. No signs of ATRA syndrome had been observed before this fatal event. Patient 5 died on day 25, from septic shock, during the phase of aplasia after the addition of chemotherapy to ATRA when WBC counts increased.

In the chemotherapy group, three of the four early deaths occurred within 8 days of inclusion in patients with high WBC counts and were due to bleeding (patients 6 through 8), whereas patient 9 died from aspergillosis on day 40, while recovering from aplasia.

In the 101 randomized patients, WBC count greater than $5 \times 10^9$/L and microgranular variant M$_3$ were significant risk factors for early death ($P = .02$ and .02, respectively, by the Fisher’s test), whereas the type of treatment had no prognostic value ($P = .90$).

**Survival**

No patient was lost to follow-up. In the ATRA group, six patients have died, and the Kaplan-Meier estimate of overall survival was 91% ± 4% at 6 months and 91% ± 4% at 12 months (Fig 3). In the chemotherapy group, nine patients have died, and the Kaplan-Meier estimate of overall survival was 91% ± 4% at 6 months, and 80% ± 7% at 12 months. The difference was not significant ($P = .25$ by the two-sided log rank test), including after Cox’s adjustment on the subset of seven prognostic variables ($P = .27$ by the score test).

**Side Effects of Treatment**

Three of the patients who received ATRA showed signs of ATRA syndrome that developed on day 14, day 20, and day 24 of ATRA treatment and when the WBC count increased 13.7, 11.6, and $3.7 \times 10^9$/L, respectively. The two first patients were receiving ATRA alone. The last patient had also received chemotherapy from day 1, because of high WBC counts, and ATRA syndrome occurred on recovery from aplasia. Signs of ATRA syndrome disappeared spontaneously in the first patient and with intravenous dexametha-
sone in the other two cases, and all three patients achieved CR.

Other side effects of ATRA included dryness of skin and mucosae in 49% of the patients, headache in 29% of the patients (severe in only one case), bone pain in 25%, and hypertriglyceridemia in 79% of the patients. In the ATRA group, 85%, 68%, and 56% of the patients (during the induction period) had an increase in liver function tests, transaminases, and serum creatinine, respectively, compared with 83%, 75%, and 49% of the patients in the chemotherapy group (differences not significant).

The time to reach WBC > 1 × 10⁹/L, neutrophils > 0.5 × 10⁹/L, and platelets > 50 × 10⁹/L after chemotherapy course I was significantly shorter in the ATRA group than in the chemotherapy group: mean 16 ± 8 days versus 23 ± 4 days for WBC; mean 19 ± 9 days versus 26 ± 4 days for neutrophils; mean 19 ± 5 days versus 22 ± 4 days for platelets (P = .001, P = .001, and P = .02, respectively, by Kruskal-Wallis’s test).

Evolution of Coagulopathy

In the ATRA group, 79% of the patients received prophylactic platelet transfusions after the onset of treatment, 46% received heparin, 7% tranexamic acid, and 17% fibrinogen concentrates, compared with 100%, 66%, 9%, and 27% of the patients, respectively, in the chemotherapy group. When present at diagnosis, coagulopathy disappeared after a mean of 4 ± 3 days (range 1 to 11) in the ATRA group and 7 ± 4 days (range 1 to 12) in the chemotherapy group (P = .001 by Kruskal-Wallis’s test). In patients without coagulopathy at diagnosis, the onset of ATRA never triggered coagulopathy, whereas in the chemotherapy group, the onset of treatment triggered coagulopathy in four patients (one of whom experienced fatal bleeding). However, significant coagulopathy appeared in six patients treated by ATRA when hyperleukocytosis developed and chemotherapy was added, although this was never associated with bleeding. By taking into account all randomized patients, the mean duration of significant coagulopathy was 3 ± 3 days (range 0 to 11) in the ATRA group, and 6 ± 4 days (range 0 to 12) in the chemotherapy group. The difference was also significant (P = .0005 by Kruskal-Wallis’s test).

Supportive Care and Hospitalization

Mean duration of hospitalization until CR in the chemotherapy group and for ATRA and course I in the ATRA group, was 38 ± 13 and 39 ± 17 days, respectively (P = .95 by Kruskal-Wallis’s test). During this period the number of days on antibiotics and the number of platelet and red blood cell transfusions were not significantly different between the two treatment groups (details not shown).

DISCUSSION

This randomized clinical trial shows for the first time, to our knowledge, a beneficial effect of ATRA in addition to

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex/Age</th>
<th>Bleeding</th>
<th>WBC (10⁹/L)</th>
<th>Morphology</th>
<th>Significant Coagulopathy</th>
<th>Fibrinogen (g/L)</th>
<th>Platelets (10⁹/L)</th>
<th>Survival From Inclusion (days)</th>
<th>Cause of Death</th>
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Table 3. Initial Characteristics of the Patients Who Had Early Death in APL 91 Trial

Fig 3. Kaplan-Meier estimate of survival (in percentage of patients) in the two treatment groups.
Patients achieving CR with ATRA rapidly generally relapse if ATRA is maintained alone or if moderate chemotherapy is administered in CR. By contrast, in the ATRA group of the present study, where intensive chemotherapy was administered in combination to or after ATRA, no patient relapsed within 6 months of CR achievement, and the estimated risk of relapse was only 19% at 1 year, confirming the low relapse rate seen in the pilot trial (10% at 1 year). The significantly higher incidence of relapse in the chemotherapy group (estimated risk of 40% at 1 year) was similar to that previously reported in APL treated with chemotherapy alone. Because follow-up is still short in the present study, one could argue that the combination of ATRA and chemotherapy only delayed relapse, compared with chemotherapy alone. However, in the pilot trial, with a follow-up in CR ranging now from 24 to 40 months, the last relapse occurred at 15 months (unpublished data).

EFS, the major endpoint of this trial, was significantly higher in the ATRA group than in the chemotherapy group. This significant difference resulted from the combination of a nonsignificant difference in CR rate between the two groups, and of a significant difference in relapse rate. On the other hand, no significant survival advantage was seen in the ATRA group, as patients randomized in the chemotherapy group who had resistant leukemia or subsequently relapsed were salvaged by ATRA. However, relapsing APL patients who are salvaged by ATRA generally relapse again, irrespective of the type of postremission therapy, with the possible exception of allogeneic BMT. In addition, our previous experience suggests that most of the patients who, after primary resistance to chemotherapy, achieve CR with ATRA will relapse: of 6 such patients, 4 relapsed within 19 months, and 2 remained in CR, but with short follow-up (unpublished data). These findings strongly suggest that the higher EFS in the ATRA group will translate into significantly longer survival with additional follow-up.

Side effects of ATRA were generally moderate in this study. With the addition of chemotherapy when WBC counts rapidly increased, the ATRA syndrome was seen in three patients only, and rapid reversal of the symptoms was obtained with intravenous dexamethasone in two patients, confirming results obtained by Frankel et al. Our preventive approach of the ATRA syndrome by starting intensive chemotherapy as soon as a rapid increase in WBC was seen thus proved very effective on a multicenter basis, but resulted in the fact that about 70% of the patients in the ATRA group received chemotherapy before CR achievement. Interestingly, however, the duration of leukopenia and neutropenia after chemotherapy was significantly shorter in patients receiving ATRA than in the chemotherapy group. This was possibly due to a stimulatory effect of ATRA on normal residual BM progenitors, shown in a previous work. Still, one patient died, from sepsis, during the phase of aplasia after the addition of chemotherapy to ATRA. Other side effects of ATRA were moderate, as previously reported, and did not require discontinuation of the treatment.

Earlier studies have suggested that treatment with ATRA lead to rapid improvement of coagulopathy whereas with chemotherapy, the improvement was slower, and often preceded by transient worsening of coagulation parameters. Results of the present randomized trial, where duration of coagulopathy was significantly shorter in patients receiving ATRA, confirmed those previous findings. Of note was that this shorter duration of coagulopathy was observed although the majority of patients in the ATRA group also received chemotherapy during the induction phase.

In conclusion, results of this randomized clinical trial show a significant improvement of EFS in newly diagnosed APL by combining ATRA with anthracycline-Ara C chemotherapy, compared with chemotherapy alone. The improvement in EFS mainly resulted from a lower incidence of relapses in patients receiving both ATRA and chemotherapy, suggesting that ATRA and chemotherapy could act synergistically in reducing the tumor burden in APL. ATRA could differentiate the majority of leukemic cells and lead to their delayed death. Chemotherapy, on the other hand, could be active on more primitive clonogenic leukemic cells, not differentiated by ATRA. Because most of the patients who were resistant to or relapsed after chemotherapy alone will not be cured by further treatments, it is very probable that the difference in EFS between the two treatment groups will translate into a significant difference of survival with longer follow-up. Overall, these findings strongly suggest that ATRA should be incorporated in the frontline therapy of newly diagnosed APL, in association with anthracycline-Ara C chemotherapy.

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APPENDIX

Dr Fenaux and Dr Degos served as cochairmen. Dr Chastang and Dr Le Deley (Department of Biostatistics, Hospital St Louis, Paris) as biostatisticians, Dr Chomienné (Laboratory of Cell Biology, Hospital St Louis, Paris) performed the molecular biology, and Dr Dan-
iel (Laboratory of Hematology, Hopital St Louis, Paris) reviewed BM slides.

The following clinical departments participated in the trial (number of patients included in parentheses).

**French APL Group**

**Hannover German AML Group**
- University Hospital of Hannover (1) (H. Link); HämatoLOGie Klinikum Nürnberg (2) (H. Wandt); Robert Bosch Krankenhaus Stuttgart (2) (B. Löffler); Krankenhauswesekverband Augsburg (2) (O. Fackler-Schwalbe); Krankenhaus St Georg, Hamburg (1) (A. Dethling); Universität Erlangen (1) (M. Gramatski); Med. Poliklinik Würzburg (1) (P. Meyer); Ev. Krankenhaus Oldenburg (1) (B. Örtemba); Bürger Hospital Stuttgart (1) (D. Hoffmann); Universität Freiburg (1) (A. Garbe); Universität Giessen (1) (H. Pralle).

**HOVON Dutch AML Group**
- University Hospitals of Rotterdam (B. Löwenberg) (2); Nijmegen (P. Muus) (1); Eindhoven (H. Hillen) (1); Groningen (E. Velenga) (1).

**Spanish AML Group**
- University Hospital of Valencia (3) (M. Sanz); Hospital Ramon y Cajal, Madrid (1) (J. Obrizozola).

**SAKK Swiss AML Group**
- Berne (M. Fey) (2); Zurich (E. Jacky) (2).

**Other Groups**
- University of Wales Cardiff UK (6) (D. Bowen, J. Whittaker); Goethe Universität Frankfurt, Germany (3) (A. Ganser, D. Hoelzer); Ludwig Maximilians-Universität München Germany (1) (U. Jehn); University of Charleroi, Belgium (1) (P. Mineur); UCL Bruxelles, Belgium (1) (J.L. Micaux); University of Mont Godinne, Belgium (1) (A. Bosy); University of Uppsala Sweden (1) (A.M. Uden).

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