AIDA (All-Trans Retinoic Acid + Idarubicin) in Newly Diagnosed Acute Promyelocytic Leukemia: A Gruppo Italiano Malattie Ematologiche Maligne dell'Adul to (GIMEMA) Pilot Study

By Giuseppe Avvisati, Francesco Lo Coco, Daniela Diverio, Michele Falda, Felicetto Ferrara, Mario Lazzarino, Domenico Russo, Maria Concetta Petti, and Franco Mandelli

From March 1993 to October 1993, 20 consecutive, newly diagnosed acute promyelocytic leukemia (APL) patients from 13 Italian institutions entered in a pilot study named AIDA, combining all-trans retinoic acid (ATRA) with idarubicin (IDA). ATRA was administered orally beginning on the first day of induction at the dosage of 45 mg/m²/d until complete remission (CR), whereas IDA was administered intravenously at the dosage of 12 mg/m²/d on days 2, 4, 6, and 8 of the induction. Patients who achieved CR were consolidated with 3 courses of chemotherapy without ATRA; thereafter, they were followed up for molecular and hematologic CR. The median age was 35.3 years (range, 6.5 to 67.6 years); 8 patients were males and 12 females; 4 had the hypogranular variant of APL (M3v), and 4 (2 with M3v) presented with leukocyte counts ≥10,000/µL. Molecular analysis for the promyelocytic leukemia-retinoic acid receptor α (PML-RARα) hybrid gene at diagnosis was performed in 16 patients by means of reverse transcription-polymerase chain reaction (RT-PCR) analysis, and all were RT-PCR+ for the hybrid gene. In the remaining 4 patients, the cytogenetic study showed the presence of the t(15;17). After a median time of 36 days (range, 28 to 52 days) 18 (90%) patients achieved CR; the remaining 2 patients died 12 and 34 days after diagnosis from myocardial infarction caused by fungal myocarditis and from massive hemoptysis, respectively. ATRA syndrome was observed in only 2 patients, and, after the prompt discontinuation of ATRA and initiation of dexamethasone, both recovered from the syndrome. However, after recovery, 1 patient achieved CR, whereas the other died at day 34 because of massive hemoptysis; other side effects were very limited. At recovery from the third consolidation course, only 3 of 14 (21.4%) tested patients were RT-PCR- for the PML-RARα hybrid gene. Of these, 2 relapsed shortly afterwards; however, in the last patient, the PML-RARα disappeared at successive testing performed 2 months later. As of September 30, 1995, after a median follow-up period from diagnosis of 27 months (range, 24 to 31 months), the overall survival and event-free survival durations are 85% and 65%, respectively; moreover, 14 of 18 (78%) patients who achieved CR are still alive and in first molecular and hematologic CR. Of the 4 relapsed patients, 3 achieved a second CR with ATRA and, after further treatment, are now in molecular and hematologic CR after 4+, 16+, and 17+ months from the second CR. These results indicate that (1) the AIDA protocol is highly effective in treating APL; (2) after consolidation courses, the majority of patients who achieved CR are RT-PCR- for the hybrid gene PML-RARα; (3) the persistence of an RT-PCR positivity for the PML-RARα hybrid gene after 3 consolidation courses is indicative of early relapse, thus these patients still require additional treatment. These results have prompted the Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA) to initiate, in cooperation with the Associazione Italiana di Ematologia ed Oncologia Pediatrica and some European Organization for Research and Treatment of Cancer (EORTC) centers, a new multicentric clinical trial named AIDA LAP 0493 for the treatment of adult and pediatric APL patients. All patients are considered eligible if APL diagnosis is confirmed with molecular or cytogenetic studies for PML-RARα hybrid gene or t(15;17) and are enrolled to receive the same induction and consolidation therapy of this pilot study. After consolidation, patients who are RT-PCR+ for PML-RARα hybrid gene are randomized to four arms, whereas patients who are RT-PCR- after consolidation undergo, if eligible, an allogeneic transplantation procedure.

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motherapy for the treatment of APL. Preliminary results published by the French group indicate that such a combination significantly improves the prognosis of the disease.26,27

The development of reverse-transcription polymerase chain reaction (RT-PCR) assays to detect PML-RARα mRNA has provided a powerful tool for rapid diagnosis and sensitive monitoring of minimal residual disease in APL. The results of several independent studies have consistently indicated that the persistence or return, during disease remission, of a PCR test for the PML-RARα hybrid gene is predictive of early relapse, whereas persistent PCR tests are associated (although not invariably) with long-term survival and cure, even in patients treated for relapse.28

Based on these clinical and biological observations, in April 1993, the Italian cooperative group Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) started a pilot study for the treatment of newly diagnosed APL. Our protocol, named AIDA, included ATRA and IDA for remission induction followed by 3 courses of intensive chemotherapy as consolidation, according to the consolidation of the GIMEMA protocol Leucemia Acuta Promielocitica (LAP) 0389, which was still active at that time.29 Thereafter, treatment was stopped, and patients were followed up generally without any further treatment. Patients were studied at diagnosis and monitored during hematologic remission by RT-PCR of the PML-RARα hybrid gene. We report here the clinical and molecular results obtained in this study as of September 1995.

PATIENTS AND METHODS

Between March 1993 and October 1993, 20 consecutive patients with newly diagnosed APL from 11 centers of the Italian Cooperative Group GIMEMA (see Appendix) entered the study. The diagnosis of APL was based on French-American-British guidelines.30 Eligibility criteria were as follows: (1) age less than 70 years; (2) karyotypic and/or molecular evidence of the t(15;17) in leukemic cells; (3) serum creatinine level less than 2.5 mg/dL; (4) serum alkaline phosphatase, serum bilirubin, and serum glutamic oxaloacetate transaminase levels ≤3 times the upper normal limit; (5) negative pregnancy test; and (6) informed consent.

AIDA Protocol (Fig 1)

Induction treatment. Induction treatment consisted of oral ATRA at the dosage of 45 mg/m²/d from day 1, associated to intravenous IDA at the dosage of 12 mg/m²/d on days 2, 4, 6, and 8. ATRA, formulated in soft gelatin capsules of 10 mg each, was rounded for remission induction followed by 3 courses of intensive chemotherapy as consolidation, according to the consolidation in leukemic cells; (3) serum creatinine level less than 2.5 mg/dL; (4) serum alkaline phosphatase, serum bilirubin, and serum glutamic oxaloacetate transaminase levels ≤3 times the upper normal limit; (5) negative pregnancy test; and (6) informed consent.

AIDA Protocol (Fig 1)

Induction treatment. Induction treatment consisted of oral ATRA at the dosage of 45 mg/m²/d from day 1, associated to intravenous IDA at the dosage of 12 mg/m²/d on days 2, 4, 6, and 8. ATRA, formulated in soft gelatin capsules of 10 mg each, was rounded up to the nearest 10 mg dose and was administered in 2 doses, approximately 12 hours apart after meals, until complete remission (CR) or for a maximum of 60 days.

Consolidation treatment. Patients who achieved CR were consolidated with 3 chemotherapy courses consisting of the following: (1) 4 days of a 6-hour infusion of cytosine arabinoside (ARA-C) at the dosage of 1 g/m² followed, 3 hours after the end of each ARA-C infusion, by a brief intravenous infusion of 5 mg/m² of IDA (course 1); (2) a brief intravenous infusion of 10 mg/m²/d of mitoxantrone on days 1, 2, 3, 4, and 5 followed, 12 hours after the start of each mitoxantrone infusion, by an intravenous infusion of 100 mg/m² of etoposide lasting 45 to 60 minutes (course 2); and (3) a rapid intravenous infusion of 12 mg/m² of IDA on day 1, associated to subsequent administration of 150 mg/m² per every 8 hours of ARA-C (total daily dosage, 450 mg/m²) and to oral administration of 70 mg/m² per every 8 hours (total daily dosage, 210 mg/m²) of 6-thioguanine

(6-TG), and ARA-C and 6-TG were also administered on days 2, 3, 4, and 5 at the same dosage of that on day 1 (course 3).

Each consolidation course was administered at recovery from the previous one, when polymorphonuclear cells were ≥1,500 μL and platelets were ≥100,000 μL. After the 3 consolidation courses, 3 patients were transplanted with autologous BM, 1 was maintained with 6-mercaptopurine (6-MP) and methotrexate (MTX), and the remaining 14 patients did not receive any further treatment. All patients were followed up at 3-month intervals for hematologic relapse with BM cytology and at variable intervals for molecular relapse with the RT-PCR analysis for the PML-RARα hybrid gene.

Induction Supportive Treatment

During the hypoplastic period after induction chemotherapy, all patients received oral anti-fungine prophylaxis, generally amphotericin B, and oral ciprofloxacin (500 mg, twice a day) until polymorphonuclear cells were greater than 1,000 μL. All febrile episodes were treated with a cephalosporin and an aminoglycoside. At the earliest manifestation of symptoms associated with the RA syndrome (dysnea, rales, fever, and/or unexplained weight gain), ATRA treatment was promptly discontinued and was replaced by the use of intravenous dexamethasone at a dose of 10 mg twice a day for a minimum of 3 days with or without furosemide for reducing body weight and blood pressure. ATRA treatment was resumed at disappearance of the symptoms associated with the RA syndrome.

Supportive platelet transfusions were administered only in the presence of overt hemorrhages or if the platelet count was less than 20,000 μL with or without laboratory signs of severe coagulopathy (fibrinogen <150 μg/mL and fibrin/fibrinogen degradation products [FDP] >40 μg/mL or D-dimer [XDP] >400 μg/mL). Platelet transfusion data are expressed as the number of platelet units transfused to each patient. In the case of single-donor apheresis, the transfusions provided the equivalent of 8 platelet units. When needed, it was common practice to transfuse 1 U/10 kg of body weight. As for prophylaxis of the coagulopathy during the induction treatment, 70% (14 of 20) of patients received tranexamic acid at a dosage of 100 mg/kg/body weight as a continuous infusion, 30% (6 of 20) received only supportive platelet transfusions as indicated, and prophylactic heparin was never used.

Packed red blood cell units were transfused to maintain hemoglobin levels ≥8 g/dL.

Laboratory Monitoring During Induction Treatment

Complete blood and platelet counts were performed daily. Prothrombin time, activated thromboplastin time, fibrinogen, and FDP or XDP tests were performed daily until normalization and twice a week thereafter. Kidney and liver function tests were performed 3 times a week during the first 15 days and twice a week thereafter. Starting from day 15 from the onset of induction treatment, BM aspirates were obtained approximately once a week until CR was documented.

Evaluation of Response

Criteria for CR were as follows: the presence of normal BM cellularity with less than 5% of leukemic promyelocytes, a normal coagulation profile, polymorphonuclear cells ≥1,500/μL, and platelets ≥100,000/μL. Resistant disease was defined as the persistence of greater than 5% leukemic promyelocytes 60 days after the start of ATRA treatment.

Toxicity

Acute and subacute toxicities were graded according to the World Health Organization recommendations.
INDUCTION

ATRA 45 mg/m² /day p.o.
IDARUBICIN 12 mg/m² /day, by brief I.V. infusion
days 2,4,6,8

3 CONSOLIDATION COURSES(1)

<table>
<thead>
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<th>COURSE # 2</th>
<th>COURSE # 3</th>
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<td>ARA-C: 1,000 mg/m² /day, by IV infusion, lasting 6 hours, days 1,2,3,4</td>
<td>MITOXANTRONE: 10 mg/m² /day, by brief I.V. infusion, days 1,2,3,4,5</td>
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<td>IDARUBICIN: 5 mg/m² /day, by brief I.V. infusion, days 1,2,3,4 (3 hours after the end of ARA-C infusion)</td>
<td>VP-16: 100 mg/m² /days, by I.V. infusion lasting 45-60 minutes, days 1,2,3,4,5</td>
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<td></td>
<td>6-ThIOGUANINE: 70 mg/m² /every 8 hours, days 1,2,3,4,5</td>
<td>6-ThIOGUANINE: 70 mg/m² /every 8 hours, days 1,2,3,4,5</td>
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</tbody>
</table>

(1): Each consolidation course will be administered at recovery from the previous course, when the PMN are ≥ 1,500 /μL and Platelets ≥ 100,000/μL.

RT-PCR of the PML-RARα Hybrid Gene

BM aspirates were obtained at diagnosis and at various intervals during hematologic remission. After isolation of the BM mononuclear fraction by centrifugation on a Ficoll-Hypaque gradient, cells were washed twice in phosphate-buffered saline and resuspended in 4 mol/L guanidium thiocyanate solution, and total RNA was extracted by the method of Chomczynsky and Sacchi. Before RT-PCR analysis, the integrity of RNAs was always assessed by running the samples on a formaldehyde minigel. The protocol and the oligo-primers used for RT-PCR of the PML-RARα hybrid gene have been reported elsewhere. In all cases, amplification of the PML-RARα hybrid gene was performed contemporaneously to the amplification of an RARα cDNA fragment including exon 2 and exon 3 sequences to further assess RNA integrity as well as the efficiency of the RT step.

Overall Survival (OS) and Event-Free Survival (EFS) Duration

OS and EFS duration were calculated by means of Kaplan Meier method.

RESULTS

Patients' Characteristics

Clinical characteristics of the 20 patients entered in this pilot study are summarized in Table 1. The median age was 35.3 years (range, 6.6 to 67.6 years); there were 12 females and 8 males; and the mean leucocyte and platelet counts were 2,200/μL (range, 300/μL to 42,700/μL) and 24,000/μL (range, 3,000/μL to 70,000/μL), respectively. Bleeding symptoms at diagnosis were present in 18 of 20 (90%) patients, whereas laboratory evidence of coagulopathy (fibrinogen < 150 mg/dL and FDP >40 μg/mL or XDP >400 μg/mL) was present in 13 of 20 patients (65%). Morphologically, 16 patients had "classical" hypergranular APL, whereas 4 had the microgranular variant. A total of 16 patients were available for molecular studies, and all 16 showed the PML-RARα chimeric transcript in BM cells; the remaining 4 patients (no. 17, 18, 19, and 20; see Table 1) had the t(15;17) translocation shown by karyotyping. As to the breakpoint within the PML gene (BCR), we were able to study 16 of 16 patients, 10 of whom (62.5%) had the long transcript (BCR₁; see Table 1) and 6 of whom (37.5%) had the short type (BCR₂; see Table 1).

Response to Treatment

A total of 18 patients (90%) achieved CR. Median time to CR, measured from the start of induction treatment, was 36 days (range, 28 to 52 days); 2 patients (10%) did not achieve CR and died during induction at 12 and 34 days from diagnosis by fungal myocarditis (Aspergillus species), as shown by autopsy, and from massive hemoptysis, respectively. No resistant disease was observed.

General Toxicity and Adverse Reactions to ATRA

During the induction, 17 of 20 (85%) patients had fever greater than 38°C. A total of 18 patients (90%) achieved CR. Median time to CR, measured from the start of induction treatment, was 36 days (range, 28 to 52 days); 2 patients (10%) did not achieve CR and died during induction at 12 and 34 days from diagnosis by fungal myocarditis (Aspergillus species), as shown by autopsy, and from massive hemoptysis, respectively. No resistant disease was observed.
to be caused by Aspergillus species myocarditis, he died of this complication at day 12 after the initiation of induction treatment. RA syndrome was observed in 2 patients (10%); in both patients there was a prompt discontinuation of ATRA and an initiation of dexamethasone. Both patients rapidly recovered from the syndrome; however, only 1 patient (in whom the syndrome manifested with weight gain and severe bone pain) achieved CR, whereas the second patient (in whom the syndrome manifested at day 12 with weight gain, lower extremity edema, fever, and dyspnea) died at day 34 because of massive hemoptysis after having recovered from RA syndrome. Details regarding the outcome of the white blood cell counts (WBCs) of these 2 patients during the induction are provided in Fig 2. Other ATRA-related adverse reactions were limited to dryness of cutis and mucous membranes (in 3 patients [15%]), hypotension (in 1 patient [5%]), headache (in 2 patients [10%]), and bone pain (in 2 patients [10%]). Finally, despite the contemporary administration of the anthracycline IDA, alopecia and ulcerative oral mucositis were never observed during the induction.

### Outcome of Coagulopathy and Supportive Transfusional Treatment

It is worth noting that, despite the combined use of IDA, ATRA was able to prevent the clinical as well as the laboratory exacerbation of the coagulopathy that frequently occurs with the initiation of chemotherapy.

In particular, coagulopathy and bleeding symptoms present at diagnosis in 13 of 20 (65%) and 18 of 20 (90%) patients, respectively, rapidly resolved within the first 7 days.

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#### Table 1. Patient Characteristics

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<th>WBC (x 10^3/μL)</th>
<th>PLTs (x 10^9/μL)</th>
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*Abbreviations: FAB, French-American-British classification; Hb, hemoglobin; WBC, white blood cells; PLTs, platelets; M3v, M3 variant; ND, not done; +, patients still alive.*
Only 1 patient, a 36-year-old female with a fibromatous uterus, continued to have grade-3 (according World Health Organization recommendations) menometrorrhagia despite adequate platelet support (total platelet units transfused, 114) and tranexamic acid treatment. However, she completely recovered and achieved CR.

As a consequence, the supportive transfusional treatment was very limited. In particular, during the entire induction treatment, the number of packed red blood cell units transfused to each patient ranged from 2 to 14 U, with 50% of patients having received less than 9 packed red blood cell units. As for platelet-supportive treatment, the number of platelet units transfused to each patient during induction ranged from 3 (in the patient aged 6.5 years) to 114, with 10 patients (50%) having received no more than 38 platelets units and 19 patients (95%) no more than 70 platelet units.

Consolidation Treatment and Clinical Follow-Up

After a median follow-up period of 120 days (range, 82 to 161 days), 17 of 18 patients (94.4%) who had achieved CR completed the consolidation phase. Only the patient aged 67.6 years, who had a prolonged hypoplasia lasting more than 3 months after the second consolidation course, did not receive the third course.

As of September 30, 1995, a relapse was observed in 4 of 18 patients (22%) after 6, 8, 8, and 23 months from hematologic CR, respectively; the remaining 14 patients (78%) are still alive and in hematologic and molecular CR. Of the 4 relapsed patients, 1 died of progressive disease 6 months later, whereas 3 were retreated with ATRA alone (45 mg/m²/d) and achieved a second CR. Then, after a single course of consolidation with mitoxantrone (6 mg/m² on days 1, 2, 3, and 4) and intermediate-dose Ara-C (1 g/m² on days 1, 2, 3, and 4), they obtained the disappearance of the PML-RARα hybrid gene. Thereafter, 1 patient received autologous BM transplantation (AuBMT), 1 refused any further treatment, and 1 is now maintained with ATRA (45 mg/m²/d) for 15 days alternated with α-interferon (3 MU/d) for 15 days. These patients are still in second hematologic and molecular CR after 16+, 17+, and 4+ months from second CR, respectively. As for the 14 patients still alive and in first CR, 10 did not receive any further treatment after consolidation, whereas 3 received AuBMT and 1 received a maintenance treatment based on the continuous administration of daily 6-MP and weekly intramuscular MTX.

The outcome of OS and EFS for the entire population of 20 patients is shown in Fig 3. As of September 30, 1995, after a median follow-up period of 27 months (range, 24 to 31 months) for the 17 patients still alive, the OS and EFS probabilities are 85% and 69%, respectively.

RT-PCR Studies of the PML-RARα Hybrid Gene

Diagnostic BM samples for RT-PCR analyses were available in 16 cases, and all had detectable PML-RARα hybrid gene. In the 4 remaining cases in which no material was available for molecular studies, the specific t(15;17) translocation was documented in leukemic blasts by conventional karyotyping.

Follow-up RT-PCR studies were always performed on BM samples. These were collected at the time of CR, before consolidation in 12 of 16 cases and at the end of the 3 consolidation courses in 14 cases. Thereafter, BM samples were collected at variable intervals during the follow-up period.

As shown in Fig 4, of the 14 patients evaluated after recovery from the third consolidation course, 11 (78.6%) were PCR− and 3 (21.4%) were PCR+. Of these 3 latter patients, 2 patients (no. 14 and 15; see Fig 4) who were PCR− for the PML-RARα hybrid gene at the end of induction phase, became PCR+ at recovery from the third consolidation course and relapsed at days +69 and +73 from the reappearance of PCR positivity. The third patient (no. 13; see Fig 4), who showed a faint positive signal at the end of consolidation, converted to PCR− at the successive control performed 2 months later and is still in molecular and hema-
AIDA IN ACUTE PROMYELOCYTIC LEUKEMIA

Patients

At diagnosis

At CR

After the 3rd consolidation

At last follow-up

Follow-Up Duration (months)

[ ]

1

2

3

4

5

6

7

8

9

10

11

12

13

14

15

16

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Legend to the figure

= Patient #1 completed only 2 consolidation courses

= PCR +; = PCR -; = PCR 0

AutBMT = Autologous Bone Marrow Transplantation

6-MP+MTX = 6-Mercaptopurine + Methotrexate

Fig 4. RT-PCR results for the PML-RARα hybrid gene at diagnosis and during the follow-up period.

tologic remission after 27+ months of follow-up from diagnosis (Fig 4). Another patient (no. 16; see Fig 4) who was PCR - at recovery from the third consolidation course, had hematologic and molecular relapse after 56 days. Of the remaining patients (no. 1 to 12), all but 1 (no. 1) are in first hematologic and molecular remission after a follow-up period ranging from 24 to 31 months from diagnosis. Patient no. 1, who completed only two courses of consolidation and tested PCR + after the second consolidation course, became PCR + 6 months later and had hematologic relapse 9 months after the molecular relapse (Fig 4). Therefore, a total of 3 of 3 (100%) PCR + patients after consolidation (patients no. 1, 14, and 15; see Fig 4) have relapsed at different intervals from PCR positivity, whereas only 1 of 11 (9%) PCR - patient (no. 16; see Fig 4) has relapsed ($P = .005$, by Fisher's exact test).

In 12 cases, PCR analysis was also performed after induction treatment, before consolidation (Fig 4). Three (25%) patients (no. 1, 4, and 7; see Fig 4) tested PCR +, and 9 (75%) tested PCR - (no. 2, 3, 6, 8, 10, 11, 12, 14, and 15; see Fig 4). All of the 3 PCR + patients at the end of induction converted to PCR - in successive controls, and only patient no. 1 again became positive after 6 months and relapsed 9 months after molecular relapse (Fig 4).

DISCUSSION

The high CR rate (90%) observed in our pilot study is similar to the best results obtained by others with ATRA, either alone or associated to standard chemotherapy, in newly diagnosed APL.18,21,24,26,27,35 However, the AIDA protocol differs from these previous studies in several aspects: (1) the very early association, since the second day of induction treatment, of IDA to ATRA; (2) the use of the anthracycline IDA as the single chemotherapeutic agent; (3) the use of three different consolidation courses after CR; and (4) the use of RT-PCR analysis for the PML-RARα hybrid gene for monitoring the quality of hematologic CR throughout the follow-up.

Before the advent of ATRA, it was already known that one peculiarity of APL was the exquisite sensitivity to anthracycline drugs used as single induction agents.13,14 This exquisite sensitivity of APL to anthracycline drugs has been very recently confirmed by a retrospective analysis of the Southwest Oncology Group, suggesting that large doses of DNR lead to long relapse-free survival in a large percentage of patients.36 Moreover, previous GIMEMA studies suggested that IDA, when used as single induction agent in APL, gave similar or even better results than DNR alone or than a combination of IDA and Ara-C.15,16-30 In particular, the CR rates in these previous GIMEMA studies were as follows: 72% using DNR alone,15 82% and 77% with IDA alone,16,30 and 66% using the combination of IDA+Ara-C.30 Therefore, to improve the quality of CR in APL, it was quite natural for us to combine IDA with ATRA. The combined clinical and molecular results after induction (90% and 75% of hematologic and molecular CR, respectively) strongly...
suggest that the AIDA regimen results in a better CR quality. In fact, so far, all patients with APL whose treatment was based on the administration of ATRA alone as the single therapeutic agent have always had a PCR<sup>-</sup> result when tested for the PML-RAR<sub>α</sub> hybrid gene.<sup>27</sup>

However, it is worth noting that, because of the patients’ wishes, 4 of 18 patients (22%) who achieved CR received additional therapy after consolidation (3 underwent AuBMT and 1 received maintenance chemotherapy with 6-MP and MTX). All of these 4 patients are among the 14 patients who remain in substantial remission. Therefore, we cannot exclude the possibility that this additional therapy may have contributed to durable, relapse-free survival.

As for toxicity, the early combination of IDA with ATRA has greatly reduced the toxic effects of both drugs. Generally, in western studies, 25% of patients treated with ATRA have experienced the ATRA syndrome. In this pilot study, only 2 patients (10%) had such a toxicity, both in the absence of hyperleukocytosis (Fig 2). This observation confirms that hyperleukocytosis is not uniquely responsible for this syndrome and that secretion of various cytokines as well as increased expression of surface adhesion molecules might be contributory<sup>38-41</sup> as indirectly shown by the observed response to early administration of high-dose corticosteroids in these patients. Therefore, the early cytoreduction produced by IDA may help to reduce the production and expression, by the leukemic cells, of cytokines and/or adhesion molecules, thus making the appearance of ATRA syndrome less probable. Contrary to what has been observed in previous studies, the other ATRA-related toxicities were very limited and were never severe. As for IDA-related toxicity, the observed cardiac death from MI was not related to IDA but to Aspergillus species myocarditis, as shown by the autopsy. Moreover, neither alopecia, nor ulcerative mucositis were observed during induction, probably because vitamin A has a role in maintaining epithelial tissues. Bleeding symptoms, observed in 90% of patients at diagnosis, rapidly disappeared within 7 days from the initiation of treatment in all but 1 patient. This behavior is in contrast with previous chemotherapeutic experiences in which the initiation of anti-leukemic drugs in APL worsened the coagulopathy causing a high rate of early hemorrhagic deaths.<sup>5,6,45</sup> It has been recently shown that ATRA has a corrective effect on the coagulation disorders associated with APL.<sup>44-46</sup> Therefore, the rapid amelioration of the coagulopathy observed in our study was certainly mediated by ATRA.

The consolidation phase was also well tolerated, and all but 1 patient who achieved CR were able to complete the proposed treatment. Moreover, the three consolidation courses administered to patients in CR have further improved the quality of CR, as shown by 90% of patients PCR<sup>-</sup> for the PML-RAR<sub>α</sub> hybrid gene at the end of consolidation phase. However, despite this high rate of PCR<sup>-</sup> CR, 3 patients relapsed shortly after the completion of consolidation at 56 days, 69 days, and 73 days respectively. Of these 3 patients, 2 were PCR<sup>-</sup> for the PML-RAR<sub>α</sub> hybrid gene at recovery from the last consolidation course, whereas the third patient was PCR<sup>+</sup>. A fourth patient, who received only two consolidation courses and tested PCR<sup>-</sup> at the recovery from the second course, had a molecular relapse 9 months before hematologic relapse. Although confirming the value of RT-PCR positivity detected during remission as the most valuable predictor of later hematologic relapse in APL<sup>29</sup>, these data indicate that a single PCR<sup>-</sup> test for the PML-RAR<sub>α</sub> hybrid gene after completion of treatment should not be associated with extended survival and possible cure.

The low relapse rate, the OS duration of 85%, and the EFS duration of 69% observed after a follow-up from diagnosis ranging from 24 to 31 months, are in agreement with previous observations.<sup>24,39,45,47</sup> In particular, in two successive studies, Fenaux et al have found EFS rates of 77% and of 79% after 18 and 12 months of follow-up, respectively.<sup>26,27</sup> The updated results of these studies indicated an EFS of 62% at 4 years and of 68% at 2 years, with OS values of 77% and 81%, respectively.<sup>47,48</sup> Similar results have been obtained also by Kanamaru et al, with an EFS of 75% after a median follow-up of 23 months. Moreover, Frankel et al<sup>24</sup> in a group of 34 newly diagnosed patients with APL treated with ATRA alone during the induction have found an OS of about 70% (value calculated from the curve) after a median follow-up of 25+ months.

Moreover, in our study, all PCR<sup>-</sup> patients after consolidation (no. 1, 14, and 15; see Fig 4) have relapsed at different intervals from PCR positivity, whereas only 1 of 11 (9%) PCR<sup>-</sup> patients (no. 16; see Fig 4) has relapsed (<i>P</i> = .005, by Fisher’s Exact Test), confirming that RT-PCR negativity is the requisite for long-term CR.<sup>29</sup> Therefore, at present, the achievement and maintenance of an RT-PCR<sup>-</sup> status is universally recognized as the main therapeutic goal in APL patients. However, only RT-PCR tests for the PML-RAR<sub>α</sub> hybrid gene that are repeatedly negative are associated with extended survival or cure. As a consequence, in our study, the use of this test has permitted us to identify early those patients who are candidates for hematologic relapse, confirming the data that RT-PCR positivity is invariably followed by hematologic relapse, usually within 6 months.<sup>27</sup> However, it is worth noting, as shown in the case of the last relapsed patient, that not all patients relapse shortly after the reappearance of an RT-PCR<sup>+</sup> test. Therefore, one of the crucial questions that needs answering in the future is whether patients with molecular relapse should or should not be treated before the occurrence of hematologic relapse. An answer to this puzzling question is not yet available, considering that 3 of 4 hematologic relapsed patients in our study have rapidly achieved a second CR with ATRA alone and are still in CR at 4+, 16+, and 17+ months from second CR.

These preliminary results have prompted the GIMEMA to initiate, in cooperation with the Associazione Italiana di Ematologia ed Oncologia Pediatrica (AIEOP) and some European Organization for Research and Treatment of Cancer (EORTC) centers, a new multicentric clinical trial named AIDA LAP 0493 for the treatment of adult and childhood APL patients. All patients are considered eligible if APL diagnosis is confirmed with molecular or cytogenetic studies for PML-RARα hybrid gene or t(15;17) and are enrolled to receive the same induction and consolidation therapy of this pilot study. Thereafter, patients PCR<sup>-</sup> after consolidation receive a maintenance therapy for 2 years, according to four randomization arms as in the European APL 93 trial.<sup>49</sup>
whereas patients RT-PCR after consolidation undergo, if eligible, an allogeneic transplantation procedure. The four randomization arms for maintenance are the following: (1) 6-MP (90 mg/m²/d) + MTX (15 mg/m²/wk); (2) ATRA (45 mg/m²/d) for 15 days every 3 months; (3) 6-MP (90 mg/m²/d) + MTX (15 mg/wk) for 3 months, alternated to ATRA (45 mg/m²/d) for 15 days every 3 months; and (4) no maintenance treatment. The preliminary results of this protocol, which is the first example of “molecularly adapted” therapy in acute leukemias, indicate that, among the first 170 patients who completed the induction phase, 159 (94%) achieved CR. Moreover, 110 of 113 patients (97%) who completed the consolidation phase are in molecular CR.80

APPENDIX

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