

Treatment of Acute Promyelocytic Leukaemia Using a Combination of All-trans Retinoic Acid and Chemotherapy

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Abstract

Introduction: The combination of all-trans retinoic acid (ATRA) with chemotherapy has improved the outcome of acute promyelocytic leukaemia (APL). Effective induction as well as maintenance therapy for APL can be achieved using this combination of anti-leukaemic agents. **Materials and Methods:** Twenty-four consecutive patients with newly-diagnosed APL were treated with ATRA daily together with either daunorubicin or idarubicin. Therapy with ATRA was continued until complete remission (CR) was achieved; thereafter, patients were treated with 2 cycles of an anthracycline-based consolidation chemotherapy (either daunorubicin or idarubicin). Maintenance therapy was achieved using 5 alternating cycles of low-dose methotrexate (MTX) plus 6-mercaptopurine (6MP) followed by ATRA alone. **Results:** Twenty-three out of 24 patients (96%) completed induction therapy and achieved haematological CR (HCR) as well as molecular remission (MR); however, 1 patient (5%) died from retinoic acid syndrome. Twenty-one out of 23 evaluable patients (91%) completed consolidation chemotherapy, and 2 patients (10%) died, 1 from neutropenic sepsis and the other from relapse following non-compliance to therapy. All 21 surviving patients in the present study received maintenance chemotherapy and are still in HCR and MR at a median follow-up of 23 months. The estimated actuarial 2-year overall survival (OS) and event-free survival (EFS) rates were both 84% ± 9%. **Conclusion:** The combination of ATRA with an anthracycline is an effective remission-induction therapy for newly-diagnosed APL. Maintenance therapy using alternating cycles of MTX plus 6MP followed by ATRA alone is effective in maintaining CR and MR as well as prolonging the survival of patients with APL.

Ann Acad Med Singapore 2001; 30:401-8

Key words: Maintenance therapy, Molecular remission, Retinoic acid syndrome

Introduction

Acute promyelocytic leukaemia (APL), or acute myeloid leukaemia (AML)-M3 under the French-American-British (FAB) classification,¹ is characterised by the following: a distinct expansion of promyelocytes and immature myeloblasts in the bone marrow; balanced reciprocal translocation between the long arms of chromosome 15 and 17, t(15;17) which results in apposition of the retinoic acid receptor (RAR) α gene of chromosome 15 telomeric to the promyelocytic leukaemia (PML) gene of chromosome 17;² and coagulopathy characterised by disseminated intravascular coagulopathy (DIC), fibrinolysis and proteolysis.³ In Singapore, approximately 5% of all newly-diagnosed cases of AML are APL. Previously, approximately 50% to 80% of patients with APL achieved complete remission (CR) after conventional induction chemotherapy which includes a combination of an anthracycline with cytosine arabinoside (AraC);⁴⁻⁷ whereas

10% to 20% of patients died from associated coagulopathy.³

⁸ More recently, all-trans retinoic acid (ATRA) has been shown to induce both the *in vivo* and *in vitro* differentiation of immature leukaemic cells in APL into more mature cells; i.e. release from a differentiation block.⁹⁻¹¹

Prior reports from several clinical trials using a combination of ATRA and chemotherapy have demonstrated good complete remission (CR) and disease-free survival (DFS) rates.¹²⁻¹⁹ Moreover, a randomised study demonstrated greater efficacy in achieving CR for simultaneous rather than sequential administration of ATRA with chemotherapy.¹⁴ The inclusion of ATRA into maintenance therapies has also resulted in significant benefits to the patient in 2 large randomised studies.^{13,14} In this study, we report our experience in the simultaneous use of ATRA with an anthracycline (either daunorubicin or idarubicin) for the treatment of 24 consecutive newly-diagnosed patients with APL in the Singapore General

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Hospital (SGH). In addition, consolidation chemotherapy with an anthracycline followed by maintenance chemotherapy using alternating cycles of methotrexate (MTX) plus 6-mercaptopurine (6MP) followed by ATRA alone was also included in our study protocol. Survival analysis was compared with a group of historical control patients treated with conventional chemotherapy without ATRA. Patients receiving ATRA were also observed for a capillary-leak syndrome (retinoic acid syndrome) associated with the use of ATRA. Historical control patients were used because previous studies had already reported favourable survival outcome in patients treated with combination of ATRA and chemotherapy. It was felt that conducting a randomised control trial in which one group of patients would be denied the potential benefit of ATRA, would be unethical.

Patients and Methods

Patients

Twenty-four patients with newly-diagnosed APL presenting to the Department of Haematology, SGH between September 1995 and April 2000 were consecutively recruited into this study. The diagnosis of APL was made on: cell morphology and cytochemistry according to the FAB classification; cytogenetic analysis demonstrating balanced reciprocal translocation between the long arms of chromosome 15 and 17, t(15;17); and transcription of PML-retinoic acid receptor (RAR) α mRNA (PML-RAR α) by reverse transcriptase-polymerase chain reaction (RT-PCR). Therapy was instituted after informed consent according to standard institutional practices.

Treatment Protocol

Induction therapy: Induction therapy consisted of daily doses of ATRA (45 mg/m²/day orally in 2 divided doses 12 h apart from day 1), and an anthracycline; either daunorubicin (60 mg/m²/day intravenously on days 5, 6 and 7) or idarubicin (12 mg/m²/day intravenously on days 5, 6 and 7). Patients expressing a total white blood cell (TWBC) count of >10,000/ μ L prior to day 5 of induction therapy, received 3 doses of the anthracycline prior at the time of TWBC count elevation. Patients >60 years old received attenuated doses of daunorubicin (45 mg/m²/day). Moreover, attenuation of anthracycline therapy was allowed for poor performance status and poor tolerance to therapy, and was done at the discretion of physician-in-charge. Since prolonged use of ATRA was associated with decreased responsiveness, therapy with ATRA was tailed-off when patients achieved CR or after 60 days of therapy, whichever came first.

Consolidation chemotherapy: Patients who achieved CR received 2 courses of consolidation chemotherapy

consisting of an anthracycline; either daunorubicin (60 mg/m²/day intravenously), or idarubicin (12 mg/m²/day intravenously) for 3 days; without ATRA. Each cycle of consolidation chemotherapy was administered when the neutrophil count exceeded 1500/ μ L and the platelet count exceeded 100,000/ μ L.

Maintenance therapy: Patients remaining in CR 3 to 4 weeks after haematological recovery following 2 cycles of consolidation chemotherapy received maintenance therapy consisting alternating courses of:

- 1) a combination of 6MP (50 to 150 mg/day orally) and MTX (15 to 25 mg weekly orally) for 2 months; adjusted to maintain TWBC between 2000/ μ L and 4000/ μ L; and
- 2) ATRA (25 mg/m² daily orally) for 1 month.

Cycles of maintenance therapy [i.e. course (1) followed by course (2)] were repeated for a total of 5 cycles over a period of 18 to 24 months.

Grading of Toxicity

Acute and subacute toxicities were graded according to the World Health Organisation (WHO) grading system for toxic reactions to drugs and therapies.

Treatment of Sepsis

Febrile neutropenia was defined as an oral temperature of at least 38°C accompanied by an absolute neutrophil count of <500/ μ L, and was initially empirically managed with broad-spectrum intravenous antimicrobial therapy (usually a β -lactamase plus aminoglycoside combination). Antibiotics were appropriately changed according to culture sensitivity reports. Granulocyte-colony stimulating factor (G-CSF, 300 μ g daily subcutaneously) was administered during life-threatening sepsis or prolonged (>14 days) neutropenia.

Transfusion Support

Transfusions of packed red blood cells were performed when haemoglobin (Hb) levels dipped below 8 g/dL. Significant coagulopathy was defined by a plasma fibrinogen level of <5.3 μ mol/L; or by the presence of at least 2 of the following 4 criteria:

- 1) D-dimer level of at least twice the maximum control value,
- 2) a positive detection of soluble fibrin monomers,
- 3) a prothrombin time (PT) greater than the control range, and
- 4) an activated partial thromboplastin time (aPTT) greater than the control range.

Judicious transfusions of fresh frozen plasma (FFP) or cryoprecipitate were used to treat coagulopathy. All patients received adequate platelet transfusion support to maintain the platelet counts >15,000/ μ L during the period of coagulo-

pathy; in the absence of coagulopathy, platelet transfusions were performed only during active haemorrhage or thrombocytopenia of $<15,000/\mu\text{L}$.

Bone Marrow Examination

Bone marrow and peripheral blood samples were obtained at diagnosis, after induction therapy, after 2 cycles of consolidation chemotherapy, 6 months after starting maintenance therapy, and upon completion of maintenance therapy. Bone marrow samples were analysed for morphology, cytochemistry, histochemistry, immunophenotype using indirect immunofluorescence flow cytometric analysis, cytogenetic analysis, and RT-PCR for the PML/RAR α mRNA transcript (sensitivity of detecting 1 positive cell in 1000 cells). Further analysis of PML/RAR α mRNA expression was performed on matched peripheral blood samples.

Haematological Parameters

A full blood count (FBC) was performed daily from a venous peripheral blood sample obtained during induction therapy. PT, aPTT, fibrinogen, D-dimer, and soluble fibrin monomer assays were also performed daily during initial period of induction therapy until normalisation of the results.

Definitions and Study Endpoints

Haematological complete remission (HCR) and haematological relapse were defined according to the National Cancer Institute criteria.²⁰ Failures were classified as resistant leukaemia and early death. Early death was defined as death occurring during induction therapy or during the period of aplasia after chemotherapy.

Molecular remission was confirmed by a normal cytogenetic profile [including the absence of t(15;17) (q22;q21)]; and negative PML-RAR α mRNA expression using RT-PCR assay with a sensitivity level of 10^{-3} .

According to Frankel et al,²¹ ATRA syndrome was defined as:

- 1) definitely present in the presence of the characteristic signs or symptoms of this syndrome²²: fever, dyspnoea, pleural and/or pericardial effusion, pulmonary infiltrates, and unexplained weight gain greater than 5 kg;
- 2) indeterminate when 2 to 4 of the above-mentioned signs and symptoms occurred; and
- 3) definitely absent in the remaining patients.

Statistical Analysis

Overall survival and event-free survival (OS and EFS) were calculated from the date of starting of induction therapy. Failure to achieve HCR, relapsed and death were considered as event, whichever occurred first.

Statistical analyses of OS and EFS were performed 4 months after inclusion of the final patient. Kaplan-Meier (KM) analysis was used to calculate OS and EFS. Patients diagnosed with APL between 1985 and 1992 received chemotherapy alone without ATRA comprised the historical control population. The log-rank test was applied to KM data sets (SPSS for Windows version 10).

Results

Patients' Characteristics

Between January 1995 and April 2000, there were 24 patients with newly diagnosed APL treated with ATRA-based chemotherapy regimen. The initial characteristics of these 24 patients were summarised in Table I. Laboratory evidence of coagulopathy was present in 16 out of 24 patients (67%). Median PT was 13.6 seconds (range 11.9 to 16.1 seconds), median aPTT was 27.5 seconds (19.5 to 36.0 seconds), and median fibrinogen levels was $7.6 \mu\text{mol/L}$ (range 2.8 to $19.0 \mu\text{mol/L}$) in patients who presented with haemorrhage. A total of 23 out of 24

TABLE I: CLINICAL AND LABORATORY CHARACTERISTICS AT DIAGNOSIS OF 24 ACUTE PROMYELOCYTIC LEUKAEMIA PATIENTS TREATED AT SGH BETWEEN 1995 AND 2000

	Median (Range)	No. (%)
Age (y)	39 (18-71)	
Male		12 (50)
Female		12 (50)
Clinical symptoms		
Haemorrhage		
Cutaneous		8 (33)
Mucosal		11 (46)
Fever		9 (38)
Asymptomatic		1 (4)
Haemoglobin (g/dL)	7.2 (4.2-13)	
White blood cell count ($\times 10^9/\text{L}$)	1.35 (0.15-65.99)	
Platelet count ($\times 10^9/\text{L}$)	36.0 (7.0-124.0)	
Coagulopathy		16 (67)
Fibrinogen ($\mu\text{mol/L}$)	7.25 (2.5-19.0)	
Karyotype		
Normal		1 (4)
t(15;17)		18 (75)
t(15;17),+8		1 (4)
t(15;17) plus other abnormality		3 (13)
Not done		1 (4)
PML/RAR α		
BCR1		14 (58)
BCR2		3 (13)
BCR3		6 (25)
Not done*		1 (4)

*Patient had t(15;17)

patients were available for molecular analysis for promyelocytic leukaemic-retinoic acid receptor α (PML-RAR α) hybrid gene. All were positive for the hybrid gene. The only patient who did not have molecular analysis done had the t(15, 17) translocation by karyotyping. All patients except 1 demonstrated the specific t(15,17) translocation. The only patient who had normal karyotype demonstrated the PML/RAR α hybrid gene by molecular analysis. As to the breakpoint within the PML gene (BCR), we were able to study 23 out of 24 patients; 14 of whom (58%) had the BCR1 breakpoint, 3 (13%) had BCR2 breakpoint and 6 (25%) had BCR3 breakpoint.

Results of Induction Treatment

Haematological response and early death: Twenty-three (95.8%) patients achieved HCR after induction. The median time to CR, as measured from the start of induction therapy, was 4 weeks (range 2 to 33 weeks). One patient (4%) had early death from intracranial haemorrhage. This patient (#5) developed ATRA syndrome on the 10th day of induction therapy which was associated with rising white blood cell count (from $0.8 \times 10^9/L$ to $14.2 \times 10^9/L$ over 3 days).

Molecular response: PML/RAR α tests were performed on 23 cases at the end of the induction (Fig. 1). Four (17.3%) patients were tested PCR-positive and 19 (82.7%) PCR-negative.

TABLE II: TOXICITY OF ATRA-BASED INDUCTION THERAPY FOR APL PATIENTS

Toxicity	No.	(%)
Fever	24	(100)
Positive microbial culture	5	(20.8)
Bacteraemia	2	(8.3)
Haemorrhage		
Pulmonary	2	(8.3)
Central nervous system	1	(4.2)
Retinal	4	(16.7)
Gastrointestinal tract	2	(8.3)
Urinary tract	1	(4.2)
ATRA syndrome		
All diagnostic criteria present	1	(4.2)
Insufficient diagnostic criteria present	3	(12.5)
Absent	20	(83.3)

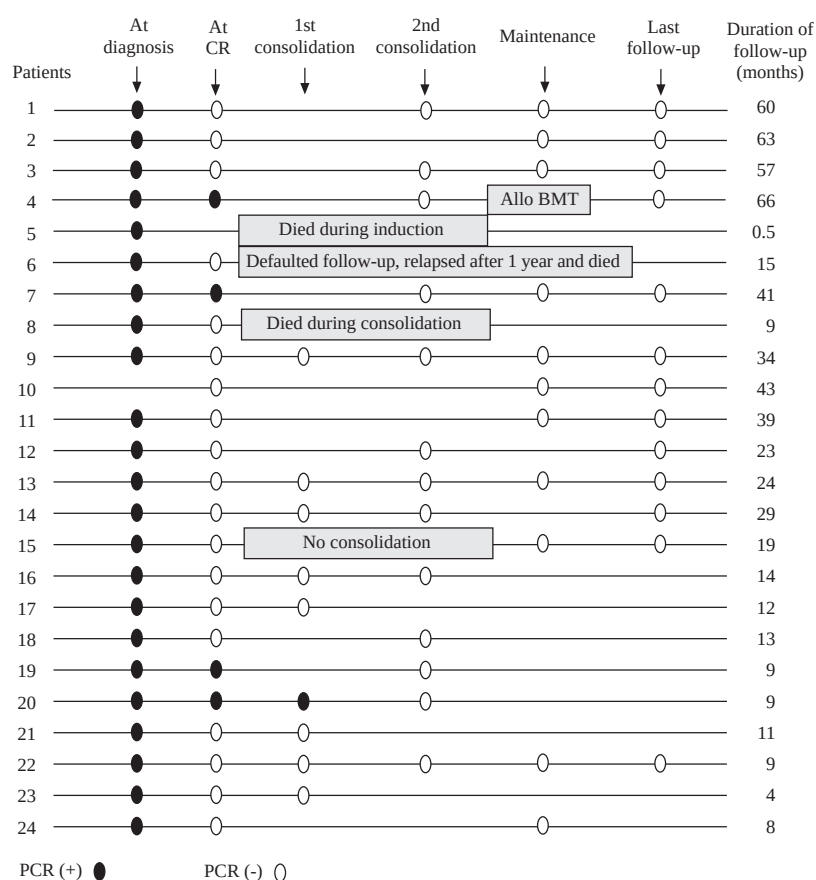


Fig. 1. RT-PCR results for the PML-RAR α hybrid gene at diagnosis and during follow-up period.

Toxicity and ATRA syndrome: All patients (100%) developed fever during induction therapy (Table II). Fever was attributed to a positive microbial culture in 5 patients (20.8%). Moreover, 2 patients (8.3%) had positive blood cultures (i.e. bacteraemia). Ten patients (41.7%) developed haemorrhage during induction therapy. Of these, 2 patients (8.3%) had pulmonary haemorrhage, 1 (4.2%) had cerebral haemorrhage, 4 (16.7%) had retinal haemorrhage, 2 (8.3%) had gastrointestinal tract haemorrhage, and 1 (4.2%) had haematuria. Fatal haemorrhage was observed in only 1 (4.2%) patient who had intracerebral bleeding. The same patient exhibited definite criteria for the occurrence of the ATRA syndrome.²¹ Three patients (12.5%) (#11, #13 and #21) were considered to have developed 'indeterminate' ATRA syndrome on the 5th, 17th and 24th day of induction therapy respectively. Patient #11 developed sudden dyspnoea and pulmonary infiltrate while the leukocyte count was $1.56 \times 10^9/L$. Patient #13 experienced chest tightness on 5th day of induction therapy and her count was rising from $4.80 \times 10^9/L$ to $20.00 \times 10^9/L$ and went up further to $29.07 \times 10^9/L$ during the onset of this symptom. Patient #21 had fever and dyspnoea of sudden onset with a leukocyte count of $5.04 \times 10^9/L$. All of them responded to prompt initiation of dexamethasone therapy. The remaining 20 patients (83%) did not exhibit any of the manifestations of the ATRA syndrome. During induction chemotherapy, all patients developed grade 3 to 4 haematological toxicity. The median time to polymorphonuclear cell count $>1 \times 10^9/L$ was 26.5 days (range 16.0 to 99.0 days) and platelet count $>100 \times 10^9/L$ was 27.0 days (range 7.0 to 99.0 days). Median red blood cell (RBC) and platelet concentrates transfused per patient were 7.5 units (range 0 to 18 units) and 20 units (range 0 to 103 units), respectively.

Consolidation Treatment and Clinical Follow-Up

Molecular response: After a median follow-up period of 23 months, 20 of 23 patients (87%) who had HCR completed the consolidation phase. Of the 4 patients who were PCR-positive after induction, 3 converted to PCR-negative after the second consolidation and the other converted to PCR-negative after allogeneic bone marrow transplant (Fig.1). All the patients who were PCR-negative after induction remained in molecular remission after consolidation therapy.

Toxicity: All (100%) patients developed grade 3 to 4 haematological toxicity. Twenty-one (91.3%) patients had neutropenic fever during this cycle. One (4.3%) patient died of neutropenic sepsis. One (4.3%) patient was deemed unfit to receive consolidation therapy and proceeded with the maintenance therapy. The remaining 1 (4.3%) patient defaulted follow-up after achieving HCR with induction therapy. He died 1 year later from acute myocardial infarct

and was in haematological and cytogenetic relapse.

Maintenance Therapy

Nineteen (79.2%) patients who completed full consolidation therapy proceeded to maintenance therapy as scheduled. One patient (#15) who had prolonged cytopenia after induction, proceeded to maintenance therapy without receiving consolidation therapy. The only patient (#4) who had allograft did not receive maintenance therapy.

After a median time of 23 months (range 4 to 66 months) after diagnosis, 9 (42.9 %) out of 21 surviving patients had completed maintenance therapy and 11 (52.4%) were still on treatment. PML/RAR α RT-PCR test was performed in all the 9 cases at the end of maintenance therapy and all of them remained in molecular remission (Fig. 1).

The maintenance therapy was generally well tolerated. Grade 1 and 2 neutropenia was commonly observed, often requiring dose reduction or temporarily discontinuation of chemotherapy. No death in CR occurred during maintenance therapy.

Outcome

As of August 2000, after a median follow-up period of 17.3 months (range 0.5 to 66.4 months), the estimated 2-year OS and EFS rates were both $84\% \pm 9\%$ (Fig. 2). No relapse was seen in any of these patients.

The results of the present series were compared with 14 APL patients diagnosed between 1985 and 1992 and were treated with chemotherapy alone (without ATRA). The median age of this historical population was 33 years (range 15 to 74 years). There were 8 male and 6 female patients. The median haemoglobin, TWBC and platelet counts were 8.2 g/dL (range 4.5 to 11.2 g/dL), $4.65 \times 10^9/L$ (range 0.77 to $88.30 \times 10^9/L$) and $17.0 \times 10^9/L$ (range 9.0 to $49.0 \times 10^9/L$), respectively. Eleven out of these 14

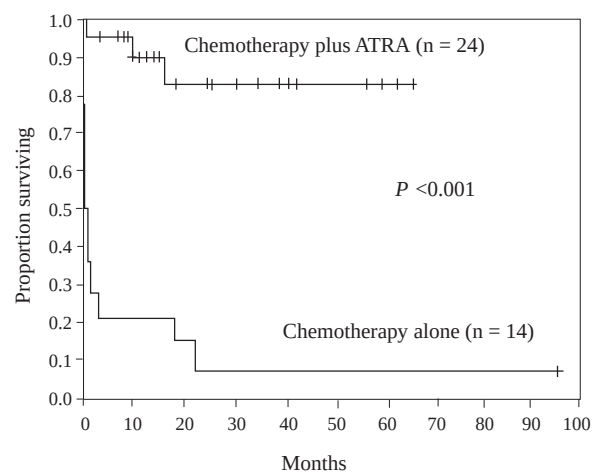


Fig. 2. Kaplan-Meier estimate of overall and event-free survival of APL patients.

patients died of haemorrhagic complications (8 intracranial haemorrhage and 3 pulmonary haemorrhage) during the induction therapy. Of the 3 patients who achieved complete remission after induction therapy, 2 patients died of relapse within 2 years from diagnosis. The estimated 2-year OS and EFS rates were both $7\% \pm 7\%$. As shown in Figure 1, the difference in OS and EFS between the present series and the control group was clearly significant ($P < 0.001$).

Discussion

In the present study, we used ATRA in combination with an anthracycline to achieve a 96% CR rate in 24 patients with newly-diagnosed APL. This greatly improved CR rate in comparison to a historical population of patients diagnosed with APL who underwent conventional chemotherapy without ATRA is equivalent to reports from other centres worldwide.^{14,15} Moreover, patients achieving CR next underwent 2 cycles of anthracycline-only chemotherapy followed by maintenance therapy consisting of 5 cycles of MTX plus 6MP followed by ATRA only; and demonstrated prolonged OS, DFS and EFS. Previous reports from 2 randomised studies, the European APL91 study group¹² and the North America Intergroup study,¹³ have demonstrated that treatment of APL with ATRA followed in sequence by chemotherapy resulted in better OS rates and lower relapsed rates than chemotherapy alone (i.e. without ATRA). These studies suggest that combination of ATRA with chemotherapy could induce additive or synergistic activity. However, relapse of APL was still observed in 30% of patients in the APL91 study and 11% of patients in the North America Intergroup study; and it was unclear whether sequential or simultaneous administration of ATRA and chemotherapy was preferable. Moreover, the possible role of maintenance therapy to prolong survival of patients with APL had yet to be established.

Recently, it was found in the European APL93 study group¹⁴ that high CR rates (>90%) could be achieved when ATRA was simultaneously administered with chemotherapy. Although CR rates were not significantly increased as compared with those previously reported by the European APL91 study group¹² and North America Intergroup study,¹³ simultaneous combination of ATRA with chemotherapy was fortuitously associated with significantly fewer relapses. Moreover, patients who received maintenance therapy with either intermittent doses of ATRA or continuous doses of chemotherapy (i.e. low-dose 6MP and MTX) demonstrated further significantly-reduced (6 out of 74; 7.4% at 2 years) relapse rates. Maintenance therapy with intermittent doses of ATRA was associated with better OS, DFS and EFS rates compared with patients who were not treated with maintenance therapy. Continuous low-dose maintenance chemotherapy was also associated with

significantly better OS and EFS rates. The high efficacy of simultaneous administration of ATRA with chemotherapy has been supported by a recent report from the Italian GIMEMA group.¹⁵ However, rates of long-term survival and late relapses have not been established.

The role of cytarabine in the management of APL has become a controversial issue. Head et al¹⁶ had shown that inclusion of cytarabine in addition to daunorubicin, with or without other chemotherapy agents (e.g. thioguanine, etoposide), does not appear to significantly affect disease outcome. Most of the recent trials^{13-15,17,18} include cytarabine and other drugs such as etoposide in combination with anthracycline as post-remission treatment. In fact, all these studies reported important significant toxicity in consolidation as well as to a death rate in remission of up to 10%. Our consolidation using 2 cycles of anthracycline alone has resulted in 1 of 21 (5%) deaths. However, the omission of cytarabine in our study has not shown any compromise in the survival rate or resulted in any disease relapse. In fact, it supports the findings of studies by Estey et al¹⁹ and Sanz et al²² that cytarabine has little therapeutic effect in APL and suggests that patients might be spared the risks associated with intensive consolidation containing cytarabine.

The role of maintenance therapy in APL continues to be an area of interest in most studies. The North America Intergroup study and the European APL93 study had both demonstrated the benefits of maintenance therapy that contains ATRA.^{13,14} We designed this maintenance therapy protocol at the time before the availability of any results from randomised trials. Following an earlier report from the MD Anderson Cancer Center group which showed that some form of maintenance therapy may be beneficial,²³ we decided to use a maintenance regimen which contained ATRA and 2 chemotherapy agents (6MP and MTX) given in an alternate manner. The rationale for the schedule of ATRA maintenance was based on the pharmacokinetic studies showing rapid decrease of peak serum levels of ATRA with its continuous use for more than 2 to 3 weeks, due to hypercatabolism of the drug. However, this metabolic pathway is reversible a few weeks after ATRA discontinuation, and high serum levels of ATRA can be obtained by new drugs intake.²⁴ Our maintenance therapy regimen was generally well tolerated, with no severe or life-threatening toxic effects. This is unlike the more severe toxic effects of the maintenance therapy reported by the North America Intergroup Study Group.¹³ In their study using continuous ATRA 45 mg/m²/day for 1 year, it was reported that 36% of patients had severe or life-threatening toxic effects, which included neurotoxicities (headache, pseudotumour cerebri, hearing loss, depression), infections and hepatotoxicity (hyperbilirubinaemia and aminotransferase elevation). Fenaux et al in their APL93 study¹⁴

showed that continuous low-dose chemotherapy (using 6MP and MTX) and intermittent ATRA had an additive effect with an estimated 2-year relapse rate at only 7.4%. All patients in our series who had completed maintenance therapy remained in molecular remission. There was no relapse at the last follow-up. In light of the short-term follow-up, we are not able to establish at present the long-term effectiveness of our maintenance regimen. However, the favourable short-term relapse-free survival in our series certainly suggests that this approach is promising and is in agreement with results of the APL93 study.

ATRA syndrome, the major and the most fearful side effect of ATRA treatment, has been reported in up to 25% of cases receiving ATRA alone.²⁵ In our series, it occurred in 17% (4 out of 24) of our patients. It occurred between the 5th day to the 17th day of induction therapy with ATRA. Hyperleukocytosis was seen in 2 of the 4 patients. All had pulmonary symptoms. One patient had a fatal outcome due to subsequent intracranial haemorrhage, whereas the other 3 responded well to steroid therapy which was started at the earliest clinical sign of dyspnoea. The incidence of ATRA syndrome was found to be 26% and 15% in the North America Intergroup study and the European APL93 study, respectively. Risk factors for ATRA syndrome have been difficult to establish.²⁶⁻²⁸ With the possible exception of CD13 expression,²⁷ no pretreatment clinical factors (age, WBC or platelet count, presence of disseminated intravascular coagulopathy) have been found to be predictive for developing ATRA syndrome.^{26,27} In light of the small sample size and the low incidence of ATRA syndrome in our patients, we were not able to find any conclusive clinical features which had direct relationship to the development of ATRA syndrome. We believed that the relatively low incidence of ATRA syndrome in our series was due to the use of concurrent ATRA and chemotherapy during the induction therapy. The induction therapy protocol required anthracycline chemotherapy to be started on the 5th day of ATRA therapy. However, anthracycline may be given prior to the 5th day of induction therapy if the leukocyte count exceeded $10.0 \times 10^9/\text{dL}$. The low incidence of "overt" ATRA syndrome (2.5%), with only 1 death in the Italian GIMEMA/AIDA study,¹⁵ suggests that concurrent ATRA/chemotherapy may prevent most cases of ATRA syndrome; perhaps by preventing or greatly ameliorating the rapid leukocytosis frequently observed with ATRA alone.

Molecular remission was achieved in 82% of our cases after induction and 100% after consolidation. All surviving patients were in molecular remission before receiving maintenance therapy and those who completed the maintenance phase remained so at the last follow-up. There was no relapse among those who were PCR negative. The

attainment of a remission status corresponding to less than 10^{-3} PML/RAR α (according to the sensitivity of our assay) in almost all the patients certainly demonstrates the efficacy of our treatment protocol.

In conclusion, simultaneous therapy using ATRA and an anthracycline (either daunorubicin or idarubicin) is highly effective as induction therapy for the newly-diagnosed APL. Moreover, maintenance therapy using cycles of 6MP plus MTX alternating with ATRA alone is associated with reduced relapse rates and increased survival of patients with APL. In future, we hope that the treatment protocol for APL using ATRA and chemotherapy will also translate to longer-term survival and fewer relapses.

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