189 in the bone marrow (75%), 16 in the CNS (6%), 24 in bone marrow and another site(s) (10%), and seven in the testis (2% of 337 boys; Table 1). Of 146 relapses in the chemotherapy group, 33 (23%) were diagnosed within 6 months from complete response. In addition, 79 (15%) of these 542 patients died during first remission at a median of 0.83 years (range, 0.1 to 6.2 years) after remission was induced. The cause of death was related to HSCT in 54 patients, chemotherapy in 15 patients, and other factors in six patients and was unknown in four patients. Second malignant neoplasms developed in four patients (0.7%) as the first adverse event. Altogether, 207 (38%) of 542 patients were in continuous complete remission on the date of the last evaluation.

Impact of Postremission Therapy on Treatment Outcome

Of the 542 patients who achieved remission by the end of induction therapy, 217 were treated with chemotherapy only, whereas 325 underwent HSCT with different types of donors (Table 1). The Cox regression model was applied to assess the effect of different postremission treatments on DFS and OS, adjusting for relevant characteristics (ie, initial leukocyte count, age, sex, and early response), as shown in Table 2. The advantage of transplantation on DFS appeared during the second year of follow-up and became significantly more evident with each successive year, suggesting greater protection against late relapses with HSCT ($P < .001$). According to the Cox model, the hazard of failure (relapse or death in remission) at 5 years was reduced by two thirds by HSCT compared with chemotherapy alone (HR, 0.32; 95% CI, 0.20 to 0.52). According to univariate comparison of the DFS curves at the 5-year time point, the advantage of transplantation was borderline significant ($P = .049$; Fig 1A). Also for survival, HSCT improved the results compared with chemotherapy alone in the long term (according to the Cox model, $P = .003$; 5-year HR, 0.42; 95% CI, 0.25 to 0.70), but the advantage at 5 years was not significant in the univariate comparison ($P = .20$, Fig 1B).

Transplantation with a matched related donor was associated with a decrease in transplantation-related mortality over the years of this survey, with a cumulative incidence of 20% ± 5.5% and 11.7% ± 4.2% before and after year 2000, respectively. However, this did not result in a significantly better outcome, with 5-year DFS rates of 38.9% ± 6.6% and 41.1% ± 6.4% before and after year 2000, respectively ($P = .39$).

Patients who underwent transplantation with a matched unrelated donor in the same time intervals had 5-year DFS rates of 41.4% ± 6.5% and 55.8% ± 5.4% before and after 2000, respectively ($P = .07$; Fig 2). This significant improvement was explained by a better disease control, as illustrated by the cumulative incidence of relapse of 38.2% ± 6.4% before year 2000 and 21.4% ± 4.1% after 2000. Mortality remained similar in the two periods (19.7% ± 4.0% before 2000 *v* 19.0% ± 5.2% after 2000).

Impact of Prognostic Factors on Treatment Outcome

In the univariate analysis of the entire cohort of 610 patients with Ph-positive ALL, age, initial leukocyte count, and response to initial treatment had a significant impact on treatment outcome (Appendix

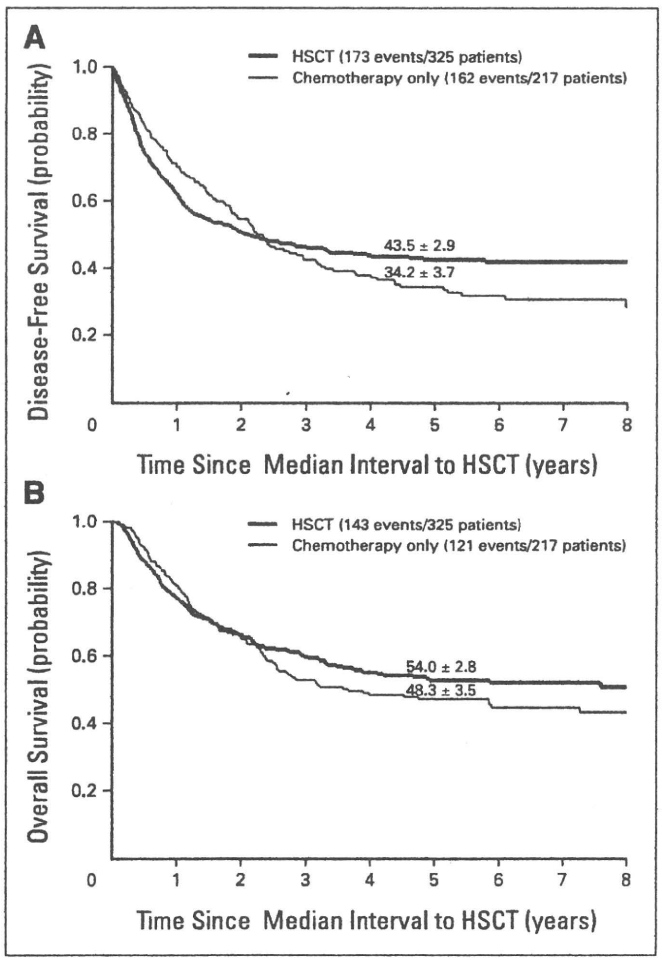

Fig 1. Estimates of (A) disease-free survival and (B) overall survival (± SE) in 542 patients treated with hematopoietic stem-cell transplantation (HSCT) or chemotherapy only. The curves have been adjusted for waiting time to transplantation, so that the zero on the time axis corresponds to the median time from first complete remission to transplantation (5.1 months); patients were assigned to this treatment group in a time-dependent fashion. Five-year estimates (from remission) are shown.

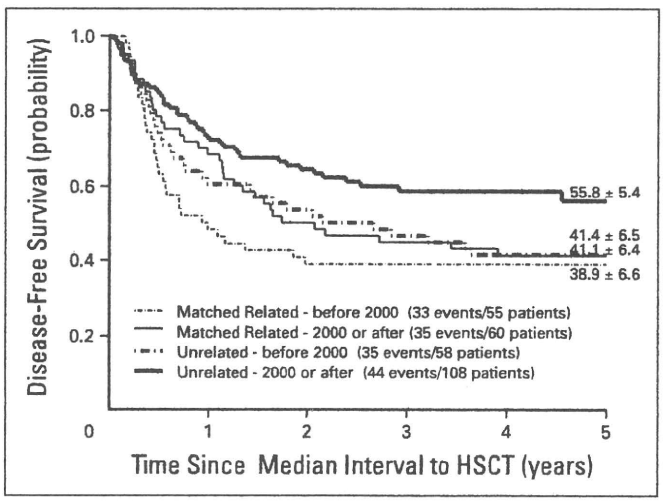

Fig 2. Estimates of disease-free survival (± SE) in 281 patients with Philadelphia chromosome–positive childhood acute lymphoblastic leukemia treated with hematopoietic stem-cell transplantation from HLA-matched related or unrelated donors before or after year 2000. Five-year estimates are shown.

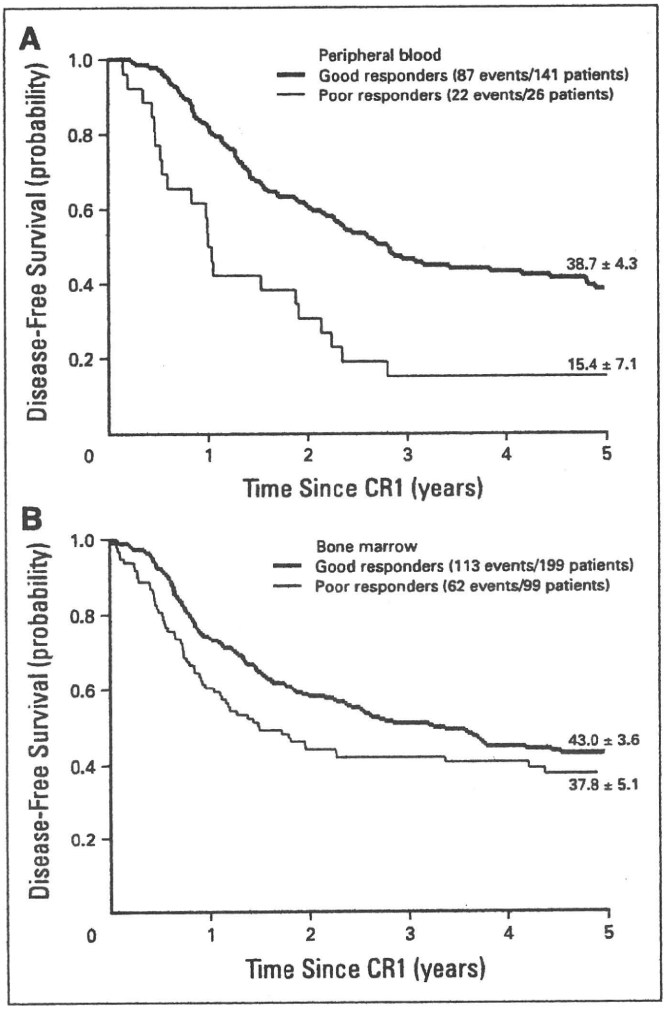

Fig 3. Estimates of disease-free survival (± SE) in good or poor responders as defined by (A) day 8 peripheral blood or (B) day 8 to 21 bone marrow evaluation. Five-year estimates are shown. CR1, first complete remission.

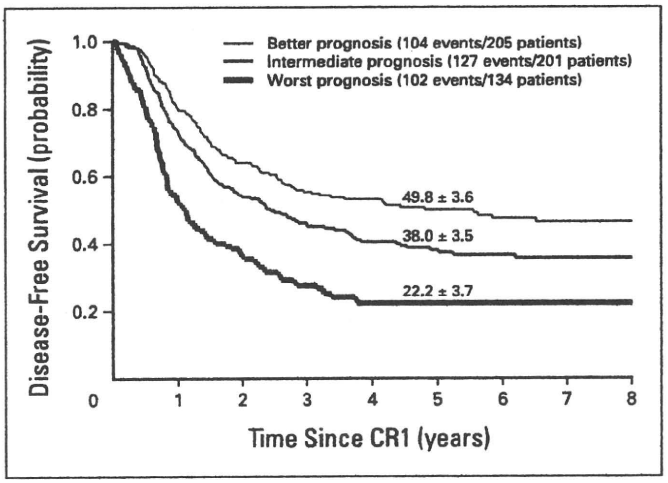

Fig 4. Estimates of disease-free survival (± SE) in 540 patients with Philadelphia chromosome–positive childhood acute lymphoblastic leukemia. The patients were classified according to modified Rome–National Cancer Institute criteria as follows: better prognosis (10 years of age or younger with a leukocyte count of < 50,000/μL), intermediate prognosis (intermediate-risk features), and worst prognosis (any age with a leukocyte count of > 100,000/μL). Five-year estimates are shown.

Table A1). On the basis of peripheral-blood blast cell count at day 8 or percent bone marrow blasts on day 8, 15, or 21 of remission induction (according to individual protocol), 348 (67.6%) of 515 evaluable patients were designated as good early responders; their 5-year EFS rate was 40.3% ± 2.7%, and 5-year DFS rate (n = 340) was 41.3% ± 2.8%. By contrast, the 5-years EFS and DFS rates for the 167 poor early responders were 24.6% ± 3.4% ($P < .001$) and 32.9% ± 4.4% ($P = .002$, n = 125), respectively.

Of the 33 patients with poor corticosteroid response, 26 achieved remission by the end of induction, but their 5-year DFS was only 15.4% ± 7.1%, a result that was inferior to the DFS rate of 38.7% ± 4.3% for the 141 good corticosteroid responders (Fig 3A; $P < .001$). Of the 134 poor responders based on the proportion of bone marrow blasts, 99 achieved remission and had a 5-year DFS rate of 37.8% ± 5.1%, compared with a 5-year DFS rate of 43.0% ± 3.6% for the 199 patients with good response who achieved remission (Fig 3B; $P = .06$).

Age and leukocyte count had prognostic significance on DFS and could be used to stratify patients into three distinct groups (Fig 4). Noticeably, within the subgroup of patients defined as better by the modified Rome-National Cancer Institute criteria (ie, ≤ 10 years of age with a leukocyte count ≤ 50,000/μL), a large subset of patients (149 of 205 patients) had a good early response and an overall 7-years DFS rate of 47.2% ± 4.5%. Their outcome by treatment is shown in Appendix Figure A1 (online only), where a nonsignificant advantage of HSCT versus chemotherapy is observed on DFS ($P = .12$) or on survival ($P = .72$). Patients with good early response but defined as intermediate or worst by modified Rome-National Cancer Institute criteria had overall 7-year DFS rates of 36.9% ± 4.4% (n = 133) and 21.4% ± 5.5% (n = 58), respectively. In the multivariate Cox regression models, treatment, age, leukocyte count, and early response retained independent prognostic significance (Table 2).

DISCUSSION

The outcome of Ph-positive ALL has steadily improved over the last three decades.[7-19,21] In this study, 45% of patients survived at 7 years, a result that compares favorably with the rate of 36% achieved in our previous cohort of 326 patients with Ph-positive ALL ($P = .017$).[21] As expected, given the large numbers, the characteristics of the patients in the two cohorts are extremely similar, with no significant difference in any of the presenting features (Appendix Table A2, online only), suggesting that there was no selection bias. As demonstrated also in our previous studies,[21,26] Ph-positive ALL represents a heterogeneous disease and can be stratified into distinct prognostic subgroups based on age, WBC count, and early treatment response. Early treatment response can be assessed by either peripheral-blood blast cell count after treatment with single-agent corticosteroid or by the percentage of bone marrow blasts after combination chemotherapy.[7-19,22,27-31] In this study, treatment response was shown to be a robust predictor of induction failure. Moreover, insufficient blast cell clearance from the peripheral blood on day 8 of single-agent prednisone treatment (poor prednisone response) was the most powerful adverse prognostic feature and was associated with a two-fold increase in the risk of failure after remission.

In our previous study, HSCT with matched related donor yielded a superior outcome compared with chemotherapy alone, but the advantage of HSCT did not extend to the use of matched unrelated donors.[21] In the present study, transplantation from matched unrelated donors produced similar outcomes to those attained with matched related donors. This finding could be attributed to improved supportive care and HLA typing, as well as to more potent graft-versus-leukemia effect on residual leukemia driven by the residual HLA disparity in unrelated donors.[32] The extended follow-up of the present cohort demonstrates that the advantage of transplantation over chemotherapy alone increased over time by preventing late relapses. The risk of failure (relapse or death) at 5 years was reduced to approximately one third for patients treated with transplantation compared with patients treated with chemotherapy alone. The significant result in the Cox model is strongly influenced by how the initial advantage of chemotherapy changes into a disadvantage in favor of transplantation as time increases. The univariate analysis, based on the single point comparison of the 5-year survival estimates, indicates that this model may overstate the late-term benefit of transplantation on survival. Our conservative interpretation is that results on survival are not so clear cut as results on DFS. Although both Cox model and survival curves agree on advantage of transplantation, this is not reflected in a fully similar measure by these two methods. We have to acknowledge that in a complex setting, such as the comparison between HSCT and chemotherapy, the Cox model and the univariate approach adjust in different ways for waiting time to transplantation and only the Cox model adjusts for patients characteristics, and this can in part explain this disagreement.

The results of the present study confirm and extend those of our former survey on patients treated a decade earlier.[21] However, although the improvements in outcome achieved in the 1996 to 2005 era were statistically significant, we observed only a small (10%) effect on OS. Treatment with either chemotherapy or HSCT in this era without tyrosine kinase inhibitor (at least during front-line treatment program) resulted in long-term survival rates of less than 50% for all groups analyzed. Overall, only 45% of children with Ph-positive ALL were alive 7 years after diagnosis, a result that remains unacceptable. Further optimization of chemotherapy or HSCT regimens is unlikely to lead to major improvements in outcome. Recent encouraging data from Children's Oncology Group study AALL0031[20] (albeit early and based on small numbers) show that outcomes for children with Ph-positive ALL were improved dramatically by incorporating a tyrosine kinase inhibitor (imatinib mesylate) into therapy. On the basis of these data and other data from adults, tyrosine kinase inhibitors are the cornerstone of therapy for children with Ph-positive ALL and should be incorporated in any future treatment schedule of childhood Ph-positive ALL. The high rates of induction failure observed with chemotherapy alone in this study emphasize the need to introduce tyrosine kinase inhibitors into treatment early during induction therapy. More study is needed to clearly define the relative roles of chemotherapy and HSCT in combination with tyrosine kinase inhibitor, and the present study will serve as a large, international historical reference for documenting any real future improvement.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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bjh research paper

Prospective study of a therapeutic regimen with all-*trans* retinoic acid and anthracyclines in combination of cytarabine in children with acute promyelocytic leukaemia: the Japanese childhood acute myeloid leukaemia cooperative study

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Summary

In childhood acute promyelocytic leukaemia (APL), the efficacy of therapy combining cytarabine with all-*trans* retinoic acid (ATRA) and anthracyclines remains unclear in terms of long-term prognosis. Between August 1997 and March 2004, 58 children with APL (median age: 11 years) were enrolled into an acute myeloid leukaemia (AML) study (AML99-M3) and followed up for a median time of 86 months. The regimen included ATRA and anthracyclines combined with cytarabine in both induction and consolidation. In induction, two patients died of haemorrhage and four patients developed retinoic acid syndrome. Of 58 patients, 56 (96·6%) achieved complete remission, two of whom relapsed in the bone marrow after 15 and 19 months respectively. Sepsis was a major complication, with an incidence of 5·6–10·9% in the consolidation blocks, from which all but one of patients recovered. Consequently, 7-year overall and event-free survival rates were 93·1% and 91·4% respectively, and cumulative incidence of relapse plateaued at 3·6% after 2 years. Follow-up survey of 54 patients revealed no patients with late cardiotoxicity or secondary malignancy, except one with asymptomatic prolongation of QTc interval. This study suggests that the combination of cytarabine with ATRA and anthracycline-based therapy may have useful implications in the perspective of long-term prognosis and late adverse effects for childhood APL.

Keywords: childhood acute promyelocytic leukaemia, all-*trans* retinoic acid, anthracyclines, cytarabine, long-term prognosis.

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The prognosis of patients with acute promyelocytic leukaemia (APL), a distinct subtype of acute myeloid leukaemia (AML) (Grignani *et al*, 1994), has been improved dramatically by the introduction of differentiation induction therapy with all-*trans* retinoic acid (ATRA) (Fenaux *et al*,1999; Tallman *et al*, 2002; Sanz *et al*, 2004). However, recent clinical trials with ATRA and anthracycline-based chemotherapy found that recurrent disease posed a major problem, especially for high-risk patients. (Sanz *et al*, 2000, 2009).

Childhood APL, which consists of only 7–10% of all patients, is often associated with risk factors such as hyperleucocytosis (Guglielmi *et al*,1998; Mann *et al*, 2001); however, few studies of paediatric patients have specifically examined its long-term prognosis. In those studies, the complete remission (CR) and overall survival (OS) rates have been improved to >80%, but event-free survival (EFS) remains at around 70–80% because of increased cumulative incidence of relapse (CIR). (de Botton *et al*, 2004; Ortega *et al*, 2005; Testi *et al*, 2005) In addition to frequent relapse in the bone marrow, extramedullary (EM) relapse involving mostly the central nervous system (CNS) occurs at incidence of 1–5%. (Ko *et al*, 1999; de Botton *et al*, 2006; Chow & Feusner, 2009) The therapeutic effectiveness of cytarabine added to anthracycline-based consolidation therapy has been reported for high-risk adult patients(Adès *et al*, 2006, 2008), but the efficacy of cytarabine in addition to the combination of ATRA and anthracyclines in consolidation remains unknown for paediatric patients.

More recently, there has been increasing concern regarding long-term adverse effects, including cardiotoxicity and secondary malignancy, for children with leukaemia. The cumulative dosage of anthracyclines may be related to the risk of late cardiotoxicity as well as therapy-related myelodysplastic syndrome (t-MDS)/AML for childhood malignancies (Nysom *et al*,1998; Le Deley *et al*, 2003). Although such effects of anthracyclines are yet undetermined for APL, the cumulative dosage of anthracyclines may be an important perspective of the long-term prognosis of children with APL.

This report describes the outcome of a prospective study for childhood APL, AML99-M3, in which patients received therapy with cytarabine in addition to ATRA and anthracyclines. The improved outcome of this study suggests that the combination of cytarabine, ATRA and anthracyclines may have useful implications in the perspective of long-term prognosis and late adverse effects for childhood APL.

Patients and methods

Patients

Between August 1997 and March 2004, 58 children with *de novo* APL (31 males and 27 females; median age of 11 years [range:11 months – 16 years] were enrolled in the AML99-M3 study of the Japanese Childhood AML Cooperative Study Group, and a follow-up survey was performed in May 2010 (Table I). Three patients with APL were not recruited to this study: two had already started another chemotherapeutic regimen for AML when APL was diagnosed; the other died of intracranial haemorrhage (ICH) at diagnosis. The relevant institutional review board approved the protocol. Written informed consent was obtained from the parents of all patients. APL was diagnosed according to the French–American–British (FAB) criteria (Bennett *et al*, 1982); the involvement of t(15;17) translocation was examined cytogenetically. APL patients with t(15;17) translocation or *PML-RARA* chimaeric gene confirmed through examinations with fluorescence *in situ* hybridization (FISH) or reverse transcription–polymerase chain reaction (RT-PCR) were registered to this study.

Treatment protocol

In remission induction therapy, ATRA was initiated (45 mg/m^2, until CR) and then daunorubicin (45 mg/m^2 per d, days 6–8) and cytarabine (200 mg/m^2, days 6–12) were added (Fig 1). For patients with a white blood cell (WBC) count $>10 \times 10^9$/l at diagnosis or after the initiation of ATRA therapy, chemotherapy was started before day 6. Consolidation

Table I. Characteristics of patients with APL (N = 58).

Characteristics	Median	Range	No. (%)
Age, years	11	0·9–16	
<5			9 (16)
5 to 10			14 (24)
>10			35 (60)
Sex			
Male			31 (53)
Female			27 (47)
WBC, $\times 10^9$/l	4·3	0·7–171	
<10			36 (62)
≥10			22 (38)
Haemoglobin, g/l	91	37–131	
<10			44 (76)
≥10			14 (24)
Platelets, $\times 10^9$/l	2·3	5–233	
<40			48 (83)
≥40			10 (17)
FAB subtype			
Typical			53 (91)
Variant			5 (9)
Cytogenetics			
t(15;17)			47 (81)
t(15;17) + others			9 (15)
Normal			1 (2)
Unknown			1 (2)
PML-RARA			
Examined			47 (81)
Long isoform (bcr1)			21
Short isoform (bcr3)			8
bcr not determined			18
Not examined			11 (19)

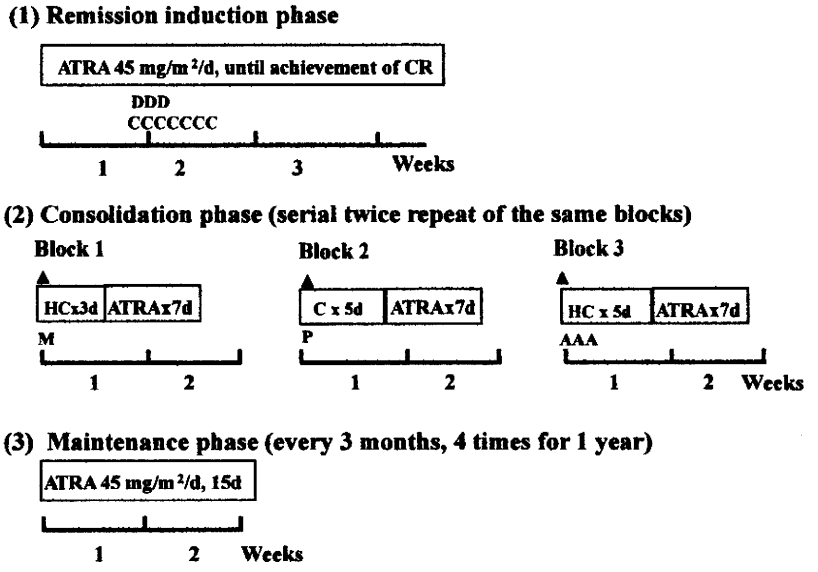

Fig 1. Scheme of AML99-M3 protocol. In remission induction, oral administration of ATRA (45 mg/m^2 per d) was combined with daunorubicin (D) (45 mg/m^2) and cytarabine (C) (200 mg/m^2). In consolidation, administration of ATRA (45 mg/m^2 per d for 7 d) was combined with mitoxantrone (M) (10 mg/m^2 per d, day 1) and high-dose cytarabine (HC) (3 g/m^2 × 2/d, days 1–3) in Block 1, with pirarubicin (P) (45 mg/m^2 per d, day 1) and cytaratibine (C) (200 mg/m^2 per d, days 1–5) in Block 2, and with aclarubicin (A) (30 mg/m^2 per d, days 1–3) and high-dose cytarabine (HC) (3 g/m^2 per d, days 1–5) in Block 3. For CNS prophylaxis, intrathecal injection (▲) of methotrexate, cyatarbine and hydrocortisone at day 1 of every consolidation block in age-adjusted doses as described in Methods. In maintenance therapy, ATRA (45 mg/m^2 per d, days 1–15) was administrated every 3 months for 1 year.

therapy consisted of six courses of block treatment (Blocks 1, 2 and 3), in which each block was performed every month and the same block was repeated serially twice. In respective blocks, chemotherapy with cytarabine and each of the different anthracycline agents was administrated respectively and then ATRA (45 mg/m^2 per d, 7 d) was administrated consecutively. Block 1 consisted of mitoxantrone (10 mg/m^2 per d × day 1) and cytarabine (3 g/m^2 × 2/d × days 1–3), Block 2 of pirarubicin (45 mg/m^2 per d × day 1) and cytarabine (200 mg/m^2 per d × days 1–5), and Block 3 of aclarubicin (30 mg/m^2 per d × days 1–3) and cytarabine (3 g/m^2 per d × days 1–5). On the first day of each consolidation block, patients received intrathecal (IT) therapy with methotrexate (3 mg for <3 months; 6 mg for 3 months to <1 year; 7·5 mg for 1 year; 10 mg for 2 years; 12·5 mg for 3 years or older), cytarabine (6 mg for <3 months; 12 mg for 3 months to <1 year; 15 mg for 1 year; 20 mg for 2 years; 25 mg for 3 years or older) and hydrocortisone (10 mg for <3 months; 10 mg for 3 months to <1 year; 15 mg for 1 year; 20 mg for 2 years; 25 mg for 3 years or older). In maintenance therapy, ATRA alone (45 mg/m^2 per d) for 15 consecutive days was given every 3 months, for a total of four times during 1 year.

Adverse effects

Retinoic acid (RA) syndrome was diagnosed based on clinical signs, including fever, respiratory distress, pulmonary infiltration, pleural and pericardial effusion and renal failure. (Ko *et al*, 1999) When RA syndrome was diagnosed or strongly suspected, ATRA therapy was stopped and the patients received administration of dexamethasone (8 mg/m^2 per d, i.v. in two doses) unless they clinically improved. Disseminated intravascular coagulopathy (DIC), bacterial infections, and other adverse effects were summarized in each phase of treatment. Long-term adverse effects, including cardiotoxicity and secondary malignancy, were surveyed through followe-up analysis. For evaluation of the potential risk of cardiotoxicity, cumulative doses of anthracyclines were converted to equivalent doses of daunorubicin using ratios in 1:3–1:5 for idarubicin/mitoxantrone, 1:1·6 for pirarubicin, and 1:0·2 for aclarubicin (Warrell, 1986; Lenk *et al*, 1990; Sakata-Yanagimoto *et al*, 2004).

Minimal residual disease (MRD) monitoring

For MDR monitoring, the *PML-RARA* chimaeric mRNA in marrow samples was detected using RT-PCR as described (Suzuki *et al*, 2001). Serial evaluation of MRD monitoring was performed every 3 months for 17 patients whose bone marrow samples were sent to the reference laboratory.

Statistical analysis

The OS and EFS were calculated from the beginning date of induction therapy to the date of events; failure to achieve CR, relapse or death of any cause. The OS and EFS were analyzed

using the Kaplan–Meier method. Statistical analyses used the Statistical Package for the Social Sciences (SPSS) software, version 16 (SPSS Japan Inc., Tokyo, Japan), estimated by the log-rank test and considered to be significant when a P value is <0·05. For patients who achieved CR, cumulative incidence functions of relapse as well as death without relapse were calculated using the competing risk method with the cmprsk software package (http://biowww.dfci.harvard.edu/~gray), ver.2.1-5 on R ver.2.10.1.

Results

Patient characteristics

The median follow-up period of 58 patients was 86 months (range: 16 d–12·1 years) (Table I). The median age of patients was 11 years (range: 11 months–16 years); 35 (60%) patients were over 10 years old; 31 patients were male and 27 were female. The WBC counts at diagnosis were 0·9–171 $\times 10^9$/l (median: 4·3 $\times 10^9$/l) and 22 patients (38%) had WBC counts >10 $\times 10^9$/l. The proportion of these high-risk patients was comparable to that (35–48%) reported by other studies for childhood APL (de Botton *et al*, 2004; Ortega *et al* 2005; Testi *et al*, 2005). Haemoglobin levels were 37–131 g/l (median: 91 g/l). Platelet counts were 5–233 $\times 10^9$/l (median: 23 $\times 10^9$/l) and 48 patients (83%) had a platelet count <40 $\times 10^9$/l at diagnosis. Haematological examination identified FAB:M3 morphology in 53 patients and five others exhibited the microgranular FAB: M3v morphology. No patient showed leukaemic infiltration in the cerebrospinal fluid obtained by lumbar puncture performed for CNS prophylaxis at the beginning of consolidation therapy.

Cytogenetic examination revealed that 47 patients had t(15;17) translocation abnormality alone, nine had t(15;17) with additional chromosomal abnormalities, one with normal karyotype, and one with no result. In the latter two patients, the involvement of *PML-RARA* chimaeric gene was confirmed using RT-PCR. Examinations for *PML-RARA* were performed in 47 patients. RT-PCR detected *PML-RARA* in 29 patients, 21 of whom showed the long type (bcr1) isoform; eight showed the short type (bcr3) isoform. Eighteen patients had *PML-RARA* detected by FISH analysis without differentiation of the isoform types. No patient had ATRA-insensitive fusion genes, such as the *ZBTB16-RARA* caused by the t(11;17) chromosomal translocation.

Clinical course and statistical analysis

In induction therapy, two patients (3·4%) died from ICH and pulmonary bleeding after 16 and 24 d respectively. CR was achieved in 56 patients (96·6%), two of whom exhibited relapse at bone marrow; one relapsed at 15 months and died of ICH, the other relapsed at 19 months and remains in second CR after treatment with marrow transplantation. For patients who achieved CR, the period of ATRA administration in induction was a median of 29 d (range 14–60 d), during which 13 patients temporarily discontinued the administration of ATRA for a median 4 d (range 1–31 d). Overall, four patients died: two of DIC with haemorrhage during induction, one of sepsis and meningitis in remission, and one of ICH after relapse. Consequently, the OS and EFS rates at 7 years were respectively, 93·1% (95% confidence interval [CI], 86·5–99·7%) and 91·4% (95% CI, 84·0–98·4%) (Fig 2A). No significant difference was found in the OS and EFS rates between patients with or without haematological risk factors, such as WBC count >10 $\times 10^9$/l or platelet count <40 $\times 10^9$/l. (Sanz *et al*, 2000) (Figs 2B, C) The CIR was 3·6% (95%CI: 0–8·5%) at 7 years, while the cumulative incidence of death without relapse, one of the competing events, was 1·8% (95%CI: 0–5·3%) at 7 years (Fig 2D).

Adverse effects and events

Table II presents the incidence of adverse effects and the duration of neutropenia. In induction therapy, DIC was observed in 10 patients (17%) and four of these patients (7%) showed haemorrhagic complications including retinal haemorrhage in two patients and ICH and/or pulmonary haemorrhages in the other two who died. RA syndrome, which occurred in 7% of cases, was resolved with cessation of ATRA and administration of dexamethasone, the incidence of which was comparable to those (7–19%) reported by other studies of childhood APL (de Botton *et al*, 2004; Ortega *et al* 2005; Testi *et al*, 2005).

Bacterial infection was the major adverse effect in induction and consolidation, and sepsis with documented microbes was determined at a higher incidence during consolidation than induction. Although one patient died in remission of pseudomonas sepsis and meningitis after Block 2 consolidation, all other patients recovered from sepsis with treatment. A proportional relationship was apparent between the periods of neutropenia (<0·1 $\times 10^9$/l) and the incidence of whole infections at any sites, including gingivitis, stomatitis, bronchopneumonia, enteritis, or cellulites during neutropenia, and herpes zoster only in maintenance. Other complications included impaired consciousness or convulsion associated with pseudotumour cerebri and aclarubicin-related dysuria in consolidation Block 3. Severe headache/nausea associated with ATRA therapy was experienced at an incidence of 8–22% throughout treatment.

Table III shows the characteristics of five patients with early death or relapse, two of whom exhibited at least one of the following: WBC count >10 $\times 10^9$/l, M3v morphology, *PML-RARA* bcr3 isoform. The proportion of these patients was not significantly different from that of the whole population of 58 patients. Because of adverse effects, Block 3 consolidation was omitted or reduced in dosage at the physician's discretion in five patients, including two with WBC count >10 $\times 10^9$/l, of whom all remained in remission for 4·9–8·9 years.

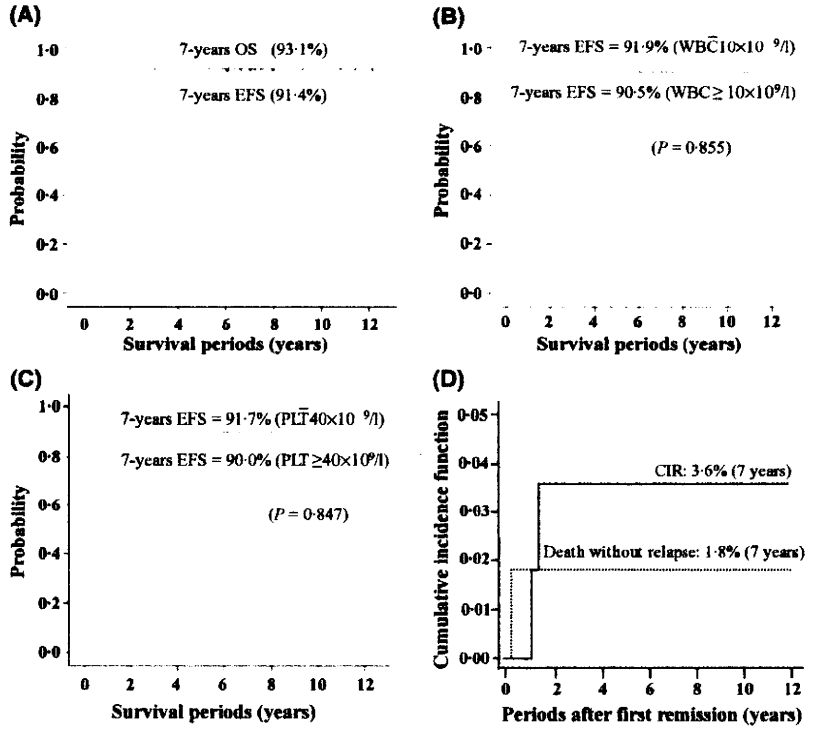

Fig 2. Analysis of the rates of OS, EFS and cumulative incidence functions of CIR and death without relapse in patients treated with the AML99-M3 protocol. (A) OS and EFS rates of total patients; (B) EFS rates of patients with WBC count > 10×10^9/l or <10×10^9/l at diagnosis; (C) EFS rates of patients with a platelet (PLT) count <40×10^9/l or >40×10^9/l at diagnosis. No significant difference was found in the EFS rates of patients with and without these risk factors. (D) the cumulative incidence functions of CIR (solid line) and death without relapse (dotted line).

Table II. Incidence of adverse effects and periods of neutropenia.

		Consolidation			
	Induction	Block 1	Block 2	Block 3	Maintenance
No of assessed patients	55	54	54	53	49
Deterioration of DIC with serious haemorrhages, %	7·2	0	0	0	0
Sepsis, %	1·8	9·2	10·9	5·6	0
Infection of any site, %	10·8	14·5	14·8	15·9	10·2
RA syndrome, %	7	0	0	0	0
Consciousness impairment and/or convulsion, %	3·6	1·8	0	0	0
Severe headache or nausea, %	23·6	11·1	12·9	13·2	8·1
Dysuria, %	0	0	0	3·7	0
Duration of ANC < 0·5, days	17·2	14·3	16·1	16·1	0
Duration of ANC < 0·1, days	6·3	10	10·3	10·9	0

DIC, disseminated intravascular coagulopathy, RA syndrome, retinoic acid syndrome; ANC, absolute neutrophil count, $\times 10^9$/l.

In the evaluation of late cardiotoxicity, echocardiography and electrocardiogram were performed in 18 patients, of whom one patient showed asymptomatic prolongation of the QTc interval in the electrocardiogram. Except for this patient, no clinical symptoms of late cardiotoxicity was seen in other patients including those who did not receive examinations. As of May 2010, no patient had developed t-MDS/AML.

MRD monitoring

In 17 patients, including six with WBC count > 10×10^9/l, MRD monitoring was performed at the initial onset and subsequently every 3 months; the monitoring period was an average of 13·6 months. As a result, MRD levels became undetectable (lower than 10^{-3}–10^{-4}) after consolidation Block 1 in 16 patients (94%) and another PCR-positive patient

Table III. Characteristics of patients with early death or relapse.

Patients	Age (years)	Sex	WBC	PLT	PT	APTT	Fibrinogen	D-dimer	FAB	Breakpoint of *PML-RARA*	Clinical course	Outcome
1	15	M	1·2	55	1·25	28·6	0·65	65 300	M3	bcr1	ICH at 15 d in induction	Death at 24 d
2	4	F	171·0	28	1·47	25·9	1·02	6200	M3v	bcr3	BM relapse at 15 months in maintenance and then BMT in 2CR	Alive at 83 months
3	14	M	62·4	4	1·46	30·4	0·79	17 400	M3v	n.e.	ICH at 2 d	Death at 16 d
4	11	F	2·1	39	1·10	27·1	1·00	35 000	M3	n.e.	Pseudomonas sepsis and meningitis after four courses of consolidation	Death at 5 months
5	12	M	1·8	16	1·45	26·9	1·10	6500	M3	n.e.	BM relapse at 19 months and then ICH during subsequent treatment	Death at 24 months

WBC, white blood cell count ($\times 10^9$/l); PLT, platelet count ($\times 10^9$/l); PT, prothrombin time (s); APTT, activated partial thromboplastin time (s) Fibrinogen, mg/dl; D-dimer, μg/ml. FAB, French-American-British classification; ICH, intracranial haemorrhage; BM, bone marrow; BMT, bone marrow transplantation; 2CR, 2nd complete remission; n.e., not evaluated.

became PCR-negative after 6 months of therapy. No patient that was monitored for MRD exhibited re-conversion to PCR-positivity.

Discussion

APL with the *PML-RARA* chimaeric gene is more homogenous than other types of AML and, for infrequent childhood APL, therapy has been often considered together with that of adult patients. However, for paediatric patients, who typically have physiological differences from adults, it has not been thoroughly understood whether the combination of cytarabine with ATRA and anthracyclines would be effective in terms of long-term prognosis.

As shown in Table IV, recent clinical studies of childhood APL, in which patients were enrolled from the mid-1990s to the early 2000s and followed up for median periods of 36 months or longer, were compared to our study (de Botton *et al*, 2004; Ortega *et al*, 2005; Testi *et al*, 2005). In all of these studies, induction therapy with administration of ATRA and anthracyclines with or without cytarabine achieved CR rates at >90% and incidence of early death at <10% respectively. In the state-of-the-art treatment guidelines (Sanz *et al*, 2009), anthracyclines should start together with ATRA (or as soon as possible) in high-risk patients. Regarding drug dosages and clinical parameters, the adjusted cumulative dosage of anthracyclines of our study (375–415 mg/m^2) was lower than other studies (390–750 mg/m^2), while that of cytarabine varied to a large extent among studies. Regarding long-term survival, other three studies presented EFS rates of 71–82% despite OS rates at around 90%, whereas our study achieved a 7-year EFS of 91·4% (Table IV). Accordingly, the 7-year CIR of our study (3·6%) was lower than reported by other studies (15·6–27%) (Table IV). Moreover, none of our patients suffered EM relapse, whereas the other studies reported five patients with EM relapse (skin, middle ear or CNS; Table IV). In our study, one patient exhibited asymptomatic prolongation of QTc interval, which may be associated with late effects of anthracyclines. One other study (Testi *et al*, 2005) reported that two patients developed t-MDS after 36 and 80 months from diagnosis.

In post-remission therapy studies including chemotherapy-based consolidation without ATRA, recurrent disease might develop late in the course, such as seven clinical relapses that occurred over 4–36 months in the APL93 study (de Botton *et al*, 2004) or 14 haematological and five molecular relapses at the median of 26 and 31 months respectively, in the AIDA (ATRA and idarubicin) study (Testi *et al*, 2005). The PETHEMA (Programa para el Estudio y Tratamiento de las Hemopatias Maligna) group reinforced the consolidation therapy of LPA96 study with single anthracycline agent by adding ATRA and increased dosage of idarubicin for intermediate and high-risk patients (LAP99 study). (Ortega *et al*, 2005) These reports indicated that addition of ATRA to anthracycline-based consolidation therapy improved the prognosis of APL patients, especially those with risk factors,

Table IV. Comparison of AML99-M3 with recent studies on childhood APL.

Reports	de Botton *et al*	Testi *et al*	Ortega *et al*	Imaizumi *et al*
Protocol	APL93	AIDA	LPA96/LPA99	AML99-M3
Year	2004	2005	2005	This study
Period of enrollment	1993–1998	1993–2000	1996–2004	1997–2004
Median follow-up time	67 months	79 months	38 months	86 months
No. of patients	31	110	66	58
Proportion of patients with WBC $\geq 10 \times 10^9$/l	48%	35%	39%	38%
Therapy				
Induction	1) ATRA → DNR + CA* 2) ATRA+DNR + CA*	ATRA + IDA	ATRA + IDA	ATRA + DNR + CA
Consolidation	1) DNR + CA 2) DNR + HCA	1) IDA + HCA 2) MIT + VP-16 3) IDA + CA + 6TG	1) IDA + ATRA† 2) MIT + ATRA† 3) IDA + ATRA†	1) ATR + MIT + HCA‡ 2) ATRA + THP + CA‡ 3) ATRA + ACM + HCA‡
Maintenance	(−) or ATRA ± MP/MTX§	ATRA or MP/MTX§	ATRA + MP/MTX	ATRA alone
Dosage of anthracyclines (mg/m^2)	DNR (495)	IDA (80), MIT (50)	IDA (80–100), MIT (50)	DNR(135), MIT(20), THP(90), ACM(180)
Anthracycline dosage converted to DNR (mg/m^2)¶	495	390–650	390–750	375–415
Cumulative dosage of cytarabine (mg/m^2)	10800	6250	0	68000
Cumulative dosage of ATRA (mg/m^2)	1350–6750	750–6150	3750–4875	5940
Incidence of headache/ pseudotumour cerebri (%)	39/16	13/9	30/6	24/5
Clinical outcome				
Early death (%)	3	3·6	7·5	3·4
CR rate (%)	97	96	92	96·6
CIR (%)	27 (5 years)	NA	17 (5 years)	3·6 (7 years)
Extramedullary relapse (sites)	1 (skin)	2 (middle ear)	2 (CNS)	0
Overall survival rate (%)	90 (5 years)	89 (10 years)	87 (5 years)	93·1 (7 years)
Event-free survival rate (%)	71 (5 years)	76 (10 years)	82 (5 years)	91·4 (7 years)
Late cardiotoxicity	No	No	No	1**
Secondary malignancy	No	2 (tMDS)	No	No

DNR, daunorubicin; IDA, idarubicin; MIT, mitoxantrone; THP, pirarubicin; ACM, aclarubicin; CA, cytarabine; HCA, high-dose CA; MP, mercaptopurine; MTX, methotrexate; CR, complete remission; CIR, cumulative incidence of relapse; CNS, central nervous sytem; tMDS, therapy-related myelodysplastic syndrome; NA, not available.

*Patients with WBC $\leq 0{\cdot}5 \times 10^9$/l were randomized to 1) or 2), and those with WBC $>0{\cdot}5 \times 10^9$/l assigned to 2).

†In LPA96 anthracyclines alone; in LPA99 ATRA was combined and IDA dose was increased for intermediate and high-risk patients.

‡Each course was repeated twice.

§Patients were randomized.

¶Equivalent DNR doses were converted using ratios in1:3–1:5 for IDA/MIT, 1:1·6 for THP and 1:0·2 for ACM.

**One patient with asymptomatic prolongation of QTc interval in the examination with electrocardiogram.

although the trial to add cytarabine to ATRA and anthracycline-based consolidation remains undetermined.

In our study, which combined cytarabine with ATRA and anthracyclines both in induction and consolidation, the long-term outcome was improved and showed a low CIR level. Moreover, by adopting prarubicin (Lenk *et al*, 1990) and aclarubicin (Warrell, 1986), two agents of anthracyclines with relatively low acute cardiotocicity, the cumulative doses of anthracycllines were lowered to levels that did not exceed moderate dosages (approximately 300–550 mg/m^2). Late abnormalities of left ventricular performance were uncommon with cumulative anthracycline doses <300 mg/m^2, but late cardiotoxicity might be an important concern in patients with moderate or higher dosages.(Sorensen *et al*, 1997; Nysom

et al, 1998) However, our study included one patient who showed asymptomatic electrocardiographic changes of QTc prolongation which may be associated with late effects of anthracyclines (Bagnes *et al*, 2010) and, therefore, cautious observation might be important for children with a long prospect of survival.

It is to be noted, however, that our regimen with six reinforced courses of consolidation led to increased risks of infectious complications attributable to the prolonged duration of neutropenia. The incidence of sepsis in our study (5·6–10·9% in each consolidation block) was higher than that (3·3–6·6% of incidence) reported by the PETHEMA study (Ortega *et al*, 2005). Although all but one of patients in remission recovered from sepsis with treatment, the compliance of the regimen was decreased in five patients with inevitable omission or dose-reduction of Block 3 consolidation because of chemotherapy-related toxicities. On the basis of the decreased MRD shown during this combined consolidation therapy, the intensity of consolidation therapy should be adjusted to ensure safety. In the ongoing trial in Japan that succeeded AML99-M3, the intensity of consolidation therapy has been reduced from six to four courses, and the effects of this will be compared to AML99-M3.

Recently, the European APL Group suggested the possibility of additional cytarabine to reduce the chance of relapse for patients with APL.(Adès *et al*, 2006) More recently, in the comparative analysis between APL2000 trial with additional cytarabine and LPA99 trial without cytarabine, the 3-year OS and CIR of high-risk patients were respectively, 91·5% vs. 80·0% and 9·9% vs. 18·5%.(Adès *et al*, 2008) Furthermore, the PETHEMA group also demonstrated that the risk-adapted treatment with ATRA, idarubicin and cytarabine for high-risk patients significantly improved the 3-year CIR (11%) when compared to that (26%) of their previous study.(Sanz *et al*, 2010) These findings suggest an importance of risk-adapted treatment and additional cytarabine for high-risk patients.

While EM relapse involving mostly CNS occurs at an incidence of 1–5% (Liso *et al*, 1998; Ko *et al*, 1999; Specchia *et al*, 2001; Breccia *et al*, 2003), at least one in 10 relapses of APL have a CNS component (Sanz *et al*, 2009). For 81 children with relapse reported in the literature, six patients (7·4%) had CNS involvement and the incidence of isolated CNS of good risk patients was as low as 2/218 (0·92%).(Chow & Feusner, 2009) In a European study, which reported 169 relapses (23%) in 740 patients (de Botton *et al*, 2006), the 3-year cumulative incidence (5·0%) of EM relapse was more frequent in patients with WBC count $> 10 \times 10^9$/l, suggesting that high-risk patients may benefit from IT therapy for CNS prophylaxis. Accordingly, IT therapy was performed for high-risk patients (Sanz *et al*, 2005; Adès *et al*, 2008), whereas IT therapy for CNS prophylaxis is not currently recommended for low-risk patients (Chow & Feusner, 2009). As high-dose cytarabine could have contributed to the prevention of CNS relapse because of a high penetration property into the CNS, TI therapy for low-risk patients would be omitted in our regimen while holding CIR at low levels.

Secondary malignancy is another emerging problem, even if at low levels, for APL patients as their survival is prolonged. The PETHEMA LPA99 study, with 560 subjects, identified nine patients with second malignancies, including six t-MDS/AML, at a median interval of 41 months.(Sanz *et al*, 2008) More recently, the European APL group reported the very long-term outcome of 578 patients with a median follow-up of 10 years, in which the cumulative incidence of secondary tumours and t-MDS was 1·4% and 0·2% at 5 years respectively, and 2·7% and 1·1% at 10 years respectively.(Adès *et al*, 2010) It is of note that the risk of t-MDS/AML may be increased by exposure to moderate or high cumulative doses of anthracyclines, which act by inhibiting DNA topoisomerase II, for children with malignant tumours.(Zunino & Capranico, 1990; Le Deley *et al*, 2003) Although the risk of secondary malignancy may not be thoroughly understood with regard to the use of anthracyclines for APL, the cumulative dosage of anthracyclines may be an important perspective of the long-term outcomes and adverse effects for childhood APL.

Recently, therapy with arsenic trioxide, which induces differentiation as well as apoptosis of APL cells, has been shown to be effective for patients not only with relapsed but also with newly diagnosed APL (Ferrara, 2010). With accumulating evidence for the efficacy and safety of therapy with arsenic trioxide alone or in combination with other agents, it would be a promising approach for treatment of childhood APL in the near future (Zhang *et al*, 2008) (Zhou *et al*, 2010).

In conclusion, although this study, without risk-adjusted stratifications or randomized approaches, is insufficient to make definite conclusions, the improved outcome of paediatric APL patients in this study may provide useful implications in the perspective of long-term prognosis and late adverse effects of childhood APL. Further investigations are needed.

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Retrospective Analysis of Non-Anaplastic Peripheral T-Cell Lymphoma in Pediatric Patients in Japan

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Background. Reports of non-anaplastic peripheral T-cell lymphoma (PTCL) in pediatric patients are relatively rare. ***Procedure.*** We performed a retrospective analysis in patients with PTCL over an 18-year period (1991–2008). ***Results.*** We could analyze clinical data in 21 patients with non-anaplastic PTCL; 10 were female and 10 male. Median age of onset was 11 years (range: 1–21 years). There were nine patients with PTCL, not otherwise specified (PTCL-NOS); ten with extranodal NK/T-cell lymphoma, nasal type; one with angioimmunoblastic T-cell lymphoma; and one with subcutaneous panniculitis-like T-cell lymphoma. Initial lesions involved cervical lymph nodes in five patients, and the skin in five patients. In five patients, hemophagocytic syndrome (HPS) was the initial clinical feature. There were 12 patients with advanced stage disease (stages III and IV). Chemotherapy and radiation was administered in 18 and 2 patients, respectively. Among the two patients who did not receive chemotherapy and radiation, one patient died while being treated for HPS but another improved spontaneously. Although 5 patients relapsed, 18 of 21 patients remained alive without disease at last follow-up. Five-year overall survival rate was 85.2%. ***Conclusions.*** Generally, the outcome results of conventional chemotherapy for high-risk PTCL are poor in adult patients. However, the excellent results in our study suggest that PTCL of childhood is quite different from that of adulthood. Although this study is first report about PTCL of Asian children, the number of patients was small in this study. Larger studies are needed to confirm these findings. Pediatr Blood Cancer 2010;54:212–215. © 2009 Wiley-Liss, Inc.

Key words: child; peripheral T-cell lymphoma

INTRODUCTION

Peripheral T-cell lymphomas (PTCLs) are a heterogeneous group of rare diseases, usually demonstrating clinical aggressiveness [1]. Because of difficulty and variability in diagnosis, improvements in diagnostic technology, and changing classification systems over time, the interpretation of studies is complicated. In addition, the response to current treatments and long-term outcome are generally poor [2–6]. Reports of non-anaplastic PTCL in pediatric patients are relatively rare [7–11]. Moreover, although geographic variation has been well documented, this may reflect exposure to specific pathogenic viruses, such as Epstein Barr (EB) virus and human T-cell leukemia virus-1 in Asian countries. There are no reports about child PTCL from Asia. We therefore performed a retrospective analysis of patients with PTCL over an 18-year period (1991–2008).

METHODS

We performed this retrospective analysis as the lymphoma committee of the Japan Leukemia and Lymphoma Study Group (JPLSG). Data were obtained from the Japan Association of Childhood Leukemia Study (JACLS), Tokyo Children's Cancer Study Group (TCCSG), Japanese Children's Cancer and Leukemia Study Group (JCCLSG), and Kyushu-Yamaguchi Children's Cancer and Leukemia Study Group (KYCCSG). In the 18-year study period, 55 patients were registered as having PTCL or NK/T lymphoma including blastic NK lymphoma and myeloid/NK lymphoma. Clinical data for 21 patients with non-anaplastic PTCL after excluding 34 patients with blastic NK lymphoma and myeloid/NK lymphoma were analyzed.

Pathologic diagnoses were confirmed by central review in 9 of 21 patients. Central review was performed using WHO classification. For the other 12 children, histopathology was performed at the treating center only and confirmed from a copy of the pathology report. In almost all reports, immunophenotyping such as CD79a, CD20, CD3, CD43, TdT, and MPO was included.

The presence of an association with EB virus was determined by detection of EB virus genome in white blood cells or plasma, or the detection of this virus in histological material by EB virus encoded small RNA (EBER) in situ hybridization [12].

Statistical Analyses

Analysis of overall survival was performed using the Kaplan–Meier method, with differences compared by log-rank test. Differences between groups were analyzed using a Fisher exact test and a Mann–Whitney *U*-test. Statistical analyses were

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performed using Dr. SPSS II for Windows (release 11.0.1J, SPSS Japan, Inc.).

RESULTS

In the 18-year study period, we were able to analyze clinical data from 21 patients with non-anaplastic PTCL (Table I). Because 1,711 child and adolescent patients with non-Hodgkin lymphoma were registered in the 18-year period, the proportion of NHL classified as PTCL was 1.2%. Of the 21 patients, 10 were male and 11 were female. Median age of onset was 11 years (range: 1–21 years). There were nine patients with PTCL not otherwise specified (PTCL-NOS); ten with extranodal NK/T-cell lymphoma, nasal type; one with angioimmunoblastic T-cell lymphoma; and one with subcutaneous panniculitis-like T-cell lymphoma. Initial lesions involved the cervical lymph nodes in five patients, and the skin in five patients. In five patients, hemophagocytic syndrome (HPS) was the initial clinical feature. With regard to stage of disease at diagnosis, eight patients were at stages I and II, six were at stage III, and six were at stage IV; this information was not available for one patient. Chemotherapy and radiation were administered in 18 and 2 patients, respectively. Two patients received no treatment. Treatment for PTCL was not consistent in this study. Eight patients received a T-cell lymphoma/leukemia regimen, and four received a B cell lymphoma/leukemia regimen. Among the two patients who did not receive chemotherapy and radiation, one patient died while undergoing treatment for HPS and another improved spontaneously. In the latter patient (patient 5), the initial clinical features were fever, cervical lymphadenopathy, and pancytopenia. He was diagnosed with HPS from laboratory data and bone marrow aspiration. Lymph node biopsy revealed PTCL and there was positive staining on EBER in situ hybridization. However, after several days, the fever abated and laboratory data improved. He received no chemotherapy at the request of his parents and remained disease-free at last follow-up, 9 months after onset.

Eleven patients received stem cell transplantation. Of these, two received an autologous peripheral blood stem cell transplant (PBSCT), five received a related bone marrow transplant (BMT), two received a related PBSCT, two received an unrelated cord blood stem cell transplant (CBSCT), and one received an unrelated BMT. Although 5 patients relapsed, 17 of the 21 patients were alive without disease at last follow-up, giving an overall 5-year survival rate of 85.2% (Fig. 1). Causes of death for the three patients who succumbed to their disease were HPS, progression of disease and complications of stem cell transplantation. Ten of the 21 patients had PTCL associated with EB virus. Compared with patients with extranodal NK/T lymphoma, nasal type, those with PTCL-NOS were younger (median 7 years vs. 15.5 years, $P < 0.05$) and had a lower relapse rate (11% vs. 40%). However, gender (male/female; 5/4 vs. 4/6), proportion with advanced stage disease (56% vs. 60%), survival rate (87.5% vs. 80.0%) and association with EB virus (44% vs. 60%) were similar and statistically non-significant differences.

DISCUSSION

Peripheral NK/T-cell neoplasms are an uncommon group of diseases that show distinct racial and geographic variation. The prognostic significance of the T-cell phenotype has been clearly defined in recent studies by using modern lymphoma classification systems. Anaplastic large cell lymphoma, not rare in childhood, is another type of PTCL. Results of conventional chemotherapy for high-risk PTCL are poor compared with those for their aggressive B-cell counterparts in adult patients.

However, although case reports of pediatric PTCL are sometimes seen [7,10,11], large case series are very rare. The only two such case series published are a report from the United Kingdom [8] and the Children's Oncology Group (COG) Study [9]. In the UK series, 25 cases were identified, 44% of children died and 5-year survival rate was 59%. On the other hand, in the 20 patients in the COG series, 5-year survival rate was 90% in patients with localized disease and 50% in those with advanced disease. In the present study, 21 patients with PTCL were identified; these included 9 with PTCL-NOS; 10 with extranodal NK/T-cell lymphoma, nasal type; 1 with angioimmunoblastic T-cell lymphoma; and 1 with subcutaneous panniculitis-like T-cell lymphoma. Surprisingly, although 57% of patients had advanced stage disease and five patients relapsed after chemotherapy, the 5-year survival rate was 85.2%. However, treatment for PTCL was not consistent in this study. Eight patients received a regimen for T-cell lymphoma/leukemia, and four patients received a B cell lymphoma/leukemia regimen. Moreover, in one patient, symptoms improved spontaneously, and this has not previously been reported. Although five patients had relapse, four patients remained disease free at last follow-up and only two patients had undergone stem cell transplantation. Our study suggests that in the present population, PTCL in childhood does not have a poor outcome compared to adult with PTCL. This reason is not clear. However, the role of stem cell transplantation might be important. Stem cell transplantation had been undergone in eight patients with first complete response or partial response, one patient with progressive disease and two patients after relapse. After stem cell transplantation, only two patients died and nine patients are surviving without relapse.

Many cases of extranodal NK/T-cell lymphoma, nasal type were seen in this study compared with previous reports. Moreover, patients with this type of lymphoma were older at initial presentation than those with PTCL-NOS. Extranodal NK/T-cell lymphoma, nasal type is mostly confined to East Asia, and it predominantly occurs in the nasal or paranasal areas and less frequently in the skin. Most of the tumors show NK-cell phenotypes, although T-cell phenotypes are occasionally seen. The EB virus genome can usually be detected in lymphoma cells. Disease was associated with EB virus in 65% of patients with extranodal NK/T-cell lymphoma, nasal type compared with 50% of patients with PTCL-NOS. Suwiwat et al. [13] detected cell-free EBV DNA in 32/38 (84%) of adult PTCL patients, but failed to find EBV in controls. Rates of EB virus were higher in that report than in our study, possibly because Suwiwat et al. examined adults rather than children. However, we found EB virus in three of four patients who had HPS as the initial clinical feature. EB virus associated with HPS is sometimes seen in childhood, and some of these patients might also have PTCL. T-cell lymphoma-associated hemophagocytic syndrome (T-LAHS) has been frequently reported in Asian countries and is considered to have an extremely poor prognosis. Tong et al. [14] retrospectively analyzed the records of 113 patients with aggressive T-cell lymphoma, of which 28 had LAHS. The therapeutic results of chemotherapy alone or in combination with other modalities were discouraging for T-LAHS and the survival time for most patients was no more than 1 year. In the present study, unlike in other reports, three of four patients with HPS remained disease-free at last follow-up.

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TABLE I. Clinical Characteristics and Outcomes for 21 Patients With Peripheral T-Cell Lymphoma

	Age	Gender	Diagnosis	Initial lesion	Stage	Treatment	Response	Relapse	Transplantation	Association of EB virus	Survival time (months)
1	6	M	PTCL-NOS	Liver, spleen	4	JACLS NHL98ER	PR	N	Y	N	68+
2	4	F	PTCL-NOS	HPS	4	ALL (T)	CR	N	N	Y	60+
3	16	M	PTCL-NOS	Cervical	3	BFM NHL-T	PR	N	Y	N	36+
4	5	F	PTCL-NOS	Skin	1	JACLS NHL98T	CR	N	N	N	12+
5	7	M	PTCL-NOS	Cervical, HPS	1	None		N	N	Y	9+
6	9	M	PTCL-NOS	Cervical, spleen	3	TCCSG NHLT01	CR	N	N	ND	57+
7	11	F	PCTL-NOS	Cervical	1	T-LBL	CR	Y	Y	N	12
8	1	F	PTCL-NOS	HPS		VP16 + DEX	CR	N	Y	Y	30+
9	12	M	PTCL-NOS	Submandibular	3	CHOP	PR	N	Y	Y	30+
10	14	F	Subcutaneous panniculitis-like	Skin	2	Steroid	CR	N	Y	N	8+
11	14	M	AITL	Cervical	4	JACLS NHL98T	CR	N	N	N	96+
12	17	M	Extranodal NK/T nasal type	Adrenal grand, HPS	3	None		N	N	N	0
13	14	F	Extranodal NK/T nasal type	Skin	4	93mix	CR	N	Y	N	132+
14	21	F	Extranodal NK/T nasal type	Sinusoidal	4	HLH94	CR	Y	N	Y	30+
15	10	F	Extranodal NK/T nasal type	Orbit, breast	3	DeVIC	PD	Y	Y	N	36+
16	18	F	Extranodal NK/T nasal type	Nasal sinus, kidney, ovary	4	ALL (B)	PD	N	Y	Y	5
17	11	M	Extranodal NK/T nasal type	Skin	3	TCCSG NHL B96-04	CR	N	N	N	107+
18	18	M	Extranodal NK/T nasal type	Nasopharynx	2	Radiation	CR	Y	N	Y	105+
19	8	F	Extranodal NK/T nasal type	Skin	1	CCLSG NHL960LB	CR	N	N	Y	94+
20	10	M	Extranodal NK/T nasal type	Nasal sinus	1	DeVIC + radiation	CR	Y	Y	Y	45+
21	18	F	Extranodal NK/T nasal type	Nasal sinus, HPS	2	CHOP	PR	N	Y	Y	147+

PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified; AITL, angioimmunoblastic T-cell lymphoma; HPS, hemophagocytic syndrome; CR, complete response. PR, partial response; PD, progressive disease; Y, yes; N, no; ND, no data. The drugs contained in remission introduction of each treatment is as follows: JACLS NHL98ER, vincristine (VCR), pirarubicin (THP-ADR), cyclophosphamide (CPM), L-asparaginase (L-asp), dexamethasone (DEX), prednisolone (PSL), JACLS NHL98T, VCR, CPM, adriamycin (ADR), L-asp, PSL, TCCSG NHLT01, VCR, CPM, ADR, L-asp, THPADR, PSL, CHOP: CPM, ADR, VCR, PSL, HLH94: etoposide (VP16), DEX, cyclosporine, DeVIC: DEX, ifosfamide, carboplatinum, VP16, TCCSG NHL B96-04: CPM, VP16, methotrexate (MTX), PSL, CCLSG NHL960LB: CPM, VCR, PRD, ADR, MTX.

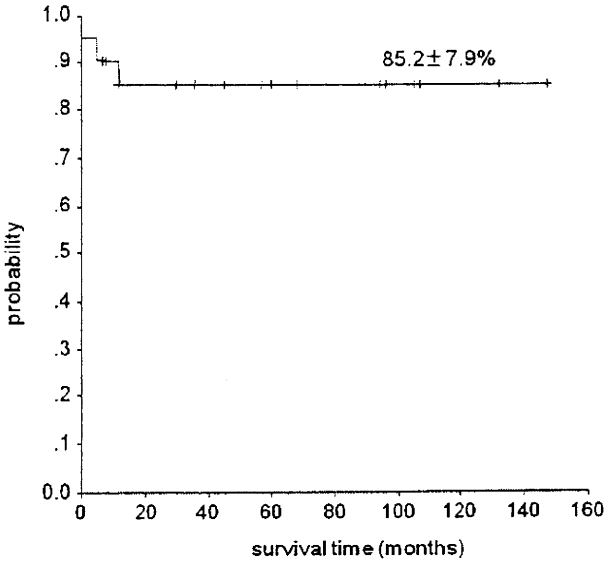

Fig. 1. Survival rate of patients with peripheral T-cell lymphoma. Five-year survival rate was 85.2%.

The findings of the present study differ from those of past reports of PTCL that included adults and children. However, the present study examined only a small number of patients. Larger studies are needed to confirm these findings.

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LETTER TO THE EDITOR

Flow cytometric analysis of de novo acute myeloid leukemia in childhood: report from the Japanese Pediatric Leukemia/ Lymphoma Study Group

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Immunophenotypic analysis has become a powerful tool for the correct identification of leukemic cell lineage. Our study evaluates the diagnostic utility of flow cytometric immunophenotyping of pediatric AML. We retrospectively collected data of immunophenotype from 375 cases of de novo AML studied from 1997 to 2007 at central laboratory institutions of the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG): Department of Pediatrics and Developmental Science, Mie University Graduate School of Medicine; Department of Pediatrics, Osaka University Graduate School of Medicine; Center for Clinical Research, National Center for Child Health and Development; and Department of Pediatrics, Aichi Medical University. The diagnosis of AML was made according to the French-American-British (FAB) classification based on morphology and enzyme cytochemical analysis as follows: M0 (acute myeloid leukemia without differentiation, $n = 11$), M1 (acute myelocytic leukemia with little differentiation, $n = 41$), M2 (acute myelocytic leukemia with differentiation, $n = 113$), M4 (acute myelomonocytic leukemia, $n = 47$), M5 (acute monocytic leukemia, $n = 54$), M6 (acute erythroleukemia, $n = 6$), and M7 (acute megakaryoblastic leukemia, $n = 61$).

Mononuclear cells of bone marrow or peripheral blood samples were stained with various combinations of fluorescein isothiocyanate (FITC)- and phycoerythrin (PE)-labeled monoclonal antibodies against the following antigens: CD4, CD7, CD13, CD14, CD15, CD19, CD33, CD34, CD36, CD41, CD42b, CD45, CD56, CD61, CD65, CD117, glycophorin A (GPA: CD235a), and HLA-DR. Cytoplasmic MPO was also detected by anti-MPO antibody after permeabilization. Two-color flow cytometric immunophenotyping was performed by collecting 10,000 ungated list mode events. An antigen was considered as

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Table 1 Immunophenotypic profile of 375 de novo cases of acute myeloid leukemia

	CD34	CD117	HLADR	MPO	CD13	CD33	CD14	CD15	CD65	GPA	CD36	CD41	CD42b	CD61	CD7	CD4	CD19	CD56	CD45
M0 (11)	72.7 (11)	90.9 (11)	63.6 (11)	45.5 (11)	54.5 (11)	90.0 (11)	0 (11)	33.3 (9)	16.7 (6)	0 (11)	9.1 (11)	9.1 (11)	9.1 (11)	ND	54.5 (11)	9.1 (11)	9.1 (11)	45.5 (11)	90.0 (10)
M1 (41)	85.4 (41)	100 (36)	73.2 (41)	100 (41)	90.2 (41)	97.6 (41)	2.6 (39)	60.7 (28)	75.0 (20)	0 (37)	18.9 (37)	10.0 (40)	0 (36)	ND	51.2 (41)	2.7 (37)	7.3 (41)	19.5 (41)	90.9 (33)
M2 (113)	83.8 (111)	94.4 (89)	89.2 (111)	96.4 (84)	91.2 (113)	92.9 (113)	7.4 (108)	55.1 (89)	33.3 (63)	0 (93)	12.0 (92)	4.5 (112)	2.2 (92)	ND	14.3 (112)	0 (95)	24.8 (113)	36.4 (110)	97.3 (74)
M3 (42)	14.3 (42)	76.3 (38)	4.8 (42)	96.9 (32)	92.9 (42)	97.6 (42)	4.8 (42)	15.6 (32)	53.8 (26)	2.8 (36)	5.6 (36)	0 (42)	10.8 (37)	ND	0 (42)	2.7 (37)	2.4 (42)	7.1 (42)	85.2 (23)
M4 (47)	53.2 (47)	76.7 (43)	78.7 (47)	94.9 (39)	87.2 (47)	93.6 (47)	29.8 (47)	80.0 (30)	80.6 (31)	2.3 (43)	51.2 (43)	10.6 (47)	4.5 (44)	ND	8.5 (47)	23.1 (39)	2.1 (47)	15.2 (46)	94.4 (36)
M5 (54)	24.1 (54)	39.6 (48)	81.5 (54)	68.6 (35)	64.8 (54)	98.1 (54)	34.6 (52)	74.5 (47)	87.1 (31)	2.3 (43)	60.5 (43)	5.6 (54)	2.1 (48)	ND	3.7 (54)	52.1 (48)	1.9 (54)	57.4 (54)	93.8 (32)
M6 (6)	50.0 (6)	66.7 (6)	50.0 (6)	80.0 (5)	100 (6)	100 (6)	0 (6)	0 (4)	25.0 (4)	66.7 (6)	83.3 (6)	0 (6)	0 (6)	ND	33.3 (6)	16.7 (6)	0 (6)	0 (6)	60.0 (5)
M7 (61)	41.1 (56)	74.5 (51)	49.1 (57)	2.8 (36)	73.7 (57)	90.0 (60)	1.9 (53)	8.9 (45)	5.7 (35)	32.0 (50)	78.0 (50)	72.4 (58)	58.5 (53)	85.7 (14)	69.6 (56)	20.0 (50)	1.7 (58)	45.6 (57)	96.8 (31)

Values indicate proportion of positive cases (%); parentheses indicate evaluable cases; *ND* not done

positive, if more than 30% of the gated cells showed specific labeling above that of controls, or if positive subpopulation was distinctively identified even in <30% positive cases.

The result is summarized in Table 1. Cytoplasmic MPO expression was found in less than half of cases with M0 (45.5%), which is consistent with other reports [1, 2]. However, M0 blasts expressed CD33 (90.0%) and CD117 (90.9%), and, less frequently, CD34 (72.7%), suggesting myeloid lineage. The low expression of CD13 as compared to CD33 in our study may reflect a more mature myeloid profile in pediatric cases [1, 3]. CD7, expressed in more than half cases, is known to be expressed in a proportion of AML-M0 and M1 cases [3–5], consistent with the fact that CD7 is expressed during early stages of normal myeloid differentiation [6]. CD56 was also expressed in nearly half of cases, but only one case co-expressed CD7 and CD56 consistent with NK/myeloid-cell precursor acute leukemic cells [7].

M1 and M2 blasts expressed CD34, CD117, HLA-DR, MPO, CD13, CD33, and HLA-DR in more than 80% of cases, and less commonly CD15 and CD65. CD7 was detected in 51.2% of M1 cases, while its expression was repressed in M2. CD19, detected in 24.8% of M2 cases, was reported to be detected in 78–81% of M2 cases with t(8;21) translocation [8, 9].

M3 cells expressed CD13, CD33, and MPO at high frequency, as for M1 or M2 cells. However, the frequency of CD117 expression was 76.7%, lower than for M1 or M2 cells. A striking feature is that the expression of CD34 and HLA-DR was low, at 14.3 and 4.8%, respectively. The lack of CD34 and HLA-DR was a feature of M3 blasts [4, 5, 10].

Leukemic cells of most M4 and M5 cases expressed monocyte markers, CD15 and CD65. The less common expression of CD14 has been reported by others, particularly in M5 cases [2, 5, 10]. M4 and M5 expressed CD33 at similarly high frequencies. The progenitor-associated antigens, CD34 and CD117, were seen in a lower proportion of M5 cases, which might reflect commitment to monocytic lineage. CD4 was expressed in 52.1% of M5 cases and 23.1% of M4 cases, in line with other reports [2, 10].

We observed only six M6 cases. Leukemic erythroblasts expressed CD36 and GPA in 66.7 and 83.3% of cases, respectively. Myeloid antigens (MPO, CD13, and CD33) and hematopoietic progenitor-associated markers (CD34 and CD117) were also expressed at variable frequencies. The expression of monocytic markers (CD14 and CD15) was absent, as well as megakaryocyte-associated antigens (CD41 and CD42b).

The expression frequencies of megakaryocyte-associated antigens, CD41 and CD42b in cases with M7, were